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BIOCIDAL POLYMERS

Edited by Narendra Pal Singh Chauhan

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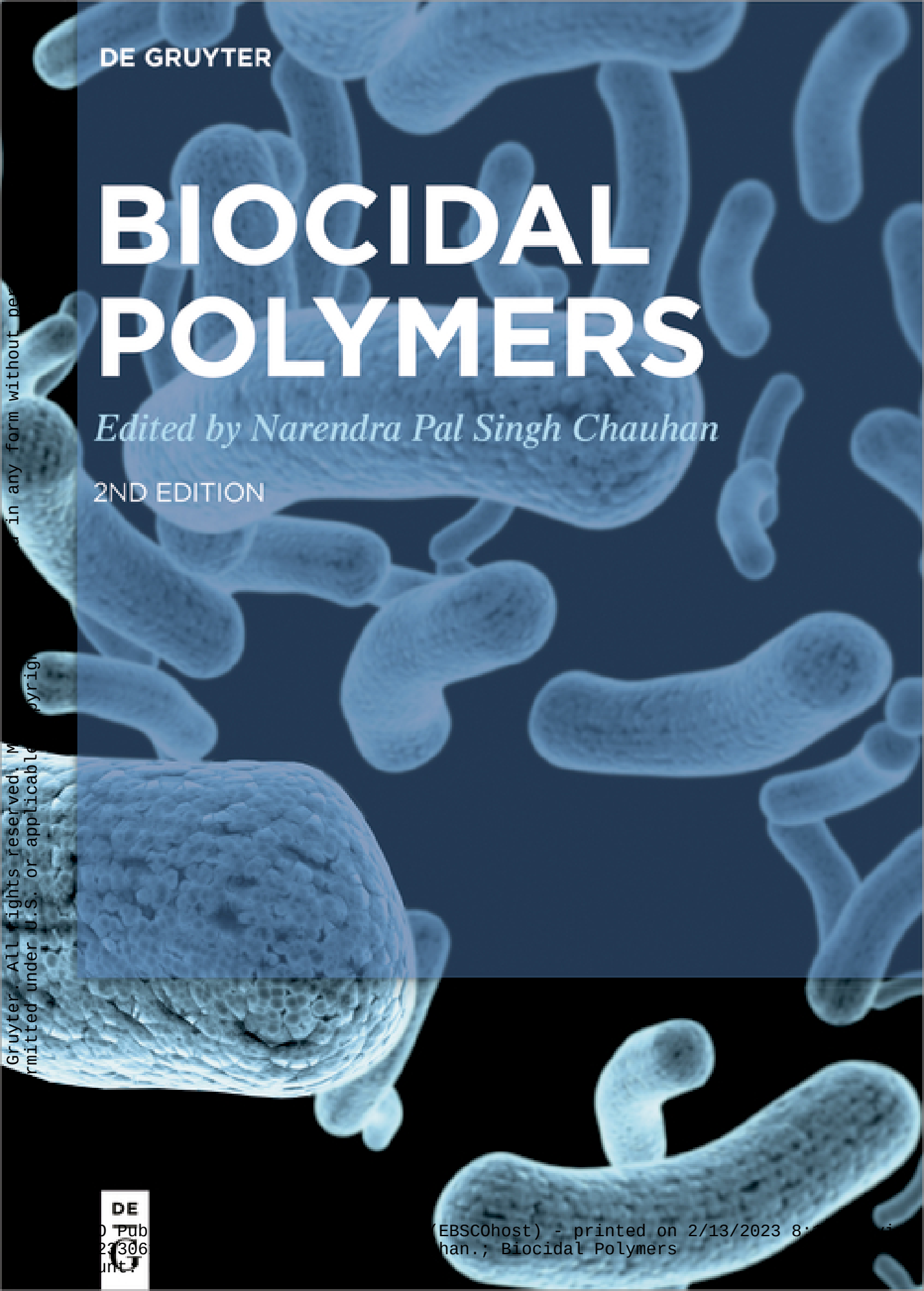

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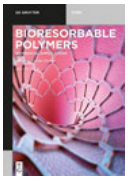

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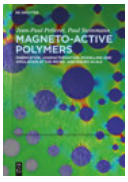

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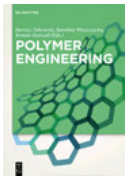

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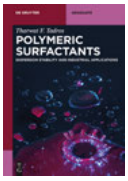

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Biocidal Polymers

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Preface

In the four years since the first edition of this book was published, I have received numerous email messages and letters from readers commenting on the book and suggesting how it could be improved. I have also built up a large file of ideas based on my own experiences in reading, writing and editing as well as in examining and reviewing many papers. With the aid of all this information I have completely revised the book. This edition provides technical corrections, updates and clarifications in all chapters; summarises new developments; and provides abstract and keywords at the beginning of each chapter.

This revised edition of the book is composed of 13 chapters which summarise the state of the art of the polymer industry, i.e., the synthetic strategy of using various antimicrobial polymers, including cationic biocidal polymers, amphiphilic biocidal polymers, biomimetic antimicrobial polymers, polymer–metal nanocomposites which exhibit biocidal properties, biodegradable polymers which exhibit antibacterial properties, polyethylene glycol- and polylactic acid-based antimicrobial polymers, conducting polymers, plastics and elastomers, functionalised antimicrobial polymers, *N*-halamine-based biocidal polymers which exhibit antimicrobial properties, different methods of antimicrobial testing, antimicrobial peptide (in a new chapter), and future perspectives in this field. The main focus of this edition is on the synthesis and mechanistic strategy of using biocidal activities of natural, biodegradable and synthetic polymers.

Now, I sincerely hope that this book will be of a general interest to microbiologists, biotechnologists, medical doctors, organic chemists, pharmacists, polymer scientists, food scientists and technologists. I also hope that this book offers a balanced, interesting and innovative perspective which is applicable to academics and industries.

Dr. Narendra Pal Singh Chauhan
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1 Cationic antimicrobial polymers

Abstract: Naturally derived and synthetic cationic polymers have been widely studied for their potential applications in biomedicine. These polymers carry positive charges in their backbone or side chains. Positive charge provides cationic polymers unique physico-chemical, biological and antimicrobial properties. These properties make them an excellent candidate for different applications, such as drug conjugation and delivery, tissue engineering and therapeutic applications. In this chapter, various types of cationic polymers, as well as their chemical structure, properties and biomedical applications are discussed in details.

Keywords: cationic polymers, biomaterials, antimicrobial polymers, tissue engineering, drug delivery, biomedicine

1.1 Introduction

The syntheses of polymeric systems bearing positive charges in the presence of novel cationic entities, incorporated either on their backbone or as side chains, are called cationic polymers. These systems exhibit unique physico-chemical properties and their ability to allow further modification renders them highly appealing for biological applications. The interest in cationic polymers results from their potential to form polyelectrolyte complexes with nucleic acids (deoxyribonucleic acid [DNA]), ribonucleic acid [RNA] and peptide nucleic acid). Cationic polymers show potential as biomaterials for the treatment of various human diseases. The properties of a cationic polymer are highly dependent upon polymer chain flexibility, hydrogen (H)-bond formation, hydrophobic interactions, electrostatic forces, charge transfer potential, amine group with its neighbouring functionalities, pKa and nucleophilic character.

Cationic polymers mediate transfection via the condensation of nucleic acids, provide protection from enzymatic degradation and facilitate cellular uptake and endolysosomal escape, making them an excellent candidate for gene delivery. However, their development has also expanded to other applications, including drug conjugation and delivery, tissue engineering and therapeutic applications.

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1.2 Naturally derived cationic polymers

Natural cationic polymers are generally non-toxic, derived from renewable resources, biocompatible, biodegradable and possess low immunogenicity. Most natural cationic polymers contain reactive sites, which can be easily modified to improve physico-chemical properties.

1.2.1 Chitosan

Chitosan (CS) is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and *N*-acetyl-D-glucosamine (acetylated unit). It is produced by treating shrimp and other crustacean shells with the alkali, sodium hydroxide (NaOH). CS has a number of commercial and possible biomedical uses. In medicine, it is used in bandages to reduce bleeding and as an antibacterial agent; it can also be used to help deliver drugs through the skin. The amino group in CS has a pKa value of ~6.5, which leads to protonation in acidic-to-neutral solutions with a charge density dependent upon pH. This makes CS water-soluble and a bioadhesive, which readily binds to negatively charged surfaces such as mucosal membranes. CS enhances the transport of polar drugs across epithelial surfaces, and is biocompatible and biodegradable.

The properties of CS also allow it to be used in transdermal drug delivery; it is mucoadhesive in nature, reactive (so it can be produced in many different forms), and most importantly, has a positive charge under acidic conditions. This positive charge comes from protonation of its free-amino groups. Lack of a positive charge means CS is insoluble in neutral and basic environments. However, in acidic environments, protonation of the amino groups leads to an increase in solubility. This molecule will maintain its structure in a neutral environment, but will solubilise and degrade in an acidic environment. This means CS can be used for transporting a drug to an acidic environment, where the CS packaging will then degrade, releasing the drug to the desired environment; the transport of insulin is one of the greatest examples of drug delivery in this regard.

CS exhibits three reactive sites, which enable modification, including one primary amine and two primary or secondary hydroxyl groups per glucosidic unit. The modification of CS using cationic moieties has been achieved via quaternisation of the amino group or by grafting small molecules or polymer chains onto the CS backbone. However, modifications do not imply a change in the fundamental properties of CS but introduce new properties. The quaternisation of CS has been investigated by several research groups [1, 2]. This strategy provides good control over the cationic character without affecting its pH independency, which is desirable for improving the stability of ionic complexes. In addition, the solubility of CS in water is increased, rendering it

soluble over a wide pH range. The reaction of CS with methyl iodide under basic conditions is the most straightforward route to quaternise CS.

Quaternisation improves the mucoadhesive properties of CS, the extent of which depends on the degree of quaternisation, which makes this CS derivative a good candidate for gene delivery. Jia and co-workers reported the synthesis and antibacterial activities of quaternary ammonium salts of CS, including *N*,*N*,*N*-trimethyl CS, *N*-propyl-*N*,*N*-dimethyl CS, *N*-furfuryl-*N*,*N*-dimethyl CS and *N*-diethylmethylamino CS [3]. In addition, quaternised chito-oligomers have also demonstrated antibacterial activity [4]. By exploiting the cationic nature of CS, several drug conjugate approaches have been developed for therapeutic applications. Yang and co-workers synthesised folic-acid–conjugated CS as a carrier for 5-aminolevulinic acid and determined its targeting and uptake efficiency in different human colorectal cancer cell lines (human colon epithelium adenocarcinoma – HT29 and heterogenous human epithelial colorectal adenocarcinoma cells – Caco-2) via folate receptor-mediated endocytosis [5].

1.2.2 Gelatin

Gelatin is a translucent, colourless, brittle (when dry), flavourless foodstuff, derived from collagen, which is obtained from various animal by-products. It is commonly used as a gelling agent in food, pharmaceuticals, photography and cosmetic manufacturing. Substances containing gelatin or functioning in a similar way are called gelatinous. Gelatin is an irreversibly hydrolysed form of collagen and is a mixture of peptides and proteins produced by the partial hydrolysis of collagen extracted from the skin, bones and connective tissues of animals, such as domesticated cattle, chicken, pigs and fish. During hydrolysis, the natural molecular bonds between individual collagen strands are broken down into a form that is more easily rearranged.

Gelatin is composed of 18 non-uniformly distributed amino acids with both positive and negative charges. The inherent cationic property of gelatin is largely due to lysine and arginine residues. The denaturation process, through which gelatin is obtained from collagen, involves acidic or basic treatment, resulting in gelatin A with an isoelectric point (IEP), the pH at which a particular molecule carries no net electrical charge, of 6–9 and gelatin B with an IEP of 4.7–5.4, respectively [6]. Gelatin exhibits cationic behaviour at pH values below its IEP via protonation of amino groups. The cationic density is higher for acidic gelatin and lower for basic gelatin. The US Food and Drug Administration (FDA) classified gelatin as a safe excipient and it is currently used as a constituent of various biomaterials [7].

Gelatin is generally cationically derivatised to enable interactions with biomolecules of an anionic nature without being dependent upon pH; for example, Xu and co-workers applied cationic gelatin nanoparticles (NP) for the non-viral delivery of

plasmid-deoxyribonucleic acid (p-DNA) encoding insulin-like growth factor-1 (IGF-1) to adult canine articular chondrocytes in vitro[8]. The results of the study revealed that chondrocytes transfected with IGF-1, using cationic gelatin NP, were able to maintain steady IGF-1 overexpression when subsequently grown in cationic gelatin scaffolds for up to 2 weeks in a three-dimensional culture.

1.2.3 Cationic dextran

Dextran is a complex, branched glucan (a polysaccharide made of many glucose molecules) composed of chains of varying lengths (from 3 to 2,000 kilodaltons). It is used medicinally as an antithrombotic (antiplatelet) agent, to reduce blood viscosity and as a volume expander in hypovolaemia.

The straight chain consists of α-1,6 glycosidic linkages between glucose molecules, while branches begin from α-1,3 linkages. This homopolysaccharide is suitable as a polymeric carrier due to its biodegradability, wide availability, ease of modification and solubility in water irrespective of the pH. A series of dextran-based cationic polymers including diethylaminoethyl–dextran and dextran–spermine have been prepared for the efficient delivery of nucleic acids [9]. As an alternative to protamine in anticoagulant therapy, Kaminski and co-workers [10] prepared cationic derivatives of dextran by substituting hydroxyl groups with glycidyltrimethylammonium chloride (GTMAC). The degree of substitution of the polymers ranged from 0.50 to 0.65 GTMAC groups per glucose unit. These cationic dextran derivatives formed complexes with unfractionated heparin and the binding efficiency correlated with the degree of cationic modification.

Dextran–spermine-based conjugates have been prepared via reductive amination between oxidised dextran and spermine [11]. Spermine, a naturally occurring linear polyamine, is involved in cellular metabolism and is a polycation at physiological pH. Dextran was initially oxidised with potassium periodate and the obtained dialdehyde derivative was then reacted under basic conditions with spermine. Dextran–spermine displayed particularly high transfection efficiency, which was attributed to the unique complexation properties between DNA and the grafted spermine moieties. Dextran– spermine and their derivatives have shown high transfection of *p*-DNA both in vitro and in vivo [12].

Cohen and co-workers combined the unique characteristics of acetyl-dextran (Ac-DEX) and spermine with small interfering RNA – a class of double-stranded RNA molecules, 20–25 base pairs in length – as a delivery system. Ac-DEX possesses several characteristics suitable for the delivery of bioactive agents such as proteins. The novel system combined ease of synthesis and biocompatibility with the advantage of controlled release, that is, sensitivity to physiologically relevant acidic conditions. Acid-catalysed hydrolysis of spermine–Ac-DEX generated spermine-modified dextran, which could be further metabolised in vivo by enzymes [13].

1.2.4 Cationic cellulose

Cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$, a polysaccharide consisting of a linear chain of several hundreds to many thousands of β(1→4)-linked D-glucose units. Cationic cellulose derivatives, which possess many useful characteristics including hydrophilicity, biodegradability and antibacterial properties, have numerous therapeutic applications [14, 15]. An important cationic cellulose derivative was prepared by the etherification of cellulose using glycidyl ammonium salts or alkylene epoxides in the presence of a suitable alkaline catalyst, usually NaOH [16]. Song and co-workers reported the homogeneous quaternisation of cellulose in an aqueous solution [17]. Cellulose was dissolved in an aqueous solution of NaOH–urea followed by the addition of 3-chloro-2-hydroxypropyltrimethylammonium chloride (CHPTA chloride), an etherifying agent under alkaline conditions. Under these conditions, an epoxide was produced in situ and quaternised cellulose was subsequently formed via the reaction between cellulose and sodium alkoxide; this reaction resulted in the formation of diols as a side product. The quaternised cellulose derivatives that were obtained in the aqueous system proved to be promising gene-carrying agents. Despite the successful preparation of cationic cellulose, cationic cellulose derivatives, prepared directly from cellulose via a homogeneous process, have been scarcely reported due to the insolubility of cellulose in water and in most organic solvents as a result of strong inter- and intramolecular hydrogen bonding. Among the other cellulose derivatives, hydroxypropylcellulose (HPC) and hydroxyethyl cellulose (HEC) are the most widely utilised [18]. HPC-based materials have been approved by the FDA and are widely used in food and drug formulations. The transfection properties of cationic HEC/p-DNA NP for gene-delivery applications were investigated by Fayazpour and co-workers [19]; they evaluated the DNA complexation properties of two types of cationic HEC, including polyquaternium-4-cellulose (PC-4) and polyquaternium-10-cellulose (PC-10). In both PC-4 and PC-10, the sugar monomers were substituted with polyethylene glycol (PEG). However, in PC-4, the quaternary ammonium groups were directly linked onto the cellulose backbone, while, in PC-10, the quaternary ammonium groups were present at the end of the PEG chains.

1.3 Synthetic cationic polymers

1.3.1 Poly[2-(dimethylaminoethyl)methacrylate]

Poly[2-(dimethylaminoethyl)methacrylate] (pDMAEMA) (Figure 1.1) is a mucoadhesive antimicrobial polymer that is cationic when dissolved in acidified media or if

quaternised with alkylating agents [20, 21]. It can be produced cheaply and at high yield and purity by atom transfer radical polymerisation (ATRP) [22]. Cationic antimicrobial agents may prevent device-associated infections caused by *Staphylococcus epidermidis* and *Staphylococcus aureus*. Research has also shown that the cationic antimicrobial polymer, pDMAEMA, is more effective at the antagonising growth of clinical isolates of *Staphylococcus epidermidis* than *Staphylococcus aureus*.

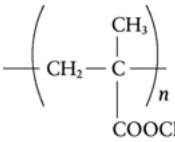

Figure 1.1: pDMAEMA [23].

1.3.1.1 Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* strains to poly[2-(dimethylaminoethyl)methacrylate]

Agar diffusion assays were performed for estimating the susceptibility of 11 *Staphylococcus aureus* and 11 *Staphylococcus epidermidis* strains. At each of the three concentrations of pDMAEMA tested, *Staphylococcus epidermidis* was significantly more sensitive than *Staphylococcus aureus*. Although pDMAEMA (20 mg/mL) partially inhibited the growth of *Staphylococcus aureus*, such high concentrations are not physiologically relevant [24].

1.3.1.2 Effect of poly[2-(dimethylaminoethyl)methacrylate] on Planktonic and Biofilm *Staphylococcus aureus* and *Staphylococcus epidermidis* cultures

pDMAEMA has a minimum inhibitory concentration (MIC) value against planktonically grown *Staphylococcus epidermidis* 1457 of 0.1 mg/mL but has no inhibitory effect against *Staphylococcus aureus* RN4220 at concentrations up to 18 mg/mL [25]. Fluorescence analysis of Alamar Blue reduction showed that pDMAEMA disrupted the 24 h biofilm growth of *Staphylococcus epidermidis* 1457 at a concentration of 1 mg/mL; however, no further decreases in viability were observed following increases in concentration from 1 to 50 mg/mL. Conversely, even at concentrations as high as 50 mg/mL, pDMAEMA had no effect on the *Staphylococcus aureus* RN4220 biofilm.

1.3.1.3 Role of surface charge in the susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to cationic antimicrobial agents

The different susceptibilities of *Staphylococcus epidermidis* and *Staphylococcus aureus* strains to the cationic agent pDMAEMA suggested a possible role for bacterial surface charge. To investigate this, zeta potentials (the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle) were measured at pH 7.5, reflecting the pH value used in antimicrobial assays. *Staphylococcus aureus* was significantly less negatively charged than *Staphylococcus epidermidis* at pH 7.5.

1.3.1.4 Role of surface hydrophobicity in the susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to poly[2-(dimethylaminoethyl)methacrylate]

Staphylococcus aureus strains are more hydrophobic than *Staphylococcus epidermidis* strains. The greater surface hydrophobicity of *Staphylococcus aureus*, along with the reduced negative surface charge on these bacteria, correlated with its increased resistance to pDMAEMA (Spearman's correlation coefficient >0.90). (Spearman's correlation coefficient is a non-parametric measure of statistical dependence between two variables and assesses how well the relationship between two variables can be described using a monotonic function. If there are no repeated data values, a perfect Spearman correlation of +1 or −1 occurs when each of the variables is a perfect monotone function of the other.) Although the *Staphylococcus aureus* isolates are more hydrophobic, as determined by mean values, some individual isolates had bacterial adhesion to hydrocarbon values similar to those of *Staphylococcus epidermidis* strains [23].

1.3.1.5 Binding of poly[2-(dimethylaminoethyl)methacrylate] to bacteria

The cationic antimicrobial agent, pDMAEMA, is more active against *Staphylococcus epidermidis* than *Staphylococcus aureus* at least in part due to the decreased hydrophobicity and increased negative surface charge on *Staphylococcus epidermidis*. pDAEMA also showed significant potential to attack established biofilms, which suggests that it may have a role as an antimicrobial coating on indwelling medical devices or in catheter lock solutions.

1.3.2 Antimicrobial cationic polyethylenimines

Pathogenic bacteria pose a significant threat to human health. Surface coatings derived from cationic, alkylated polyethylenimine(s) (PEI+) (Figure 1.2a) have

demonstrated antimicrobial activity against a wide variety of bacteria [26, 27]. A suggested mechanism by which these polymers kill bacteria involves membrane disruption. The biocidal effects of PEI+ are influenced by the cationic charge of PEI+ [28], the size of the polymer and length of the alkyl side chain [29]. Gram-negative bacteria such as *Escherichia coli* possess extracytoplasmic stress responses that allow them to both sense and respond to perturbations of their cellular envelope, including potentially lethal environmental stresses. There are three envelope stress responses of *Escherichia coli*: the Cpx two-component envelope stress response system comprised of an inner membrane histidine kinase CpxA, the cytosolic response regulator CpxR and the extracytoplasmic function (ECF) sigma-factor-regulated responses are involved in the sigma (E) envelope stress response of *Escherichia coli*. Each of the three characterised envelope stress responses (rE, CpxRA and Bae) exhibits unique inducing signals, as well as signals that overlap with the other two responses. The rE stress response detects stresses that perturb the outer membrane, such as heat and ethanol [30], the CpxRA stress response detects changes in pH and certain misfolded periplasmic proteins [31, 32], and the Bae stress response is sensitive to both sodium tungstate and some flavonoids [33]. The existence of these three known stress responses and their correlation with specific types of inducing signals allow us to employ stress response activation as an assay to assess potential mechanisms by which PEI+ causes bacterial cell death. For comparison, stress response activation was also studied using two other compounds as inducers, a non-antimicrobial polymer polyethylene oxide (PEO) (Figure 1.2b) and a small molecule with demonstrated antimicrobial activity, vanillin (Figure 1.2c). PEO was chosen as a comparison for PEI+ to elucidate if any observable stress response induction by PEI+ was due solely to its polymeric structure and, therefore, unrelated to its biocidal properties. Vanillin provided contrast as a small antimicrobial molecule with an established mechanism of action involving membrane disruption [34].

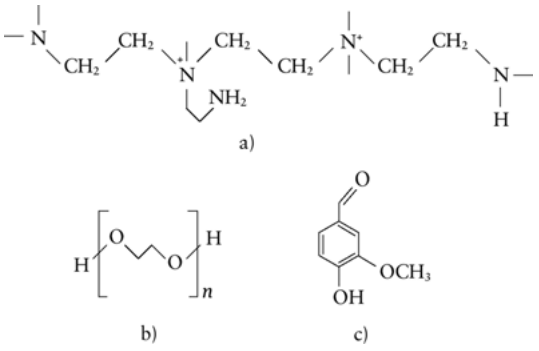

Figure 1.2: Chemical structure of (a) PEI+, (b) PEO and (c) vanillin [35].

Antimicrobial, cationic PEI+ is synthesised from a commercially available, branched polyethylenimine (PEI) by alkylating with dodecyl bromide and methyl iodide. Although methods for preparing antimicrobial surface coatings from PEI+ exist, studies of *Escherichia coli* stress response induction required formulating new protocols to allow PEI+ to exert its antimicrobial effect in solution as adhesion to surfaces is known to induce one of the three extracytoplasmic stress responses in *Escherichia coli* [36]. PEI+ is minimally soluble in water and Lysogeny broth growth media, but is soluble in *n*-butanol; hence, solutions of PEI+ were prepared in *n*-butanol and added to cultures of *Escherichia coli* such that the amount of *n*-butanol was less than 0.4% v/v. Growth inhibition was observed after 3 h starting at 80 μg/mL and the bacterial density was reduced by approximately 50% at 320 μg/mL. In contrast to PEI+, PEO was substantially more soluble in growth media and demonstrated no growth inhibition at 80–320 μg/mL of polymer. The small antimicrobial molecule, vanillin, was highly soluble in ethanol and allowed the introduction of vanillin to growth cultures using volumes of ethanol that did not affect bacterial densities. Three different strains bearing reporter gene fusions were used to assess the stress response induction. Each gene fusion couples the expression of stress response proteins, CpxRA, rE or BaeSR, to the production of a reporter protein, β-galactosidase (a fusion gene is a hybrid gene formed from two previously separate genes). The induction of each stress response was assessed by measuring the amount of β-galactosidase produced by the bacteria after exposure to PEI+, PEO or vanillin. Quantification of the stress response induction by PEI+, PEO and vanillin was obtained by introducing 80–320 μg/mL of each polymer or 320–640 μg/mL of vanillin, dissolved in appropriate alcohol, if needed, to bacterial cell cultures. This method of analysis insured that the magnitude of the stress response induction of each compound was independent of any effect due to the alcohol alone. For experiments involving PEO, a water-soluble polymer, the induction ratio was derived from comparing the quantified stress responses of PEO to that of a sample where an equivalent volume of water had been added.

Induction of the CpxRA stress response by PEI+ was found to be dose dependent. The polymeric character of PEI+ appears not to be solely responsible for its ability to induce the CpxRA stress response. The non-antimicrobial polymer, PEO, failed to induce any of the three extracytoplasmic stress responses at concentrations identical to those of PEI+, which were sufficient to induce CpxRA. This result suggests that the stress response induction by PEI+ is not simply attributable to its polymeric nature since a polymer of similarly high molecular weight (MW), but with no antimicrobial activity, did not produce the same effect. In contrast to PEI+ and PEO, the small antimicrobial molecule, vanillin, only induced the rE stress response, approximately 12–14-fold in the presence of 640 μg/mL vanillin. Induction of rE is associated with perturbations that specifically disrupt the folding of the outer membrane protein. Further evidence for different interactions of PEI+ and vanillin with the bacterial membrane were seen in electron paramagnetic resonance (EPR) studies of spin-labelled liposomes, an established method for observing

changes in membrane stability [37]. The spin-labelled liposomes were exposed to PEI+ (320 μg/mL), PEO (320 μg/mL) and vanillin (640 μg/mL). The effects of PEI+ on the EPR spectral parameters were compared with those of the corresponding solvent controls (*n*-butanol for PEI+, buffer for PEO and ethanol for vanillin). Both PEI+ and PEO decreased the mobility of the spin-labelled liposomes, consistent with the notion that these agents exert a stabilising effect on the membrane. Conversely, vanillin had a negligible effect on spin-labelled liposome mobility.

Overall, it was determined that antimicrobial, cationic PEI is a specific inducer of the CpxRA stress response in *Escherichia coli*. In contrast, the small antimicrobial molecule, vanillin, known to disrupt membranes, specifically induced the rE stress response. These results suggest that extracytoplasmic stress response induction can provide an intriguing window into the differences of the bactericidal action of antimicrobial compounds [35].

1.3.3 Antimicrobial polymethacrylamide derivatives

Antimicrobial peptides (AMP) show great potential as they are small biopolymers (~20–50 amino acids in length) that can selectively bind and eliminate pathogenic bacteria, within a certain therapeutic range, without harming eukaryotic cells [38]. They are produced naturally by many complex, multicellular organisms and play a role in immunity processes. AMP have diverse amino acid sequences, however they typically display a net positive charge at physiological pH. This cationic net charge results from an abundance of basic lysine and arginine residues. Most eukaryotic cells are rich in zwitterionic phospholipids such as phosphatidylethanolamine, phosphatidylcholine and sphingomyelin. The net positive charge of AMP and the net negative charge of bacterial cell membranes drive the initial attraction of the peptide to the cell surface [39]. Specifically, lysine and arginine residues interact strongly with negatively charged phospholipids, allowing electrostatic interactions to govern the initial binding to targeted cell membranes [40]. After binding to the negatively charged phospholipid, hydrophobic moieties on the peptide interact with the inner hydrophobic core of the bacterial membrane leading to a disruption in integrity and subsequent cell death. AMP show great promise as therapeutic agents to combat infections and biocidal applications in general; however, they are produced naturally at low levels and synthesising them in the laboratory is quite expensive. For this reason, there is extensive interest in developing synthetic systems that mimic AMP behaviour. Several groups have synthesised low MW oligomers that possess antimicrobial properties [41, 42]. Tew and co-workers [43] utilised ring-opening metathesis polymerisation to prepare polynorbornene derivatives with pendent primary amines resembling the functionality of lysine. Mowery and co-workers [44] prepared statistical β-lactam copolymers to form an amphipathic, antimicrobial polymer. In an attempt to further improve the biocompatibility of antimicrobial polymers,

Venkataraman and co-workers [45] used reversible addition-fragmentation transfer (RAFT) polymerisation to create antimicrobial PEG functionalised polymers from methacrylate derivatives. The authors statistically copolymerised *N*-[3-(dimethylamino)ethyl]methacrylate (DMAEMA) and oligo(ethylene glycol)-methylether-methacrylate and then performed postpolymerisation quaternisation of the pendent DMAEMA nitrogen with different halide functional species, testing the relationship of chain length and chemical group for antimicrobial properties. It was concluded that shorter hydrophobic chain lengths (i.e. methyl and ethyl) produced polymers with the best antimicrobial properties. De-Grado and Kuroda [46] synthesised antimicrobial polymethacrylate derivatives via free-radical polymerisation and obtained a system exhibiting microbial toxicity. The effect of a pendantamine structure on antimicrobial activity was described for low MW (polydispersity index [PDI] = 6–10) random copolymers prepared from similar methacrylate derivatives [47]. Within this system, it was concluded that primary amines helped to maximise antimicrobial properties, while quaternary ammonium-containing polymers required the addition of excess hydrophobic functionality to realise potent activity. The goal of the study was to define the roles of cation structure and distribution of hydrophobic moieties of protonated amines to determine the selective toxicity behaviour of polymethacrylamide derivatives. The use of aqueous RAFT to copolymerise *N*-(3-aminopropyl)methacrylamide (APMA), which mimics the cationic amino acid lysine, with *N*-[3-(dimethylamino)propyl]methacrylamide (DMAPMA) or *N*-[3-(diethylamino)propyl] methacrylamide (DEAPMA), both of which impart a hydrophobic property to amines while maintaining the water solubility of each of the individual monomers, can be explained by the fact that aqueous RAFT facilitates the polymerisation of primary amines without the need for protecting group chemistry or organic solvents [48]. A series of copolymers were prepared with the systematic variation of (1) the ratio of primary to tertiary amines and (2) the concentration and structure of hydrophobic groups (DMAPMA and DEAPMA comonomers) to gain greater understanding of the effects of the individual components on selective toxicity.

1.3.3.1 Synthesis of homopolymers and copolymers

Methacrylamide monomers were chosen due to their hydrolytic stability, structural similarity to amino acids found in naturally occurring AMP, incorporation of hydrophobic and hydrophilic moieties and pKa values. APMA was chosen due to its similarity to lysine. While ionic bonding facilitates initial polymer–cell interactions, it is the hydrophobic substituents that act to disrupt the lipid membrane of bacteria. DMAPMA and DEAPMA were chosen due to their hydrophobic dimethyl and diethyl amino groups, respectively. Copolymers were formed by copolymerising APMA with DMAPMA and APMA with DEAPMA at varying ratios.

1.3.3.2 Antimicrobial activity

The biocidal nature of the polymers was initially investigated using the broth microdilution method, which is used to simultaneously test the susceptibility of bacteria to multiple antibiotics and is highly accurate. All polymers were tested against *Escherichia coli* and *Bacillus subtilis*; the MIC values were reported as a function of saline concentration, incubation time (6 and 24 h), polymer concentration (0–2 mg/mL), polymer composition (effect of hydrophobic functionality and cation architecture) and bacterial cell line. The presence of salt is known to induce the antipolyelectrolyte effect [49]; thus, it was necessary to determine if physiological salt conditions would influence the polymer–cell electrostatic interaction. In general, high antimicrobial activity (defined as an MIC value of 100 μg/mL or lower) was exhibited by all of the polymers against *Escherichia coli*. On the other hand, a generally lower antimicrobial efficiency was observed against the Gram-positive bacteria, *Bacillus subtilis*.

1.3.3.3 Haemolysis

An effective way to test eukaryotic cell toxicity is to study the haemolytic behaviour of a system. While not conclusive, haemolysis experiments are used to indicate the possibility of eukaryotic cell toxicity [50]. It was found that, at the highest concentration tested (3 mg/mL), minimal haemolysis was observed. The high selectivity of these polymers is attributed to the use of fully water-soluble monomers, which allow the incorporation of hydrophobic moieties while maintaining the water-solubility of the polymer. A common practice within the antimicrobial polymer community is to report polymer selectivity toward bacterial cells over eukaryotic cells as a ratio of HC_{50}/MIC, where HC_{50} is the polymer concentration required to lyse 50% of the red blood cells (RBC). At 3 mg/mL, haemolysis was near 5% for each polymer, which is well below 50% [51].

1.3.4 Biocidal activity of Polystyrenes

The development of a new class of antibiotics has become increasingly important. Among the possible candidates, antimicrobial cationic peptides have attracted increasing research and clinical interest. Antimicrobial cationic peptides, including magainins, defensins, cecropins, melittin and thionins, have been found in a variety of sources, such as amphibians, mammals, insects, plants and bacteria [52]. These cationic peptides, and many others, exhibit potent activities against a rather broad spectrum of microbial organisms, including Gram-positive and Gram-negative bacteria, fungi and enveloped viruses [53]. Based on their secondary structure, cationic peptides can be categorised into four major groups, including α-sheet structures

stabilised by 2–3 disulfide bridges, R-helices, extended structures with a predominance of one or more amino acids and loop structures containing only one disulfide bridge.

The topic of discussion in this chapter arose from an interest in determining whether conformational preorganisation is required for antimicrobial activity in synthetic oligomers or polymers. The helical antimicrobial α-peptides produced were composed of cyclically constrained α-amino acids and were quite rigid [54]. As a result, antimicrobial activity was completely lost upon sequence scrambling to give a non-amphiphilic helix. In contrast, R-helical host-defence peptides (HDP) can be scrambled with only modest loss of activity [55]. This difference in the effect of residue scrambling can be explained by the increased flexibility of the R-peptide backbone, which might allow scrambled sequences to populate non-helical but nevertheless globally amphiphilic conformations. The highly preorganised α-peptide backbone cannot adopt such alternative, non-helical conformations. This speculation led to the question of whether a sufficiently flexible synthetic polymer backbone might be able to display a random sequence of cationic and lipophilic side chains in a manner resulting in global amphiphilicity and, therefore, antimicrobial activity. This was addressed by comparing polymers that contained 4-(dimethylaminomethyl)-styrene [poly(**1**)] (Figure 1.3) linked to an R-peptide, such as Ala8,13,18-magainin 2 amide, which is known to display potent antimicrobial activity [56]. Polymers that are cationic by virtue of quaternised nitrogen and structurally related to poly(**1**) have long been studied as antimicrobial agents [57]; polymers that contain poly(**1**) differ from the previously produced quaternised polymers in that, like HDP, polymers containing dimethylaminomethyl groups require protonation to develop a positive charge, as shown in Figure 1.3. Poly(**1**) and copolymers containing poly(**1**) and 4-octylstyrene [poly(**2**)] (Figure 1.3) via azobisisobutylonitrile (AIBN)-initiated radical polymerisation were prepared [58]. Most samples had a number average molecular weight (*M*n) near 3,000, comparable to the MW of the magainin derivative (2,478). The PDI values of these samples were close to 3.0, indicative of the broad MW distributions typically associated with AIBN-initiated radical polymerisations. MIC for poly(**1**) and a series of copolymers containing poly(**1**) and poly(**2**) (up to 40 mol% poly(**2**) as estimated by ^{1}H-nuclear magnetic resonance [NMR]) were determined with *Escherichia coli*, [59] *Bacillus subtilis* [60], methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* [61]. Turbidity-based assays for bacterial growth inhibition were conducted at polymer concentrations up to 50 μg/mL, a limit determined by polymer solubility in the assay media. Poly(**1**) displayed significant antimicrobial activity against all four bacteria. Poly(**1**) is less active than the magainin derivative, particularly toward the pathogenic *Staphylococcus aureus* and *Enterococcus faecium* strains; however, this variation seems relatively modest in light of the profound difference in molecular structure between the peptide and poly(**1**) and the difference in effort required for the chemical synthesis of the peptide versus the homopolymer. Comparison of poly(**1**) with poly[trialkyl-3-(and 4-)vinylbenzylammonium chloride] [poly(**3**)] (Figure 1.3) was performed for evaluating the

difference between *N*-protonation and *N*-quaternisation as a source of positive charge. HDP, such as magainins, are much more effective at disrupting bacterial cell membranes than disrupting eukaryotic cell membranes. Poly(**1**) is highly haemolytic, somewhat more so than pure melittin (on a weight basis); hence, this polymer is more a mimic of melittin than of an HDP. Both non-polar and electrostatic forces are believed to be important in the interactions of HDP, such as magainins, and general toxin peptides, such as melittin, with cell membranes. It therefore seemed curious that the introduction of hydrophobic units, derived from poly(**2**), did not cause a significant increase in lytic activity relative to poly(**1**).

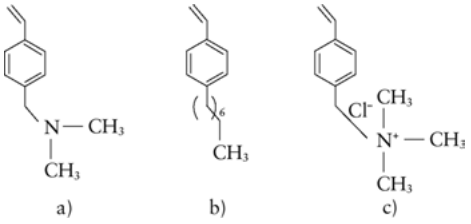

Figure 1.3: Structure of (a) poly(**1**), (b) poly(**2**) and (c) poly(**3**) [66].

Monomeric quaternised ammonium compounds containing sufficiently hydrophobic organic groups are toxic to bacteria, presumably via a detergent mode of membrane disruption [62]. To evaluate the possibility of poly(**1**) forming micellar subdomains that disrupt cell membranes in a similar way, that is, whether this material is simply a polymeric detergent, the solubilisation of orange OT in an aqueous solutions of poly(**1**) was attempted. Orange OT is a highly hydrophobic dye that does not dissolve in water. This substance can be used to determine critical micelle concentrations of surfactants because orange OT dissolves in the non-polar core of surfactant aggregates [63]. However, orange OT was not soluble in aqueous solutions of poly(**1**) or copolymers formed from poly(**1**) and poly(**2**); thus, the biocidal properties of these polymers do not arise from a detergent-like membrane disruption mechanism. Poly (**1**) is broadly biocidal, killing bacteria and lysing human RBC at relatively low concentrations, and bears some similarity to a number of polystyrene (PS)-derived antibiotics that are cationic by virtue of *N*-quaternisation. Poly(**1**) displays comparable or slightly reduced activity toward both Gram-positive and Gram-negative bacteria, including human pathogens that are resistant to clinical antibiotics, relative to a potent derivative of the HDP, magainin 2. Poly(**1**) is much less expensive to prepare than a peptide such as magainin 2 or synthetic oligomers [64] that have been shown to display magainin-like activity. The lack of discrimination between prokaryotic and eukaryotic cells by poly(**1**) diminishes its potential for biomedical applications relative to HDP or their oligomeric mimics, but structure–activity relationships established

among peptides suggest strategies for improving the cell selectivity of synthetic polymers [65]. In particular, decreasing the hydrophobicity of the polymeric backbone seems to be a promising approach [66].

1.3.5 Cationic antimicrobial peptide derived from Polyhydroxyalkanoates

Polyhydroxyalkanoates (PHA) are well-studied biodegradable polymers, which are produced by bacteria. PHA can be divided into two broad groups: short chain length (scl)-PHA and medium chain length (mcl)-PHA [67]. The monomers of scl-PHA are made up of between 3 to 5 carbons, while the monomers of mcl-PHA are between 6 and 12 carbons long [68]. The medical applications of PHA are basically focused on sutures, drug delivery and tissue-engineering scaffolds [69]. The cytotoxic effect of cationic antimicrobial peptides (CAP) on microorganisms and neoplastic cells is largely due to their amphipathic nature and secondary structure [70]. CAP are a class of natural macromolecules and their synthetic analogues exhibit selective cytotoxicity against a broad spectrum of human cancer cells, including neoplastic cells that have acquired a multidrug-resistant phenotype. Conjugation of fatty acids to known antitumour chemotherapeutic agents resulted in improved killing potential when administered in a conjugated form [71]. The conjugation of a hydroxylated fatty acid [(R)-3-hydroxydecanoic acid] offers an advantage over conjugation of a fatty acid as the former contains a chiral centre, which can be used as a synthetic chemistry focal point for future modification of the drug molecule. Monomers of the biodegradable polymer mcl-PHA are being used to enhance the anticancer activity of a peptide and the mechanism of enhanced activity will now be described. In an attempt to improve upon naturally occurring anticancer peptides, hybrid peptides have been synthesised, which combine the best qualities of individual anticancer peptides [72]. DP18 (synthetic peptide) is the D isomer of the hybrid L peptide P18{P18[KWKLFKKIPKFLHLAKKF-NH(2)]}, an α-helical AMP designed from a cecropin A- magainin-2 hybrid, known to have potent antimicrobial activity against bacteria as well as fungi and does not exhibit any haemolytic activity. LP18 (a leader peptide) is cytotoxic to human cancer cell lines (IC_{50} values 3.5–8 μM) of breast (MD-MB-361-human breast adenocarcinoma) and leukaemic [Jurkat and K-562-leukaemia cell lines)] origin, while not exhibiting as pronounced an effect on non-neoplastic cells, namely fibroblasts NIH 3T3 (mouse embryonic fibroblast cell lines; 3T3 refers to the abbreviation of 3 day transfer inoculum 3×10^5 cells) [73]. The IC_{50} values of DP18 and P18 containing L amino acids were almost identical but higher than those reported by Shin and co-workers [74]. The substitution of isoleucine with leucine (15× less expensive) in DP18 generated DP18L, which generally showed greater cytotoxicity, compared with the parental DP18 peptide, and a more pronounced effect against a number of cell lines tested. The branching of the side chain occurs on the β-carbon of leucine and on the γ-carbon of isoleucine, which may result in less steric hindrance by

leucine, consequently affecting the conformational structure or folding within the peptide which, in turn, could affect biological activity. The specificity of potential drug molecules toward cancer cells and not normal cells is a highly desired property, for example, CAP have been reported to be specific to cancer cells over normal cells. The modification of DP18 through the conjugation of (R)-3-hydroxydecanoic acid unfortunately resulted in greater cytotoxicity toward both cancer and normal cells. However, the new peptide derivative was still 3× more toxic to cancer cells compared with human umbilical vein endothelial cells.

While other peptides have been shown to be haemolytic [75], P18 had little or no lytic effect on erythrocytes as determined by Shin and co-workers [74]. Work performed by the authors' group has shown that DP18L, and its derivatives, also lacked haemolytic activity with concentrations up to 66-fold higher than the IC_{50} values needed to observe any degree of haemolysis. Biotin-labelled peptides were able to transverse the cell membrane and were localised to the mitochondria. R10DP18L (R)-3-hydroxyecanoic acid was internalised faster than DP18L. A highly negative transmembrane potential and the negative mitochondrial surface could contribute to their susceptibility to attack by CAP [76]. The high net negative charge, due to the presence of the anionic lipid cardiolipin, could mediate electrostatic interactions with the basic residues of DP18L and its derivative, R10DP18L. An increased cardiolipin content of the outer and inner mitochondrial membrane in cancer cells relative to healthy cells has also been reported [77].

Furthermore, the induction of apoptosis in HeLa (a cell type in an immortal cell line used in scientific research; it is the oldest and most commonly used human cell line and was derived from cervical cancer cells from Henrietta Lacks, a patient who eventually died of cancer) and MiaPaCa cells (human pancreatic carcinoma cells) was evident within 2 h, with the progression of cells through early and into late apoptosis. The levels of apoptosis occurring suggested that this was the dominant mode of cell death and was evident by the levels of late apoptosis/necrosis at 24 h. Mitochondria are involved in both apoptotic and necrotic death processes, influenced by the level of adenosine triphosphate and the activation of caspases [78].

Loss of mitochondrial potential is commonly associated with destabilisation of the outer mitochondrial membrane, which leads to release of cytochrome c [78]. Caspase-9 is an initiator caspase for the mitochondria-dependent pathway of apoptosis and activation of Caspase-9 leads to activation of downstream executioner caspases, including Caspase-3, Caspase-6 and Caspase-7 [79]. The involvement of Caspase-9 and the lack of death receptor involvement suggest that the mitochondria-associated pathway of apoptosis plays an important role in peptide-induced cell death of HeLa and MiaPaCa cells. This finding is consistent with reports that other CAP are able to trigger apoptosis in human carcinoma cells [80]. Synergy between emerging and established anticancer agents creates the possibility of developing new combinational approaches to disease treatment. The use of combinations of drug

candidates can result in increased effectiveness compared with the administration of single drug candidates alone and can decrease the required dosage of chemotherapeutic drugs to achieve a positive response. P18, or a derivative, was the first to be reported to show a synergistic action with another anticancer agent; as P18 is a hybrid of cecropin A and magainin 2 [81], it is important to note that cecropin A has been shown to act in synergy with known anticancer drugs (e.g., 5-fluorouracil and cytarabine) against acute lymphoblastic leukaemia cells [82] but has not been tested for activity with taxotere, cisplatin or gemcitabine. In addition, analogues of magainin 1 and 2, namely magainin A and G, are reported to have an additive effect with chemotherapeutic agent, such as cisplatin, on small lung cancer cells [83]. However, in this study it was shown that the P18 derivative R10DP18L had a more desirable (i.e. synergisitic) effect with cisplatin on A549 lung cancer cells [84].

1.3.6 Cationic Polysiloxane biocides

Polymer biocides containing biocidal groups permanently bonded to their chains encompasses a very broad class of polymers that can efficiently inhibit the growth of bacteria and other microbes without releasing low MW toxic products into the environment [85, 86]. Biocidal polymers of polycationic structure have been developed, in particular, those containing quaternary tetraalkylammonium (QAS) [87–89], tetraalkylphosphonium [90] and *N*-alkylpyridinium salt groups [91] pendent to the polymer chain (Figure 1.4). These polycations bear considerable positive charge and may violently interact through electrostatic forces with the negatively charged bacterial walls and membranes leading to bacterial death [92]. Polycationic antimicrobial agents which have been used include organosilicon polymers, polysiloxanes and polysilsesquioxanes [93]. Polysiloxanes are particularly attractive as they show exceptionally high static and dynamic flexibility of their polymer chains, which gives them high solubility in many solvents, high permeability and unusual surface properties. Polysiloxanes containing pendent quaternary ammonium salt groups (QAS) show high biocidal potency. Recently, polysiloxanes containing imidazolium salt (ImS) groups have shown some antibacterial effect due to the uncharged imidazole ring attached to organic polymers [94].

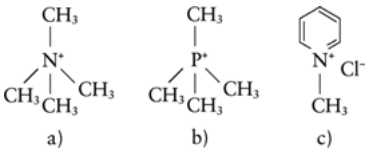

Figure 1.4: Structure of (a) QAS, (b) tetraalkylphosphonium and (c) *N*-alkyl pyridinium salt [95].

1.3.6.1 Synthesis of biocidal polycationic polysiloxane with N,N′-dialkylimidazolium groups

Polysiloxanes with *N,N′*-dialkylimidazolium groups can be synthesised via 3-*N*-imidazolopropyl substituted polysiloxanes. The monomer used in the synthesis of the precursor polymer was 3[(*N*-imidazolo)propyl]methyldiethoxysilane, obtained via the hydrosilylation of *N*-allylimidazole. Imidazole-substituted olefins are not readily reactive during hydrosilylation as the nucleophilic imidazole ring deactivates the catalyst by forming complexes with transition metals. The hydrolytic polycondensation of 3[(*N*-imidazolo)propyl]methyldiethoxysilane in the presence of ammonia led to a polysiloxane with pendent 3-(*N*-imidazolo)propyl groups. This polymer can be purified via the dialysis of water-soluble low molar mass imidazole derivatives, including lower hydroxyl-ended oligomers. The polymer generated by this method had, on average, about 10 imidazole-substituted siloxane units and was characterised by ^{1}H-NMR spectroscopy and size exclusion chromatography (SEC). The polymer was subjected to a reaction with *n*-octylbromide and *n*-octyl chloride in dimethylformamide; the progress of the reaction was monitored using ^{1}H-NMR spectroscopy.

This polymer was also characterised by SEC. Its bimodal molar mass distribution pointed to a significant contribution from the cyclic polysiloxane fraction. The dialysis of this polymer in water permitted the separation of cyclics into the lower molar mass fraction, while the linear polymer constituted the higher molar mass fraction. The gel chromatograms of both these fractions were also compared. The two-step dialysis performed for the precursor and biocidal polymer led to a narrow polydispersity of the former, Mw / Mn = 1.21 [95].

1.3.6.2 Synthesis and antibacterial properties of polysiloxanes-bearing quaternary ammonium salt groups

Precursor polysiloxanes containing 3-chloropropyl groups pendent to the siloxane chains were generated via the hydrolytic polycondensation of (3-chloropropyl) methyldichlorosilane. This polymer can be subjected to equilibration or coequilibration with octamethylcyclotrisiloxanes catalysed by trifluoromethanesulfonic acid. A known amount of hexamethyldisiloxane is introduced to control the MW and to terminate the chain by trimethylsilyl groups. This procedure leads to a statistical distribution of units in the case of copolymers.

All of the synthesised polycationic polysiloxanes described in this chapter are soluble in water. The antibacterial properties of these materials are determined in aqueous solutions using the broth dilution method and the biocidal powers of the various structures are compared by determining their MIC values. Studies were performed using two Gram-positive bacteria strains, *Enterococcus hirae* and *Staphylococcus aureus*, and three Gram-negative bacteria strains, *Escherichia coli*,

Proteus vulgaris and *Pseudomonas aeruginosa*. For each bacteria strain–biocide pair, the MIC was evaluated using three independent measurements and good agreement between the results of these measurements was attained (the mean deviation from the average value was 12%). Polysiloxanes containing pendent ImS functions have proved to be powerful bacteriocides against a broad spectrum of bacterial strains. Their antibacterial activity is comparable to polysiloxanes bearing antibacterial QAS functions. Although the biocidal behaviour of these two classes of biocides is similar, some differences are observed in their action against various bacterial species and in the structure–activity relationships. The density of the biocidal functions along the chain is not as crucial for the QAS polysiloxane as it is for the ImS derivative. With the exception of *Escherichia coli*, the QAS copolymer containing dimethylsiloxane showed similar activity to the QAS homopolymer. The antibacterial power of the QAS- and ImS-substituted polysiloxanes is stronger against Gram-positive bacteria than Gram-negative bacteria, which is related to the difference in the structure of their cell walls. It is generally accepted that the mechanism of the bacteriocidal action of polycationic biocides involves their destructive interaction with the cell wall and/or cytoplasmic membranes.

1.3.7 Cationic Polyarylene Ethynylene Conjugated Polyelectrolytes

Cationic polymers are widely recognised as effective long-lasting antimicrobials and biocides [86]. Cationic biocidal polymers function by associating with negatively charged bacteria, permeating the bacterial cell wall and physically disrupting the underlying cell membrane [96]. Conjugated polyelectrolytes (CPE) have gained significant attention due to their unique characteristics and photophysical properties [97]. In particular, CPE are soluble in water and polar organic solvents, and the presence of ionic solubilising groups allows the polymers to interact strongly with ions in solution and with charged planar or colloid surfaces. Because of their relatively high carbon atom to ionic group ratio, CPE are polymer amphiphiles; they self-assemble into nanoscale aggregates in aqueous solution and adsorb strongly to charged substrates forming films that can be tailored on the nanoscale. In addition to these interesting and useful material properties, CPE also have favourable photophysical properties such as efficient light-harvesting ability (large absorption coefficients), rapid singlet exciton diffusion along the conjugated backbone, high fluorescence quantum yields and they display the amplified fluorescence-quenching effect [98]. Such desirable characteristics of CPE have led to their use in a variety of applications, including chemo- and biosensors [99], photovoltaic devices [100] and light-activated antimicrobial materials [101]. In earlier reported work, cationic CPE poly(**1**) and poly(**2**) showed little biocidal activity against *Cobetia marina* or *Pseudomonas aeruginosa* strain PAO1, when microspheres bearing physisorbed or surface-grafted CPE were mixed with bacterial suspensions in the dark for short periods of time, 15

min/ h [102]. Prolonged incubation in the dark led to slow killing of the bacteria. However, when the same suspensions were irradiated with visible light in the presence of oxygen, there was a relatively rapid light-activated biocidal activity that likely involved interfacial generation of singlet oxygen and perhaps the subsequent formation of more corrosive reactive oxygen species. The study of a third CPE in this series, **3**, containing a thiophene ring, replacing one of the phenyl rings in the polyphenylene ethynylene (PPE) repeat unit, has been reported [103].This polymer exhibited somewhat different photophysical behaviour from CPE poly(**1**) and poly(**2**). The new behaviour resulted in dramatically reduced light-activated biocidal activity; however, poly(**3**) showed remarkable biocidal activity in the dark. This difference in behaviour provides insight into the mechanism, which occurs in the dark and the way dark- and light-activated biocidal activity may be tuned.

1.3.7.1 Photophysical Properties

The structures of CPE are illustrated in Figure 1.2. Poly(**2**) and poly(**3**) are PPE-based CPE that feature quaternary ammonium salts on their side chains. In a good solvent, such as methanol, CPE exist in a 'molecularly dissolved' state with minimal aggregation. In a poor solvent, on the other hand, the polymer chains exist as aggregates [104], which results in changes in photophysical behaviour of the polymers in solution [105]. In general, PPE-based CPE exhibit red-shift and narrowing of the absorption spectrum, and red-shift and broadening of the fluorescence spectrum upon switching from a good solvent to a poor solvent, respectively.

A methanol solution containing poly(**3**) exhibits a broad absorption band with λmax at 422 nm. In aqueous solution, the absorption maximum red-shifts to 432 nm and the absorption coefficient also decreases. The emission properties are greatly dependent upon the nature of the solvent. In a methanol solution, poly(**3**) exhibits a narrow emission band with λmax = 475 nm and a vibronic band at λmax = 502 nm. In aqueous solution, the emission band becomes broader and the fluorescence quantum yield (Φ_{fl} = 0.016) is lower than that in methanol (Φ_{fl}= 0.045). Interestingly, the emission maximum is at the same position as in methanol (475 nm) with a shoulder at 502 nm. Such behaviour is different from other PPE-based CPE, which exhibit large red-shifts of emission band in a poor solvent. The lack of a spectral shift for poly(**3**) is likely to be due to the fact that the aggregated state of the polymer has a much lower quantum yield, and therefore its contribution to the total emission spectrum is small.

1.3.7.2 Microsphere-based biocidal activity studies

A series of experiments to test biocidal activity were carried out using poly(**3**) as a physisorbed coating on 5 μm silicon dioxide microspheres. These microspheres

were exposed to PAO1 and the association and biocidal activity were observed using confocal microscopy. The bacteria attached to the surface-associated polymers in an adsorptive process, likely driven by hydrophobic and electrostatic interactions, analogous to the adsorption of polyelectrolytes to an oppositely charged colloidal particle. This process occurs in the dark or light and does not, in itself, result in bacterial death in the short term, although some physical damage (and longer term killing) of the bacteria may occur. This association is reversible to some extent and real time observation of a single particle–bacteria cluster revealed that some bacteria associate briefly and are then released while others appear to be captured irreversibly, with clusters of microspheres and bacteria agglomerating as the bacteria are killed, presumably due to the release of microbial agglutinants as the cell membranes are compromised. In solution, these agglomerates tend to be a rough indicator of the antimicrobial activity over time, with samples starting out with fairly monodisperse solutions of coated microspheres to which the bacteria are added. In the samples using poly(**3**), large aggregates with pronounced antimicrobial activity appeared within 15 min of the introduction of bacteria. Light-exposed samples tended to have less aggregation and actually showed diminished biocidal action, even with the bacteria intimately associated with the coated microspheres.

This is in contrast with the behaviour of physisorbed poly(**1**) and poly(**2**), suspensions of which show some dark- and significant light-activated biocidal activity against PAO1 but less agglomeration. Microspheres with physisorbed poly(**1**) and poly(**2**) also showed much stronger fluorescence than physisorbed poly(**3**) even when coated at the same density. Interestingly, when microspheres of physisorbed poly(**3**) were overcoated with a phospholipid bilayer the fluorescence levels became much stronger [106]. It should be noted that it is very difficult to keep dark suspensions rigorously dark and some irradiation does occur during subsequent confocal fluorescence imaging. Unexposed PAO1 typically remained viable for more than 6 h, longer than the total experimental time. For microspheres with physisorbed poly(**3**), incubation with PAO1 in the dark for 2 h resulted in greater than 95% of the bacteria being killed.

1.3.7.3 Biocidal activity of poly[trialkyl-3-(and 4-)vinylbenzylammonium chloride] in solution

The addition of poly(**2**) and poly(**3**) solutions to suspensions of PAO1 results in biocidal activity in dark conditions of both polymers; however, there are clear differences in the behaviours of the two polymers. The treatment of PAO1 with poly(**3**) results in clustering of bacteria and rapid death. In contrast, the treatment of PAO1 with poly(**2**) results in very little bacterial agglomeration. It is believed that this behaviour can be attributed to the lipophilic character of poly(**3**).

1.3.7.4 Fluorescence studies of 4-octylstyrene and poly[trialkyl-3-(and 4-) vinylbenzylammonium chloride] with *Pseudomonas aeruginosa* Strain PAO1

Fluorescence spectra of both poly(**2**) and poly(**3**) in an aqueous solution of 0.85% sodium chloride were measured in the absence and presence of PAO1 bacteria using a plate reader. As the rod-shaped PAO1 used were approximately 2 μm in length and 0.7 μm in diameter, the calculated surface area per PAO1 was approximately 4.7×10^{-12} m^2. According to the literature, the surface area per PPE repeat unit is approximately 2.8×10^{-18} m^2. Therefore, about 1.7×10^6 PPE repeat units can be associated on the surface of each PAO1. 10 μL of poly(**2**) and poly(**3**) stock solutions (1.3 μM in PPE repeat unit) were added, respectively, to 500 μL of PAO1 solution (approximately 2.3×10^8 PAO1). This resulted in 20× full coverage of PPE polymers on the PAO1 surface. While mixing with PAO1 resulted in a moderate decrease (30%) in the fluorescence intensity of poly(**2**), the fluorescence intensity of poly(**3**) decreased dramatically (~80%) under the same conditions. This result could be attributed to the fact that clusters were formed between PAO1 and poly(**3**) and not many clusters were formed between PAO1 with poly(**2**). Within the clusters, poly(**3**) aggregated around PAO1 and resulted in a higher local concentration, which could lead to an apparent quenching of the polymer fluorescence. Since poly(**2**) was well distributed in the solution in the presence of PAO1, as shown in the confocal data, it is logical to observe less decrease in fluorescence for poly(**2**). These results once again confirmed that the structural difference between poly(**2**) and poly(**3**) results in different interaction between CPE and bacteria, and differing dark biocidal activity.

1.3.7.5 Influence of negatively charged phospholipids

A negatively charged phospholipid, 1,2-dioleoyl-*sn*-glycero-3[phospho-*rac*-(1-glycerol)] (DOPG), was chosen to interact with poly(**2**) and poly(**3**) either in solution or on microspheres. The mixtures of the polymer with DOPG liposome were prepared by adding 10 μL of stock PPE solutions to 1 μL of DOPG liposome solution followed by 30 min of vortexing. The resulting ratio between the DOPG liposome and PPE polymer in a repeat unit was 1:2,000 (the hydrodynamic radius of DOPG liposomes prepared by the method mentioned earlier is approximately 110 nm as determined by dynamic light scattering. This results in a calculated surface area of approximately 1.5×10^{-13} m^2 for each spherical liposome. As the surface area of each DOPG lipid is approximately 55×10^{-20} m^2, there are approximately 2.7×10^5 DOPG lipids in each DOPG liposome. Therefore, 1 mL of 2 mM DOPG lipid solution can yield 7 nM of DOPG liposome. In 1 μL of the liposome–polymer mixture, there are approximately 7×10^{-12} mol of DOPG liposome and approximately 1.3×10^{-8} mol of PPE repeat units, giving a ratio of 1:2,000 between DOPG liposome and PPE repeat

unit). A control sample of polymer solution without DOPG liposome was also prepared by adding the same amount of polymer into 1 μL of phosphate buffered saline solution. Fluorescence enhancement and blue-shifts of the emission maximum were found for both poly(**2**) and poly(**3**) in the presence of DOPG, however, the extent of enhancement differs. In the case of poly(**2**), the emission maximum shifted from 506 to 460 nm and fluorescence intensity increased by less than 2× in the presence of DOPG; on the other hand, in the case of poly(**3**), the emission maximum shifted from 524 to 482 nm and the fluorescence intensity increased by almost one order of magnitude. As described elsewhere and investigated in detail, this fluorescence enhancement could be attributed to the interactions between cationic CPE and negatively charged phospholipids, which induce association and further insertion of PPE CP into the DOPG liposome and results in some level of deaggregation of polymers and gives rise to increased fluorescence. The dramatic difference in the fluorescence enhancement between poly(**2**) and poly(**3**) could be due to the difference in their structures, as poly(**3**) has shorter and less sterically hindered pendent quaternary ammonium groups than poly(**2**). Clearly, poly(**3**) is much more lipophilic than poly(**2**) as indicated by the different interactions with negatively charged phospholipids. The enhanced lipophilicity of poly(**3**) is a reasonable source of the dark biocidal activity for poly(**3**) [106]. Cationic polymer based on dimethylaminoethyl methacrylate has found an excellent antibacterial activity against gram-positive mycobacteria (Figure 1.5). These kind of bacteria has unique cell wall composed of mycolic acid–arabinogalactan–peptidoglycan complex (mAGP) interdispersed in lipid makes it quite persistent in the host [107].

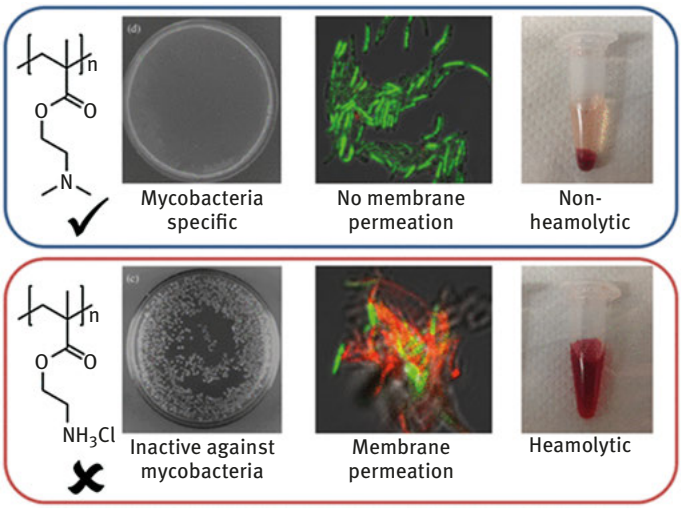

Figure 1.5: Antibacterial activities of deimethylaminoethyl methacrylate based polymers [107].

1.3.8 Cationic polymers based on imoidazolium units

An imoidazolium-based conjugated polymers (PFPhim) with and cationic positively charged backbone pendant groups have been synthesised (Figure 1.6) and exhibit photodynamic antibacterial activities (Figure 1.7) [108]. Imidazolium (Im), quaternary ammonium (Qa) and 1,4-diazabicyclo[2.2.2]octane-1,4-diium (DABCO-diium)–based cationic polymers with both main chain and pendant polymers have been prepared with excellent antibacterial activities [109].

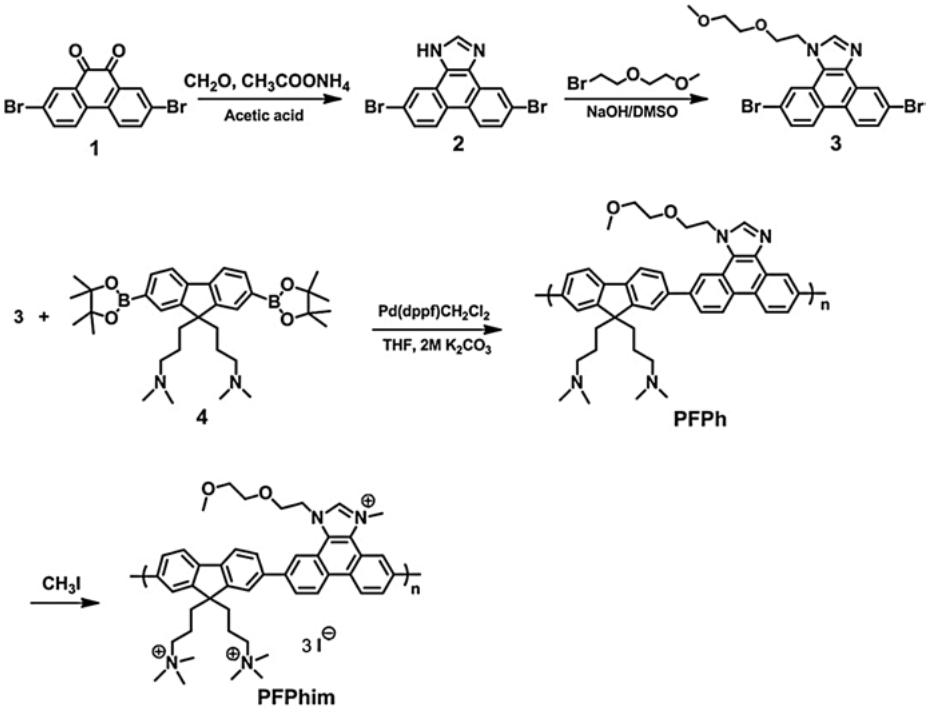

Figure 1.6: Synthesis and chemical structure of PFPhim.

Conclusion

The central aim of this chapter is to highlight some of the recent advances in the field of cationic polymer-mediated biomedical applications. Cationic polysaccharides and cationic natural polymers display biodegradability and low toxicity, and may be widely used in the future. Cationic antimicrobial agents may prevent device-associated infections caused by *Staphylococcus epidermidis* and *Staphylococcus aureus*. From the above-mentioned discussion it can be concluded that the cationic

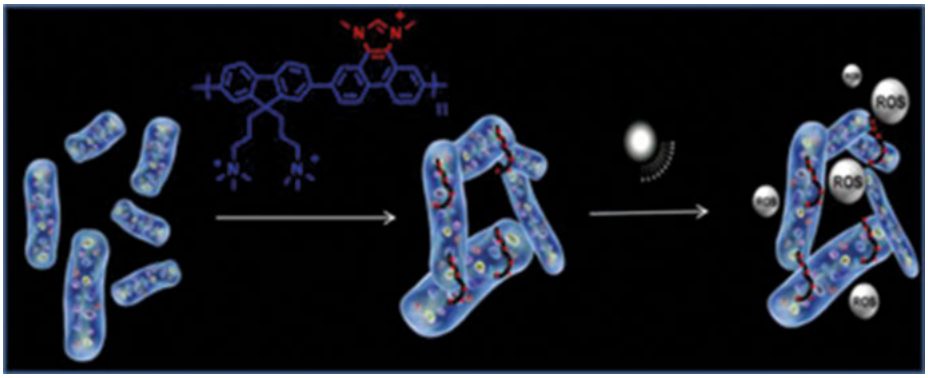

Figure 1.7: Biocidal activities of PFPhim.

antimicrobial polymer, pDMAEMA, is more effective at antagonising the growth of clinical isolates of *Staphylococcus epidermidis* than *Staphylococcus aureus*. An antimicrobial, cationic PEI is a specific inducer of the Cpx stress response in *Escherichia coli*. The small antimicrobial molecule, vanillin, known to disrupt membranes, was observed to specifically induce the rE stress response. These results suggest that the induction of the extracytoplasmic stress response can provide an intriguing window into the differences in the bactericidal action of antimicrobial compounds. The biocidal activity of PS poly(**1**) is broadly biocidal, killing bacteria and lysing human RBC at relatively low concentrations. The conjugation of (R)-3-hydroxydecanoic acid to DP18L resulted in a modified peptide with improved antiproliferation activity that appeared to be mediated through greater uptake into the cells. It is not only the presence but the position of the hydroxyl moiety that is important for activity enhancement. New biocidal polymers, polysiloxanes with *N,N′*-dialkylimidazolium halide groups pendent to the polymer chain are very potent bacteriocides for a broad spectrum of bacteria. They show particularly high bacteriocidal activity against Gram-positive bacteria and are also very effective against Gram-negative bacteria. The thiophene polymer has remarkable dark biocidal activity against the *Pseudomonas aeruginosa* strain PAO1 but very little light-activated activity. The low light-activated biocidal activity of the thiophene polymer is attributed to a highly aggregated state of the polymer in aqueous solutions and on microspheres as a physisorbed coating.

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2 Antibacterial activity of amphiphilic polymers

Abstract: Increasing prevalence of multi-drug resistant bacteria has remained as a main challenge in medical care units. Several studies are being conducted to develop an effective and novel antibacterial agent with broad-spectrum antibacterial activity against resistant bacteria and non-cytotoxicity for human cells. Among them, amphiphilic polymers have many interesting antibacterial, biological and mechanical properties such as target-based activity against microorganisms, biodegradability, biocompatibility, good mechanical strength and cell adhesion, which make them a promising candidate for biomedical applications. Amphiphilic polymers possess both hydrophilic (water-loving, polar) and lipophilic (fat-loving) or hydrophobic (water-hating) properties. In this chapter, different amphiphilic polymers, their synthesis procedures, antibacterial and biological properties, as well as possible applications as antibacterial agents in biomedicine are discussed in details.

Keywords: amphiphilic polymers, antibacterial agents, biomaterials, resistant bacteria, tissue engineering

2.1 Introduction

Because of their broad range of properties, polymers play an essential role in our everyday life in the form of plastics, fibres, rubbers, deoxyribonucleic acid (DNA), cellulose, polyvinyl chloride and many others. For plants and animals to survive, it is essential to control the growth of bacteria, fungi, yeast and algae, and as a result, living organisms have developed different mechanisms to limit microbial growth. However, these mechanisms are not fully effective, especially in humans, which has necessitated finding new methods to control infections. At the present time, we use antimicrobial agents to manage this situation but conventional medicines are toxic and create other problems; furthermore, microbes are resistant to some of

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them. Because of the modern challenges of bacterial growth, infections and resistance of bacteria to traditional medicine, the applications of polymers in medicinal chemistry is emerging. Amphiphilic polymers as antimicrobial agents play a vital role in the field of biology and medicinal science.

The word amphiphile is derived from two Greek words 'amphis' meaning both and 'philia' mean love or friendship. Thus, a chemical compound possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) or hydrophobic (water-hating) properties is called amphiphilic or an amphipathic compound; amphiphilic polymers contain a hydrophilic and lipophilic part in their structure (Figure 2.1) [1, 2].

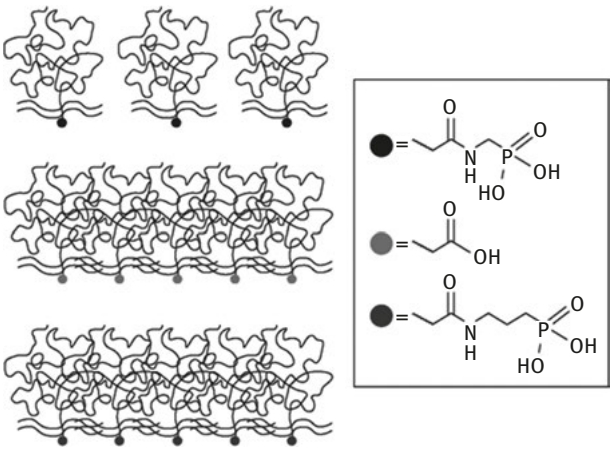

Figure 2.1: An example of an amphiphilic polymer. Reproduced with permission from J.W. Chan, Y. Zhang and K.E. Uhrich, *Bioconjugate Chemistry*, 2015, 26, 7, 1359. ©2015, American Chemical Society [2].

Peptides are also amphiphilic polymers, that is, mostly positively charged molecules with short amino acid chains, and are a key component of the innate immune system. The focus on amphiphilic polymers is due to some reports suggesting that the current global drug pipeline is woefully inadequate due to bacterial resistance to antibiotics [3]. The application of amphiphilic polymers and their block polymers to stop the microbial growth of infected tissue has been reported in the literature [4]. Amphiphilic polynorbornene derivatives can retard bacterial infection by disrupting the lipid membrane activity of liposomes. Further benefits of amphiphilic polymers include their target-based activity against bacteria and infected tissue; in addition, these polymers are biodegradable [5], biocompatible [6], a non-irritant and exhibit good film-forming properties, such as high mechanical strength and adhesion. Furthermore, they have a short lifetime and do not negatively affect the biological system. The mechanism of

antimicrobial activity depends largely on the hydrophilic– hydrophobic balance and altering this balance is a novel method of improving the target accuracy of polymers. To control this balance, amphiphilic copolymers can be synthesised by incorporating specific amounts of hydrophobic comonomers to the hydrophilic polymers. Balanced polymers enable controlled drug release, which can be used in gene therapy and cancer therapy [7]. Water-soluble amphiphilic cationic polynorbornene derivatives exhibited the highest level of activity against liposome membranes and contain phospholipid building blocks in their membrane assemblies; these phospholipid blocks change their supramolecular order by incorporating other amphiphilic polymers within their membrane assemblies.

2.2 Synthesis of amphiphilic polymers

Amphiphilic polymers have wide-ranging significance in medicinal chemistry and demand for them in the public healthcare sector is very high. Therefore, considerable research activity is focused on diverse approaches for the synthesis of amphiphilic polymers. The major challenge during their synthesis is to control the reaction chain, that is, management or balancing of the hydrophobic and hydrophilic portions of the polymer as per requirement. Group transfer polymerisation, reversible addition-fragmentation transfer (RAFT) and atom transfer radical polymerisation (ATRP) are a few methods which can be used for the preparation of amphiphilc polymers. The synthesis of amphiphilic polymers as a result of the sulfonation of polystyrene (PS) has been described by Barros and co-workers [7, 8]. All these processes involve many diverse and critical pathways. A few different methods for the preparation of amphiphilic polymers and their derivatives are described in the following sections.

2.2.1 Synthesis of amphiphilic hyperbranched polymers

Hyperbranched polymers (HBP) are highly branched polymers with three-dimensional arrangements. The encapsulation performance of amphiphilic HBP is good due to their micelle-like properties and unimolecular micelle behaviour. HBP can be used in various fields, including coatings, additives, macromolecular building blocks, supramolecular science, drug and gene delivery, phase-transfer agents, extractive distillation, solvent extraction and nanotechnology [9].

Chen and co-workers [10] synthesised amphiphilic hyperbranched core–shell polymers with floated moieties as the targeting groups (Figure 2.2); Boltorn H40, a hyperbranched aliphatic polyester, was the core of the polymer. Hydrophobic poly (ε-caprolactone) (PCL) segments were used to form the inner part of the shell while

the outer shell was composed of hydrophilic polyethylene glycol (PEG) segments. As a consequence of the coupling of the carboxylic group of the folic acid and the hydroxyl group of PEG, folic acid is added to the polymer surface. Inclusion of folic acid is a valuable way to achieve tumour cell-targeting properties.

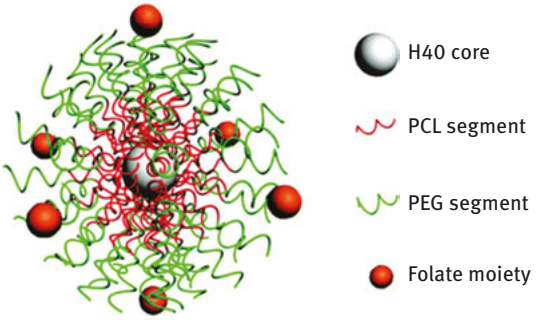

Figure 2.2: An amphiphilic hyperbranched core–shell polymer. Reproduced with permission from S. Chen, X-Z. Zhang, S-X. Cheng, R-X. Zhuo and Z-W. Gu, *Biomacromolecules*, 2008, 9, 10, 2578. ©2008, American Chemical Society [10].

Smeets and co-workers reported the synthesis of amphiphilic HBP from the copolymerisation of a vinyl and divinyl monomers [11]. Grafting of HBP has also been reported by other researchers [12]. Hou and Yan reported the synthesis of a star-shaped copolymer using in situ grafting, which contained a hyperbranchedpoly(3-methyl-3-oxetanemethanol) core and tetrahydrofuran arms [13].

2.2.2 Atom transfer radical polymerisation

During ATRP, alkyl halides function as initiators while transition metal complexes (ruthenium, osmium, iron, copper and so on) act as the catalyst. Metal complexes are used for generating radicals (such as peroxide) via a one electron transfer process and during this process the transition metal becomes oxidised. Thus, ATRP is a reversible-deactivation radical polymerisation and can be employed to prepare polymers with similar molecular weight (MW) and low MW distribution. Advantages of ATRP are the ease of preparation, use of commercially available and inexpensive catalysts and initiators [14, 15]. The synthesis and process development of ATRP, as well as some new hybrid materials made of amphiphilic polymers, have been reported in the literature (Figure 2.3) [16, 17].

Methyl methacrylate (MMA) and sodium styrene sulfonate (SSNa) are water-soluble. These polymers behave like a low MW surfactant as they form micelles in aqueous solution in which the hydrophobic part is directed toward

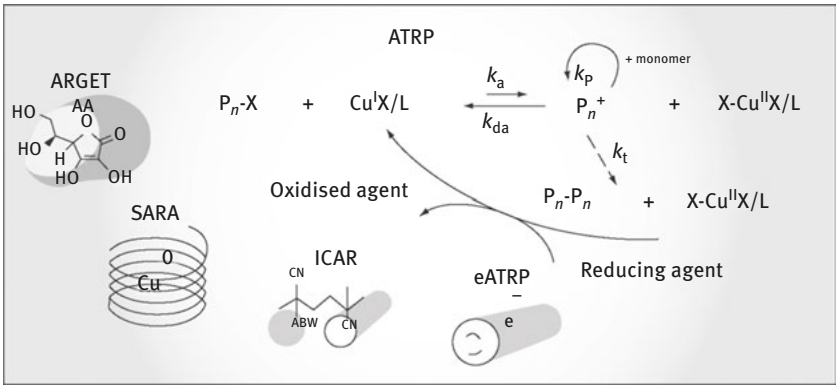

Figure 2.3: ATRP: current status and future perspectives. ARGET: activator regenerated by electron transfer, eATRP: electron-atom transfer radical polymerisation, ICAR: initiators for continuous activator regeneration and SARA: supplemental activator and reducing agent. Reproduced with permission from K.Matyjaszewski, *Macromolecules*, 2012, 45, 10, 4015. ©2012, American Chemical Society [16].

the centre and the hydrophilic part is situated on the periphery of the micelle. Owing to such features, amphiphilic block copolymers have wide-ranging applications in drugs, pharmaceuticals, coatings, cosmetics and paints. They also exhibit very high antibacterial activities. Oikonomou and co-workers used ATRP to prepare amphiphilic block copolymers, consisting of polymethyl methacrylate (PMMA) and poly(sodium styrene sulfonate) (PSSNa) blocks [18]. The synthesis methods are described below.

2.2.2.1 Synthesis of macroinitiators

Block copolymers are synthesised with PSSNa and PMMA macroinitiators as the starting material.

- Synthesis of the I-poly(sodium styrene sulfonate) macroinitiator
  Prepare a solution of SSNa (23.0 mmol), methyl-4-(bromomethyl) benzoate (initiator I, 0.332 mmol), copper (I) bromide (0.33 mmol) and 2,2′-bipyridine (0.66 mmol) in a methanol/water (50/50) solution in a dry round-bottom flask. Stir the reaction mixture at 25 °C. After 24 h, precipitate the product using tetrahydrofuran and dry under vacuum.
- Synthesis of the polymethyl methacrylate-II-polymethyl methacrylate macroinitiator.
  Add MMA (29.9 mmol), α,α′-dibromo-*p*-xylene (initiator II, 0.60 mmol), copper (I) bromide (1.20 mmol), 2,2′-bipyridine (1.20 mmol) and dimethylformamide (DMF) (solvent) to a dry round-bottom flask. Degas the reaction mixture, flush

with argon and stir in an oil bath at 110 °C for 24 h. Precipitate the product in water and dry under vacuum.

- Synthesis of the III-polymethyl methacrylate macroinitiator
  Prepare a solution of MMA (9.4 mmol), dimethyl 5-(4-(bromomethyl) benzyloxy) benzene-1,3-dioate (initiator III, 0.187 mmol), copper (I) bromide (0.037 mmol), *N*,*N*,*N*′,*N*′′,*N*′′-pentamethyldiethylenetriamine (PMDETA) (0.0370 mmol) in DMF and keep the reaction mixture in an oil bath at 110 ºC under an argon atmosphere. Product recovery is achieved using water.

2.2.2.2 Synthesis of block copolymers

- Synthesis of the I-poly(sodium styrene sulfonate)-*b*-polymethyl methacrylate (PIa) block copolymer.
  Prepare a solution of I-PSSNamacroinitiator (0.050 mmol), MMA (5.0 mmol), copper (I) bromide (0.050 mmol) and 2,2′-bipyridine (1.00 mmol) in a mixed solvent composed of methanol and water (80/20 ratio) and stir at 25 °C for 24 h, under an argon atmosphere. The copolymer is purified by dialysis and recovered via freeze-drying.
- Synthesis of the poly(sodium styrene sulfonate)-*b*-polymethyl methacrylate-II-polymethyl methacrylate-*b*-poly(sodium styrene sulfonate) (PIIa) block copolymer
  Prepare a solution of PMM-II-PMMA macroinitiator (0.074 mmol), SSNa (0.73 g, 3.57 mmol), copper (I) bromide (0.020 g, 0.15 mmol) and PMDETA (0.025 g, 0.15 mmol) in a mixed solvent composed of DMF and water (50/50 ratio) in a dry flask under an argon gas atmosphere. Stir the reaction mixture at 85 °C in oil bath for 24 h, under an argon atmosphere. Recovery of the product is similar to the method described above.
- Synthesis of the III-polymethyl methacrylate-*b*-poly(sodium styrene sulfonate) (PIII) block copolymer.
  Prepare a solution of III-PMMA (0.0128 mmol), SSNa (1.28 mmol), copper (I) bromide (0.026 mmol) and PMDETA (0.026 mmol) in DMF in a dry flask under argon. The reaction mixture is stirred at 110 °C in an oil bath. Recovery of the product is similar to the method described above.

2.2.3 Microwave-assisted synthesis

Microwave-assisted synthesis provides an environmentally friendly route for synthesis as it reduces the reaction time from days and hours to minutes and increases product yield [19]; consequently, this method is also applied to the synthesis of amphiphilic polymers. The *N*-deacetylated derivative of chitin is known as chitosan

(CS) and is found in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. CS is an eco-friendly compound as it is biodegradable and biocompatible. CS has wide-ranging applications in agricultural, horticultural, natural biocontrol and elicitor (molecules that can attach to special receptor proteins located on plant cell membranes) sectors, and as a fining agent in wine making. CS is also useful in the medical field for drug delivery, as an antibacterial agent and in bandages for blood clotting to reduce bleeding.

Furthermore, CS applications can be improved by synthesising some of its diverse derivatives. CS has an amino group, which allows the opportunity for *N*-alkylation, *N* -carboxylation, cyclisation and crosslinking (Figure 2.4). These derivatives possess good rheological and autoassociative and surface-active characteristics [20–23].

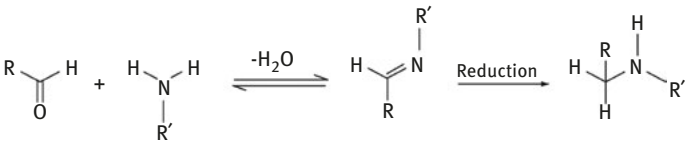

Figure 2.4: Synthesis of an amphiphilic derivative of CS. DCC: *N,N′*-dicyclohexylcarbodiimide, EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, mPEG: methoxy polyethylene glycol and NHS: *N* –hydroxysuccinimide.

Two commonly used modes for microwave-assisted synthesis are pulsed power mode and dynamic mode. One is a temperature-controlled mode and the other uses power, as well as the temperature control mode [24].

2.2.3.1 Synthesis of amphiphilic derivatives of chitosan

Mix ethanol (12 g), a CS solution (25 mL, around 15 mg of CS per mL of 0.2 M acetic acid) and an octanal solution in ethanol. Transfer the reaction mixture to a 100 mL round-bottom flask, capped with a rubber septum. Then add excess sodium cyanoborohydride to the flask. Irradiate the reaction mixture with microwave irradiation at 25 to 40 °C for 1 min to 1 h. Progress of the reaction can be checked by thin layer chromatography using a suitable solvent. The product is precipitated using a 10% sodium hydroxide solution (final pH around 8.5). Wash several times in an ethanol/water (70/30) solution; the centrifugation supernatant has a conductivity value lower than a few dozen μS cm^{-1}, which corresponds to a salt concentration lower than 0.001 mol/L. Finally, wash the product with pure ethanol [25].

2.2.4 Solid-phase synthesis of glutamate decarboxylase-1 and glutamate decarboxylase-2 peptides

A solvent-free or solid support reaction may be carried out using the reactants alone or incorporating them in clays, zeolites, silica, alumina or other matrices. Solvent-free reactions are eco-friendly as a reaction solvent is not required, which decreases pollution. Moreover, solid-phase synthesis lowers handling costs due to simplification of the experimental procedure, work-up technique and reduced labour requirement [26–29].

Peptides exhibit the highest antimicrobial activities of amphiphilic polymers and also possess antibacterial, antiviral, antifungi and anticancer activities [30–32]. In view of the potential applications of peptides, we will now discuss the synthesis of some important antibacterial peptides. Gad-1 and Gad-2 are peptides with amino acid chains and are prepared using *O*-fluorenylmethyloxycarbonyl (Fmoc) chemistry.

Putative mature Gad-1 and Gad-2 can be synthesised under solvent-free conditions [33]. For each peptide, weigh 5× excess Fmoc amino acids and place into a CS biopeptide synthesiser using 0.43 g of a 0.47 mmol/g rink amide resin. The amino acids are dissolved in 0.4 M 1-hydroxy-benzotriazole and DMF, and deblocking of amino acids is performed with 20% piperidine/DMF. Resin washes are carried out using DMF. Upon completion of peptide synthesis, the resin containing the peptides is transferred to a 10 mL syringe (BD Diagnostics Co.) equipped with a filter and washed thoroughly with methanol under vacuum. The resin is then air dried for 30 min followed by vacuum desiccation for 60 min. Cleavage of the peptide from the resin is carried out using a solution of 9.4 mL trifluoroacetic acid (TFA), 0.25 mL 1,2-ethanedithiol, 0.1 mL thioanisole (Sigma-Aldrich Co.) and 0.25 mL distilled water. The cleavage solution (5.0 mL) is added to the resin and stirred for 2 h. The resulting solution, which contains the C-terminally amidated peptide, is then extruded through the syringe into a 50 mL Falcon tube. The peptide is then precipitated with the addition of 40 mL of 20 °C diethyl ether and the tube is incubated at 20 °C overnight. The precipitate is then pelleted by centrifugation at 4 °C at 2,000 g for 5 min, the supernatant removed and two further ether precipitations are performed, each for 4 h. The resulting pellet is air-dried overnight, resuspended in double distilled water with 0.1% TFA (Sigma-Aldrich Co.) and filtered using a glass fibreAcrodisc® syringe filter. Peptide purification is performed using high-pressure liquid chromatography equipped with a reverse-phase DYNAMAX C-8 preparatory column.

2.2.5 Surface modification

In 1972, scientists from Dow Corning prepared antibacterial glass using a surface-bonded quaternary ammonium salt. The antibacterial glass was produced by heating the glass at 70 °C for 30 min with a 0.1% solution of 3-(trimethoxysilyl)

propyldimethyloctadecyl ammonium chloride. This surface was very active in killing *Streptococcus faecalis* bacteria even after extensive rinsing with water. Kotek and co-workers applied the same reagent on polyethylene (PE) terephthalate fibres and reported that treated fibres had an excellent antibacterial effect against *Escherichia coli*. The antibacterial activity strongly depends on the alkyl chain length. In another study, glass was treated with PS-*b*-poly(4-vinyl-*N*-alkylpyridinium bromide) copolymers (where the alkyl is hexyl or 6-perfluorooctyl-1-hexyl), which was also sprayed onto PS-*b*-poly(ethylene-ran-butylene)-*b*-PS–coated glass slides and showed excellent bactericidal properties. This process showed that fluorinated pyridinium surfaces are more biocidal than their non-fluorinated analogues. The bactericidal effect was found to be related to the molecular composition and polymer organisation in the top 2–3 nm of the surface and improved upon increasing the hydrophilicity and pyridinium concentration at the surface. This technique has bactericidal efficiency against *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli*; this process can be applied to glass and stainless steel surfaces.

PE is extensively used in many industrial, biological and medical applications; however, PE suffers from some drawbacks such as concerns involving the volume properties and lower surface free-energy, which reflect its low wettability. These drawbacks can be overcome by surface modification, that is, introducing hydrophilic and polar functional groups onto the surface. Cold plasma treatment is one of the best procedures to achieve this, in addition to plasma technology is an environmentally clean and dry process. Plasma treatment introduces polar functional groups onto the surface which raises the free-energy and makes the surface more hydrophilic. PEG was covalently grafted onto poly(ethylene-*co*-acrylic acid) by Zhang and co-workers (Figure 2.5) [34].

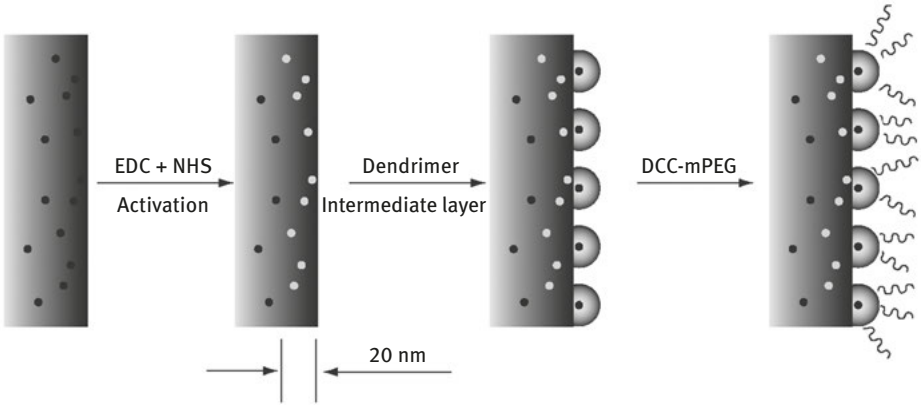

Figure 2.5: Surface grafting of PEG onto poly(ethylene-*co*-acrylic acid) films. Reproduced with permission from C. Zhang, N. Luo and D.E. Hirt, *Langmuir*, 2006, 22, 6, 6851. ©2006, American Chemical Society [34].

2.2.6 Plasma treatment

Low-density polyethylene (LDPE) foils are cleaned with dichloromethane to remove the impurities. The foils are then activated, under dynamic conditions at atmospheric pressure and room temperature, with diffuse coplanar surface barrier discharge equipment. The treatment is carried out for 15 s under an air atmosphere using a 200 W power supply. Plasma is generated by two parallel banded system of electrodes (1 mm wide, 50 micron thick, with 0.5 mm spacing between the strips, made of silver paste) embedded in 96% aluminium oxide of high purity, while the electrodes are supplied with a high frequency sinusoidal voltage (~15 kHz, Um~10 kV).

2.2.7 Grafting by polyacrylic acid

Instantly after plasma treatment, the LDPE foil is immersed in an aqueous solution of acrylic acid (AA) (10 vol%) and sodium metabisulfite (0.1 wt%) for 24 h at 30 °C, to initiate the radical graft polymerisation of AA onto the activated surface. Following AA polymerisation, polyacrylic acid (PAA) brushes are created on the LDPE surface, which are suitable for binding antibacterial agents. To remove weakly bound PAA and unreacted AA species on the surface, the grafted foils should be washed with deionised water at 30 °C.

2.3 Biological activity of amphiphilic polymers

Amphiphilic surfactants and polymers possess a membrane-forming ability and self-assembling property allowing the creation of a bilayer structure. These polymers strongly interact with biological membranes by insertion of the hydrophobic part into the lipid membrane, while exposing the hydrophilic part to the aqueous medium [35]. They also create a core structure on bacteria or infected tissue to stop microbial growth; therefore, amphiphilic polymers are an important part of the biocidal polymer. We will now explore amphiphilic polymers, which exhibit significant activity in biological systems.

2.3.1 Peptides

Peptides are long, continuous and unbranched chain polymers formed by the polymerisation of amino acid monomers. During the polymerisation process, two units are linked together via a peptide bond (–CO–NH–), which is formed by the reaction

of a carboxylic group (–COOH) of one amino acid and an amino group ($–NH_2$) of another. Peptides naturally occur in animals and plants, and can also be synthesised in the laboratory. Peptides play a significant role in the prevention of bacterial infections and, to date, more than 5,000 antimicrobial peptides (AMP) have been discovered or synthesised.

Peptides are classified on the basis of their antimicrobial activities and include cationic amphipathic peptides, cationic host-defence peptides (HDP), cationic AMP, anionic AMP, HDP and α-helical AMP. Most peptides exhibit antibacterial activity in tissues and organs, and some positively charged peptides with short amino acid chains have good target-based antibacterial properties, for example, liver-expressed antimicrobial peptides (LEAP). LEAP are found in human blood and urine. LEAP-1 is involved in the regulation of iron usage and is related to severe juvenile haemochromatosis, a genetic disease of severe iron overload. The viral infections hepatitis-C and immunodeficiency in humans are caused by LEAP deficiency [36]. LEAP-2 is constitutively expressed in the liver tissue of fish [37, 38]. The isomers of LEAP have also been reported in various fish species and each isomer shows a different spectrum against Gram-positive and Gram-negative bacteria. Assessing the minimum inhibitory concentration (MIC) is the best method to understand the variable activities of AMP. In this method, Gram-positive and Gram-negative bacteria are treated with amphiphilic polymers. Lia and co-workers carried out an experiment in which they injected LEAP-2 into fish and put them in a chamber overnight and the bacterial activity after 24 h was determined. They found that the brain, gills, heart, kidney, liver and spleen exhibited minimum infection. To study the harvested ribonucleic acid and DNA of the fish tissue, antimicrobial activities of the synthetic PaLEAP-2 (derived from LEAP-2) were determined against *Vibrio vulnificus, Pseudomonas putida, Escherichia coli* DH5, *Edwardsiellatarda, Vibrio alginolyticus, Vibrio parahaemolyticus, Vibrio anguillarum* and *Pseudomonas aeruginosa*. All fish used in the experiment died within 7 days; hence, it is also important to know the LEAP-2 concentration that is suitable for antibacterial activity and is not harmful.

Gad-1 and Gad-2 are rich in histidine which suggests their antimicrobial activities are pH dependent [39–41]. McDonald and co-workers synthesised Gad-1 and Gad -2 under solvent-free conditions and determined their structure–function relationships. They measured the MIC for Gad-1 and Gad-2 against *Escherichia coli* at pH 5 and pH 7; it was found that the MIC for Gad-1 is 5.1 μM (12.5 μg/mL) at pH 5 and 7; thus, the activities of Gad-1 are not pH dependent. When the same experiments were carried out with Gad-2, it was found that the MIC is 11.5 μM (25 μg/mL) at pH 5 and at pH 7 the MIC is greater than 23.0 μM. It is obvious from the above-mentioned experiments that Gad-2 is highly pH dependent and showed greater activity at pH 5 than pH 7.

McDonald and co-workers reported that Gad-1 and Gad-2 exhibited concentration-dependent cytotoxicity against Lewis lung carcinoma (LLC) cells, HEY ovarian cancer cells and PC3 prostate cancer cells. The cytotoxic effect of Gad-1 is greater than Gad-2 [42–47]. Both peptides exhibited considerably higher killing of ovarian and prostate

cells under acidic conditions, but Gad-2 showed a significant pH-dependent change in activity with all three carcinoma cell lines [48a].

Rapsch and co-workers have discussed the antimicrobial activities of different peptides [48b]. They developed a rapid and efficient screening procedure to identify and investigate which biologically active peptides, in an immobilised state, could produce an effective antimicrobial surface coating. In addition to this, an antimicrobial surface coating of peptides exhibited no cytotoxic activity against a eukaryotic cell line (Figure 2.6).

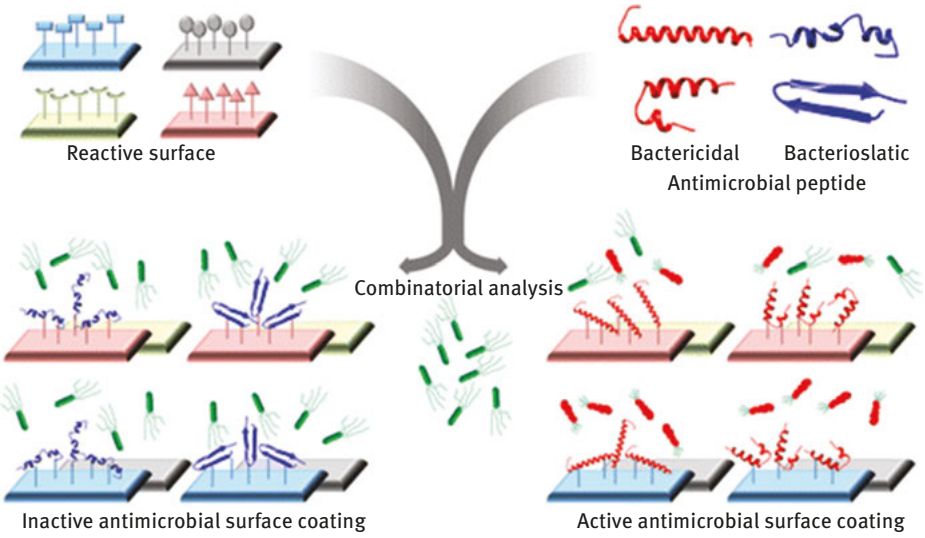

Figure 2.6: Identification of AMP and an immobilisation strategy suitable for a covalent surface coating with biocompatible properties. Reproduced with permission from K. Rapsch, F.F. Bier, M. Tadros and M. von Nickisch-Rosenegk, *Bioconjugate Chemistry*, 2014, 25, 2, 308. ©2014, American Chemical Society [48].

2.3.2 Hyperbranched polymers and cationic polymers

RAFT polymerisation is a useful technique to synthesise HBP with pendent norbornene functionalities (Figure 2.7) [49]. HBP are well-known for their multiple and simultaneous bacterial activities. They can capture multiple bacteria via the core–shell covering. As mentioned in the synthesis section, HBP are able to form micelles via self-assembly in solution and, as a result, they act as HDP. They are positively charged polymers and non-toxic to mammalian cells as are cationic polyamino acids and cationic polyelectrolytes. It has been reported that to retard the harmful microbial growth of infected tissue, HDP make a neutral core–shell on it [50, 51].

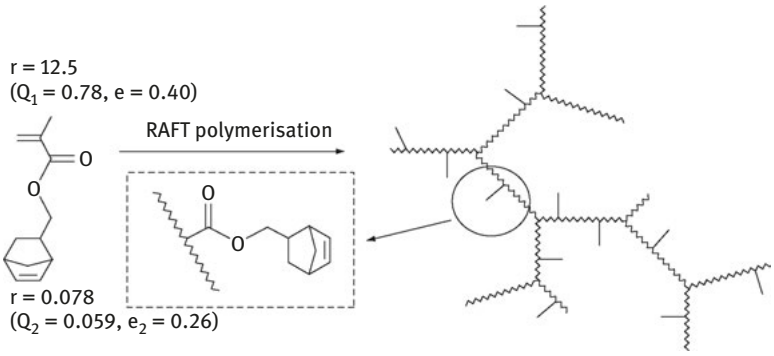

Figure 2.7: Synthesis of HBP with pendent norbornene functionalities via RAFT polymerisation of a novel asymmetrical divinyl monomer. Adapted with permission from Z-M. Dong, X-H. Liu, X-L. Tang and Y-S. Li, *Macromolecules*, 2009, 42, 13, 4596. ©2009, American Chemical Society [49].

Haemolytic and antibacterial activities, that is, efficacy toward both Gram-negative and Gram-positive bacteria, of cationic polymers such as polynorbornene are significantly affected by the hydrophobicity of the repeat unit. Ilker and co-workers reported the activity of a homopolymer with a similar MW (near 10,000 g/mol, M_n) against Gram-negative bacteria (*Escherichia coli*), Gram-positive bacteria (*Bacillus subtilis*) and human red blood cells (RBC). Cationic polymers without a substantial hydrophobic group did not show any antibacterial or haemolytic activity; however, there was an increase in the antibacterial, as well as the haemolytic activity when a hydrophobic group was introduced.

To measure the antibacterial activities of amphiphilic cationic polynorbornene derivatives, they developed a slightly different method based on optical density (OD). In this method, bacteria were grown in suspensions (*Escherichia coli* D31 and *Bacillus subtilis* ATCC® 8037™) of Müeller–Hinton broth (MHB) overnight at 37 °C, diluted with fresh MHB to an OD of 0.1 at 600 nm and further diluted by a factor of 10. This suspension was mixed with different concentrations of freshly prepared polymer solutions in *tris*-buffered saline (pH 6.5–7.0), by serial dilution in a 96-well plate, and incubated for 6 h at 37 °C. The OD 600 nm was measured for bacteria suspensions that were incubated in the presence of a polymer solution or *tris*-buffered saline only. After 6 h, the MIC for 90% inhibition of growth was determined.

2.3.3 Antimicrobial activities of biodegradable amphiphilic polymers

Amphiphilic polymers demonstrate a wide range of antibacterial activities; however, a major drawback associated with them is their non-biodegradability. Owing

to environmental concerns it has been necessary to develop amphiphilic polymers which are biodegradable, that is, do not cause pollution. Therefore, we will now discuss some highly biodegradable polymers and their antibacterial activity, for example, a copolymer of acryl sucrose, which can be synthesised using styrene and tested by applying the *Aspergillus niger* fungus to the polymer; the culture incubation method is used for detecting the biodegradation potential of the copolymers.

During the 90 days of incubation with a fungal culture, the fungal colonisation/ growth (visual growth) on the surface of the polymer film was assessed. Initiation of fungal growth could be seen using a microscope within 15–20 days; however, visual growth could only be seen after 90 days of incubation. A complete absence of carbon in the nutrient agar was observed, which indicated that the fungus used the copolymer as the sole source of carbon, as incubation progressed, the extent of fungal colonisation increased [52]. The higher colonisation rate could be attributed to the easy consumption of short chains as an energy source by the fungus as incubation progressed, which leads to the conclusion that the copolymer was bioassimilated during microbial attack. Microbial adhesion onto the surface was fairly evident after 30 days of incubation. Once the microbes were adsorbed, their penetration into the polymer was rapid as they consumed it [53]. Polar optical microscopy was used for analysing the samples. Ikeda and co-workers tested the toxicity of the remaining lower MW PS fragments of the sugar fraction after fungal degradation [54]. They reported that when soil was treated with the oligostyrene fragments, no toxic effects were observed on the earthworms living in the soil. Similarly, after putting the residual product of degradation in a fish tank, the fish were unaffected by the presence of the fragments in the tank over an extended period. Polyguanidinium oxanorbornene (PGON) is an example of a biodegradable polymer, which exhibits extremely strong antibacterial properties against Gram-negative, as well as Gram-positive bacteria, and non-haemolytic activity against human RBC. The PGON polymer is lethal and not bacteriostatic; however, it did not disrupt membranes in vesicle-dye leakage assays and microscopy experiments.

Polycarbonates (PC) are biodegradable polymers. They can be synthesised by the organocatalytic ring-opening polymerisation (ROP) of functional cyclic carbonate monomer (MTC–OCH_2BnCl). Cationic polymers containing quaternary ammonium groups of various pendent structures (e.g., imidazolinium, alkyl and aromatic) are produced via a postpolymerisation functionalisation strategy of PC. Chin and co-workers investigated the biological properties of these polymers using microbial growth inhibition assays against Gram-positive and Gram-negative bacteria, fungus, as well as haemolysis assays using rat RBC (Figure 2.8) [55]. They reported that the amphiphilic balance of the polymer is an applicable determinant in providing substantial antimicrobial strength and low haemolysis. For example, significant antimicrobial potency is exerted by polymers containing *N,N*-dimethylbutylammonium and *N,N*-dimethylbenzylammonium groups in a 1:1 molar ratio.

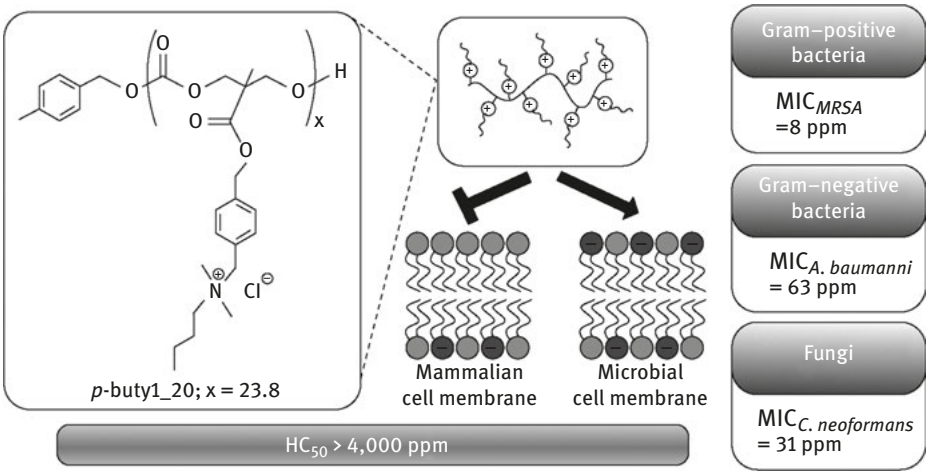

Figure 2.8: Biodegradable broad-spectrum antimicrobial PC: investigating the role of chemical structure on activity and selectivity. Reproduced with permission from W. Chin, C. Yang, V.W.L. Ng, Y. Huang, J. Cheng, Y.W. Tong, D.J. Coady, W. Fan, J.L. Hedrick and Y.Y. Yang, *Macromolecules*, 2013, 46, 22, 8797. ©2013, American Chemical Society [55].

2.3.4 Carbohydrate-derived amphiphilic macromolecules

Spirocyclic C-aryl glycosides and styryllactones are carbohydrate-based lactones. Spirocyclic C-aryl glycosides are isolated from a strain of *Paplarias sphaerospermum* and constitute a family of novel antifungal antibiotics. They serve as an excellent antifungal agent against *Candida tropicalis*, *Candida albicans* and *Pneumocystis carinii*. Styryllactones are enantiomers of (þ)-altholactone, a natural product which possesses antitumour and cytotoxic activities.

Bera and co-workers created a total of six polycationic amphiphiles (PA) to study the effect of lipid hydrophobicity on the activity of amphiphilic neomycin B conjugates; four of the compounds incorporated either palmitic or arachidonic dilipid lysine tails and the other two had single fluorinated undecanoic acid tails. They selected 10 bacteria to determine antimicrobial activity, that is, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, *Escherichia coli* (ATCC® 25922™), *Escherichia coli* (CAN-ICU 61714), *Escherichia coli* (CAN-ICU 63074) and *Pseudomonas aeruginosa*. The antimicrobial activity of neomycin B-based bilipids and fluorinated monolipids was found to be higher against Gram-positive bacteria than Gram-negative bacteria; palmitoylguanidylated dilipids were the most active compounds overall. The greatest activity was against Gram-negative bacteria, that is, *Pseudomonas aeruginosa*. The high lipid membrane activity of carbohydrate-derived amphiphilic macromolecules makes them very useful as a drug-delivery system.

Poly-amido-saccharides (PAS) (Figure 2.9) are synthetic carbohydrates that can mimic natural polysaccharides as they can bind lectinconcanavalin A (ConA) at the same site as natural carbohydrates. PAS are synthesised via anionic ROP of a β-lactam sugar monomer. In PAS, the pyranose rings are linked through the 1- and 2-positions by an amide with α-stereochemistry. β-lactam sugar is synthesised from benzyl-protected D-glucal [56].

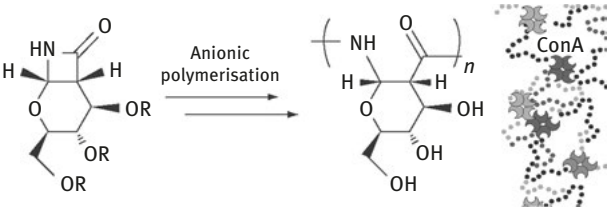

Figure 2.9: PAS: synthesis via anionic polymerisation of a β-lactam sugar monomer. Reproduced with permission from E.L. Dane and M.W. Grinstaff, *Journal of the American Chemical Society*, 2012, 134, 39, 16255. ©2012, American Chemical Society [56].

2.3.5 pH-sensitive polymers

pH-sensitive polymers are widely used by the pharmaceutical industry due to their target-based selectivity against bacteria. We will now discuss an important pH-dependent polymer, pluronic P123-docetaxel (DTX), which is synthesised by the conjugation of Pluronic P123 and the drug DTX via acid-cleavage hydrazine bonds. The critical micelle concentration of Pluronic P123-DTX is low and it forms nano-sized polymeric micelles in aqueous solution via self-assembly. These micelles exhibited their stability against rat plasma via pH-dependent drug release behaviour. Furthermore, they have the potential to balance drug stability and release, and hence control release of the drug [57a, 57b] (Figure 2.10). The presence of hydrophobic and hydrophilic properties confers the special ability to entrap the anticancer drug and form a covered drug. The formation of a covered drug increases the water-solubility capacity and the drug will be protected from enzymatic degradation and uptake by mononuclear phagocytes, reticuloendothelial and macrophage systems in the bone marrow, liver and spleen. Consequently, the blood circulation time of the drug is prolonged and it therefore has a permeation and retention (electron paramagnetic resonance) effect which makes it stable in tumour tissue. The covalent bonds in the polymer–drug conjugates are able to keep the drug in the circulation, hence less anticancer drug is released into healthy tissue. pH-responsive micelles have attracted great attention due to the existence of a mildly acidic pH in tumour tissues compared with normal tissues, which may provide a tissue-specific stimulus that can be exploited for selective drug release.

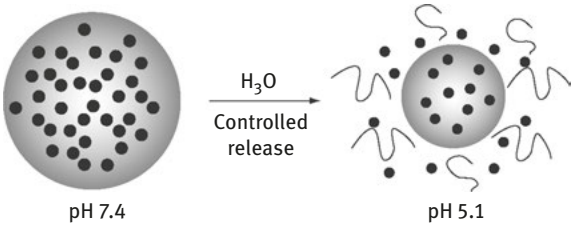

Figure 2.10: pH-sensitive polymer nanospheres for use as a potential drug-delivery vehicle. Reproduced with permission from J. Jung, I-H. Lee, E. Lee, J. Park and S. Jon, *Biomacromolecules*, 2007, 8, 11, 3401. ©2007, American Chemical Society [57].

Zhu and Zhao prepared pH-sensitive polymeric vesicular aggregates using comb-shaped amphiphilic polymers, that is, cholate grafted poly(L-lysine) (PLL-CA), with an amphiphilic PEG–doxorubicin (DOX) conjugate (Figure 2.11) [57c]. The pH sensitivity leads to better uptake of the vesicles by cancer cells (MCF-7) under conditions close to the extracellular environment of a solid tumour (pH = 6.5) and subsequent escape from endosomes after endocytosis. Moreover, if the pH value is lower the vesicles destabilise.

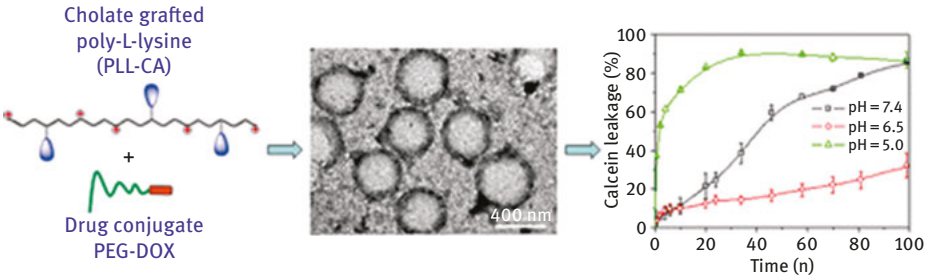

Figure 2.11: pH-sensitive polymeric vesicles from the coassembly of amphiphilic PLL-CA and the acid-cleavable polymer–drug conjugate. Reproduced with permission from L. Zhu, L. Zhao, X. Qu and Z. Yang, *Langmuir*, 2012, 28, 33, 11988. ©2012, American Chemical Society [57].

Recently, a novel acid-cleavable prodrug of DOX was prepared using hydrazone, which is quite useful in the treatment of a broad range of solid tumours. In the same way, DTX is another most effective chemotherapeutic agent, which demonstrates a more effective antitumour activity than paclitaxel. DTX is applicable to a variety of tumours, particularly for the treatment of prostate, gastric, breast, ovarian and non-benign lung cancer.

2.3.6 Polyethylene

PE is a synthetic thermoplastic polymer, which has the chemical formula $(C_2H_4)_n$. PE can be classified on the basis of branching and density, for example, high-density

PE, LDPE, high-density crosslinked PE, high MW PE, ultra-high MW PE, ultra-low MW PE and so on. PE is resistant to strong acids, strong bases, gentle oxidants and reducing agents. Moreover, PE and its copolymers are inexpensive and easy to process. Therefore, PE is useful in packaging and in the coating and production of catheters for percutaneous, transluminal, coronary angioplasty in medical and pharmaceutical industries. However, the clinical complications of employing PE include infections resulting from its use. The biocompatibility of PE can be achieved by surface modification to manage the surface free-energy and low wettability. Desai and Singh reported the different methods used for the surface modification of PE, such as chemical treatment, that is, corona treatment, grafting and plasma treatment. Low-temperature plasma treatment of the surface can increase the free-energy and make the PE surface more hydrophilic [58]. Treatment of the PE surface with substances containing antibacterial groups, such as triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] and chlorhexidine {1,1′-hexamethylenebis [5-(4-chlorophenyl)biguanide]} can enhance the antimicrobial properties [59]. These antibacterial substances are immobilised on LDPE via PAA grafted onto LDPE using low-temperature barrier discharge plasma [60]. Another problem with the use of PE is biofilm formation on the surface, which leads to various infections, usually urinary tract infections, bacterial vaginosis, catheter infections and middle-ear infections [61]. To prevent this, antiinfection modification can be performed on polymers used for medical applications [62, 63]. Antiinfective properties of polymers can be achieved through the following methods:

- Antiinfection agents mixed in the polymer.
- Copolymerisation of antiinfection agents with monomers.
- Appropriate surface treatment of medical polymers.

As antibacterial agents are not released from the polymer volume, the bulk properties of PE are not affected by the physico-chemical interaction between bacteria and the polymer [64]. Adhesion also depends upon the surface morphology (roughness) [65–67]; as the surface roughness increases, adhesion also increases. The contact angle is measured to estimate the extent of wettability. As the contact angle for any surface decreases, the wettability increases; for a hydrophobic surface, the contact angle will be more than 90°, with complete wettability achieved when the contact angle is near to 0°.

Popelka and co-workers treated an LDPE surface using cold plasma to increase its wettability, adhesive properties and roughness, and consequently, resistance toward microbial infection [68]. They reported that the contact angle for untreated PE was highest and decreased after plasma treatment. For surfaces covered with PAA, triclosan and chlorhexidine, the contact angle decreased and the largest decrease was observed for the surface covered with PAA.

From an assessment of antibacterial activity, the results show that untreated, plasma treated and acrylic-acid grafted samples did not display any antibacterial

activity against strains of *Escherichia coli* and *Staphylococcus aureus*. The sample coated with triclosan met the expected antibacterial requirements and similar results were obtained for chlorhexidine-coated samples, with the average inhibition zone of 42.2 mm^2 for *Escherichia coli* and 288.1 mm^2 for *Staphylococcus aureus*. It is worth mentioning that both antibacterial agents are more active against Gram-positive bacteria. Finally, triclosan-coated samples showed the best results of the two antibacterial substances used.

Bacterial behaviour and antibacterial properties on the polymer surface have been described in the literature [69–71].

2.3.7 Neomycin B-based bilipids

Neomycin is an aminoglycoside antibiotic, which acts as a polycationic amphiphile. It is used in topical creams, ointments, lotions, eye preparations and eardrops, and as an antibacterial preservative agent in some vaccines. Neomycin exhibits exceptional activity against Gram-negative bacteria and also has partial activity against Gram-positive bacteria [72].

The lipid bilayer is a thin polar membrane made of two layers of lipid molecules. These membranes are flat sheets that form a continuous barrier around the cell, and thus acts as a good antibacterial agent. Derivatives of neomycin B are examples of bilipids [73, 74].

Bera and co-workers synthesised six polycationic amphiphiles with the backbone of neomycin B [75]. In their experiment, they converted all six amine groups on neomycin B to guanidine and all six compounds were tested against a panel of clinically relevant and ATCC Gram-positive and Gram-negative bacteria to evaluate the effect of basicity. Guanidinylation was found to increase activity 2–8 fold, with dipalmitoylguandilyated neomycin B displaying good activity against a number of Gram-positive bacteria.

2.4 Conclusions

Amphiphilic polymers have wide-ranging applications in various fields, including medicinal chemistry, and are synthesised using different methods. Polymer synthesis can cause environmental pollution; however, there are some methods, such as solvent-free synthesis and microwave-assisted synthesis, which help to minimise pollution. Amphiphilc polymers possess different antimicrobial activities against many microbes as a result of their micelle-forming ability; however, these polymers are non-biodegradable, which is a major problem. To overcome this issue, biodegradable polymers such as CS, acryl sucrose, peptides and so on, are synthesised. Moreover,

these biodegradable polymers are non-toxic to cells. The balance of hydrophobic and hydrophilic groups has a big impact upon the antimicrobial properties of polymers and methods are available, which can achieve the desired balance. Some amphiphilic polymers are pH sensitive and their antibacterial properties may vary with pH, thus pH dependency is a major field of research. It is hoped that in the future, amphiphilic polymers will be utilised in biology as potential antibacterial agents with non-toxic properties.

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3 Design of biomimetic antimicrobial polymers

Abstract: There have been several attempts toward synthesis and design of biomimetic antimicrobial polymers. Many cationic antimicrobial polymers have been proposed sofar to act in a manner similar to membrane-active antimicrobial peptides, such as magainin.A literature search indicates that there are different synthesis strategies and polymers for this purpose, methacrylate-based copolymers, phenylene ethynylenes, polynorbornene by-products, amphiphilc arylamide polymers.In this chapter, the basic principles and recent developments of polymeric antimicrobials has been reviewed, along with a mechanistic approach to better understand the role of this class of materials in different applications.

Keywords: Antibacterial, polymer, magainin, biomimetic

3.1 Introduction

Microbial infections continue to threaten human health and pose a great economic burden to society. The management of microbial infections has become problematic due to the number of resistant microbial strains and the fact that the increase in the number of antibiotic-immune patients has risen a lot quicker than the number of functional antibiotics available [1, 2]. Antimicrobial agents are materials, which are capable of killing pathogenic microorganisms [3]. In general, antibiotics, antifungals or antivirals are based on natural materials or low molecular weight (MW) components; however, they result in residual toxicity, even when correct doses of the agents are administered [4, 5].

Low pharmacokinetics, susceptibility to proteolysis and high production costs, have limited the large-scale pharmaceutical use of antimicrobial peptides (AMP) for clinical applications [6–8]. Consequently, the use of antimicrobial polymeric materials offers an attractive alternative to increase the effectiveness of some existing antimicrobial agents while simultaneously reducing the environmental problems

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that are associated with conventional antimicrobial agents by decreasing their residual toxicity, increasing their efficiency and selectivity and extending their lifetime. Furthermore, these antimicrobial polymeric materials are non-volatile and chemically stable in certain applications [9].

In addition to a passive function (e.g., packaging or structural), polymer products also display an active function (e.g., protective and/or indicative). Polymers which exhibit resistance to microbial colonisation and prevent the growth of pathogenic microorganisms (antimicrobial polymers) have been one of the models employed to achieve active material functionality. Antimicrobial polymers are designed to defend against the negative effects of pathogenic microorganisms, as these microbes can cause extreme disturbance to society due to health impairment and undesirable economic load [10]. Antimicrobial polymers, known as polymeric biocides, are a category of polymers, which exhibit antimicrobial properties, or the ability to prevent the growth of microorganisms such as bacteria, fungi or protozoans. These polymers have been designed to mimic AMP, which are used by the immune systems of living things to kill bacteria. Biomimetic polymers, in the form of functional building blocks, are synthetic materials inspired by nature, in which a biologically active object is associated with a polymer in a designed chemical reaction. The translation of biological basics to synthetic polymers offers critically enhanced mechanical properties of the materials for tissue engineering, drug delivery and so on.

The synthesis of polymers and oligomers that mimic the complex constructions and notable biological properties of proteins is crucial research with both fundamental and practical implications. Therefore, considerable work has gone into designing polymers, which mimic important physico-chemical properties, such as the existence of both cationic and hydrophobic groups, of naturally occurring AMP [11–14].

3.2 Mechanisms of antimicrobial polymers

Antimicrobial polymers usually destroy bacteria via a series of steps (Figure 3.1). First, the polymer must adsorb onto the bacterial cell wall. Most bacterial surfaces are negatively charged, and consequently the adsorption of polymeric cations exhibits higher effectiveness than the adsorption of polymeric anions. The antimicrobial agent must then diffuse through the cell wall and adsorb onto the cytoplasmic membrane. Small molecule antimicrobial agents excel at the diffusion step because of their low MW, while antimicrobial polymers achieve better adsorption. The interruption of the cytoplasmic membrane and subsequent leakage of cytoplasmic constituents leads to cell death. A comparison of small molecule antimicrobial agents and antimicrobial polymers is presented in Table 3.1 [9].

Three separate, likely non-exclusive, modes of membrane disruption have been discussed and these are barrel-stave, [15] toroidal [16, 17] and carpet mechanisms

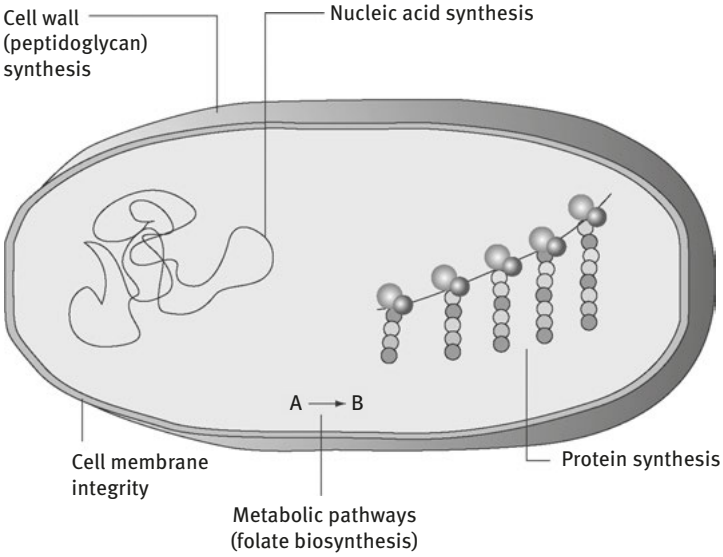

Figure 3.1: Mechanism of action at different sites.

Table 3.1: Comparison of small molecule antimicrobial agents and antimicrobial polymers.

Step	Small molecule antimicrobial agents	Antimicrobial polymers
Initial adsorption	Weak	Strong
Diffusion through the cell wall	Strong	Weak
Binding onto the membrane	Weak	Strong
Disruption and disintegration of the membrane	Weak	Strong

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[18, 19]. The latest studies have proposed that, along with these approaches, a more detailed mechanism of antimicrobial action may involve the induced sequestering of negatively charged lipid molecules followed by coarsening of the bilayer and consequent phase boundary deficiencies, which could exert a negative effect on the uniformity of the bacterial membranes leading to lysis [20, 21]. Furthermore, AMP-induced phase separation in bacterial cell membranes occurs on a noticeably shorter timescale, hence the membrane is unlikely to accommodate such rearrangement, and thus is detrimental to the integrity of the cell [20]. This mechanism is thought to be the mode of action in polymers containing flexible backbones and a random arrangement of cationic and hydrophobic moieties [17, 22].

The result of the interactions of some copolymer mimics of AMP with model bacterial membranes has been studied via atomistic molecular dynamics simulation

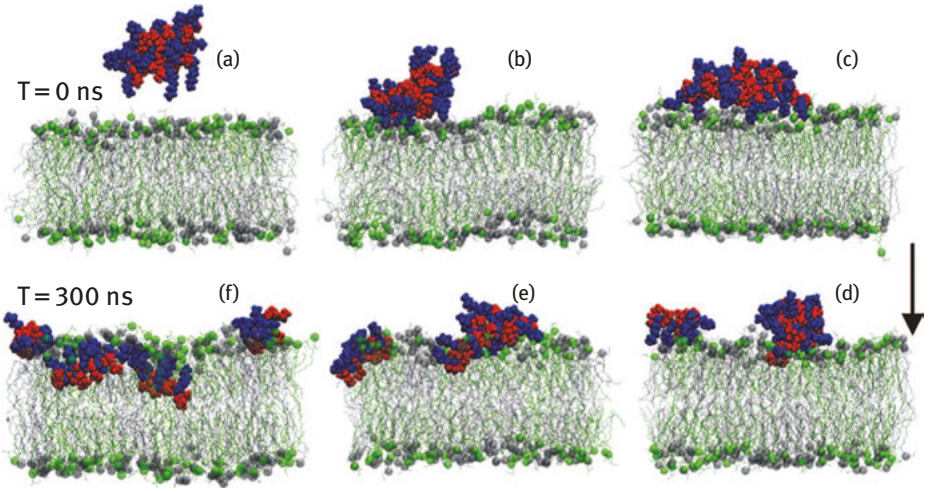


Figure 3.2: Snapshots (a) to (f) of the partitioning of antimicrobial polymers E4 ([m = 4] polymer, with aminobutylene cationic side chains) aggregating into the bilayer. Water and ions are not shown for clarity. The cationic and hydrophobic groups of E4 polymers are shown in blue and red, respectively. Reproduced with permission from U. Baul, K. Kuroda and S. Vemparala, *Journal of Chemical Physics*, 2014, 141, 084902. ©2014, AIP Publishing LLC [23].

(Figure 3.2). The model bacterial membrane expands homogeneously in a lateral manner in the membrane thickness profile compared with the polymer-free system. The individual polymers taken together are released into the bacterial membrane in a phased manner and the simulations propose that the most possible location of the partitioned polymers is near the 1-palmitoyl-2-oleoyl-phosphatidylglycerol clusters. The partitioned polymers preferentially adopt facially amphiphilic conformations at the lipid–water interface, although they lack intrinsic secondary structures, such as an α-helix or β-sheet, found in naturally occurring AMP [23].

3.3 Design and synthesis of methacrylate-based copolymers

Based on the peptide-mimetic design, Kuroda and Degrado [24] studied antimicrobial polymers, using the conventional polymer platform of methacrylate, which have no basic secondary construction as found in peptides. They synthesised polymethacrylates (PMA) containing cationic and hydrophobic groups in the side chains, which were randomly dispersed in a polymer chain (Figure 3.3). These methacrylate random copolymers contained primary ammonium groups as cationic bases, representing the cationic functionality of lysine remnants, which are available in naturally occurring

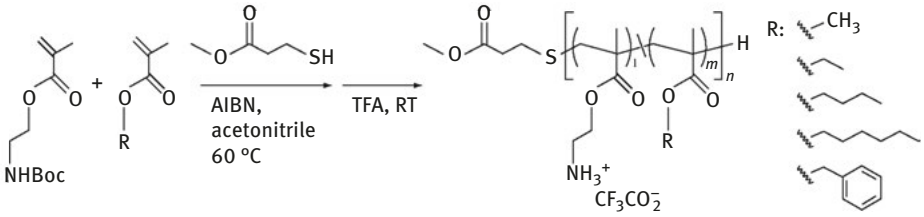


Figure 3.3: Synthesis of methacrylate random copolymers with cationic amphiphilic structures containing R: hydrophobic groups (methyl, ethyl, butyl, hexyl and benzyl groups). AIBN: azobisisobutylonitrile, RT: room temperature and TFA: trifluoroacetic acid. Reproduced with permission from H. Takahashi, E.F. Palermo, K. Yasuhara, K. Kuroda Gregory and A. Caputo, *Macromolecular Bioscience*, 2013, 13, 1285. ©2013, John Wiley and Sons [26].

and designed AMP sequences, while quaternary ammonium groups have been traditionally used in the production of antimicrobial polymers [25]. The cationic functionality of the copolymers was thought to improve the binding to bacterial surfaces via electrostatic attraction, as in the AMP design template. Takahashi and co-workers [26] produced copolymers in the presence of a chain transfer modifier, methyl 3-mercaptopropionate, to synthesise low MW copolymers (MW = 1,000–10,000 g/mol) to correspond to the reasonably low MW of AMP (a few kDa). The mole percentages of hydrophobic groups were changed from 0 (cationic homopolymers) to 60 mol% to control the balance between net cationic charge and hydrophobicity.

The polymers were synthesised from boc-protected amine monomers and alkyl or benzyl methacrylate monomers. The polymerisation was performed by applying AIBN, as a radical initiator, and methyl 3-mercaptopropionate, as a chain transfer agent, in acetonitrile. The treatment of boc-protected polymers with TFA produced amphiphilic random copolymers with cationic ammonium groups in the side chains.

Dizman and co-workers [27] produced new methacrylate monomers enclosing pendent biquaternary ammonium moieties based on 1,4-diazabicyclo-[2.2.2]-octane (DABCO) [27]. The monomer was synthesised by reacting DABCO with bromoalkanes, such as butyl and hexyl chains, to be involved in one nitrogen of DABCO; the other nitrogen of DABCO was modified using 11-bromoundecanoic acid. The carboxylic group of the product was moved to the sodium salt by reacting it with potassium carbonate. The salt was reacted with ethyl R-chloromethyl acrylate resulting in a methacrylate monomer with two quaternary ammonium groups. The antimicrobial activities of the prepared polymers were observed using *Staphylococcus aureus* and *Escherichia coli* as test organisms. The mixture of both ionic and van der Waals interactions improved the binding of the polymers to the cytoplasmic membrane of the bacteria.

Pumyani and Singh [28] described the synthesis of iodine-containing quaternary amine methacrylate (QAMA) copolymers. The monomers were prepared

via a two-step reaction: (i) the reaction of ethylene glycol dimethacrylate with piperazine in methanol at 35 °C and (ii) the quaternisation of the synthesised monomer with 1-iodooctane. The antimicrobial activities of the QAMA-containing copolymers were evaluated against *Escherichia coli* and *Staphylococcus aureus*.

Imazato and co-workers [29, 30] prepared a novel monomer, methacryloyloxydodecylpyrimidinium bromide (MDPB). A water-soluble homopolymer of MDPB and copolymer of MDPB with acrylamide were also synthesised and the bactericidal activity against oral streptococci was studied. The mechanism of action of these quaternary compounds is assumed to be due to direct cationic binding to cell wall components, which results in disruption of the cell wall membrane, and subsequently results in leakage of critical cell contents and cell death.

The PMA design platform was applied to obtain non-haemolytic antimicrobials by changing the hydrophobic nature of the random copolymers [31]. Gellman and co-workers showed that polymers containing tertiary amine groups exhibited biocidal activity, whereas analogous polymers containing quaternary ammonium groups were inactive [32]. Amphiphilic random copolymers containing cationic and hydrophobic side chains were synthesised via the copolymerisation of amine-functionalised methacrylate monomers using different ratios of an alkyl methacrylate. Primary or tertiary amine groups, or quaternary ammonium groups, were used as the source of cationic charge in each copolymer series. It was shown that the copolymer composition of amphiphilic copolymers, containing primary or tertiary amine groups, could be changed to achieve potent antimicrobial activity whilst minimising red blood cell(s) (RBC) lysis. Furthermore, the copolymers containing quaternary ammonium groups needed a greater amount of hydrophobic comonomer to express activity and displayed, in general, a lower selectivity for *Escherichia coli* versus human RBC. A reduction in the fraction of amine groups that are cationic, from R = 1.0 to 0.7, led to an improvement of antimicrobial and haemolytic activity. As this value was reduced further, that is, to R = 0.5, loss of activity was detected. The activities of polymers containing quaternary ammonium groups were shown to be pH-independent.

The activity and mechanism of AMP-mimetic [33] cationic amphiphilic PMA derivatives make them a potentially novel class of synthetic antimicrobials. PMA have been proved to be active against a panel of pathogenic bacteria, including a drug-resistant strain of *Staphylococcus aureus*, in comparison with the natural AMP magainin, which did not show any activity against the same strain. The PMA studied here showed a broad spectrum of activity against clinically essential bacterial pathogens, including an antibiotic-resistant strain of *Staphylococcus aureus*, with minimal inhibitory concentration (MIC) values less than that of the natural host-defence peptide(s) (HDP) magainin-2.

3.4 Design and synthesis of polyphenylene ethylene polymers

Structures that are similar in chemical composition to AMP include stereoisomers of natural peptides [34], β-peptides [35, 36], cyclic α-peptides [37] and peptoids [38]. Many α-peptides, β-peptides and peptoids also mimic the helical structure found in AMP such as magainin. The decoration of aromatic backbones, containing arylamides [39] and phenyleneethynylenes (PPE) [40], with cationic groups led to structures, which possess both antibacterial activity and the capability of separating prokaryotic cells from eukaryotic mammalian RBC. The smallest members of this series are the cationic oligomeric PPE, where *n* ranges from 1 to 2. The applications of cationic PPE polymers have been studied for use in fluorescence-based sensing [37, 38] and as light-activated antimicrobials [39, 40].

Sudeep and co-workers [41] synthesised three series of well-defined cationic oligomers, that is, oligomeric *p*-phenyleneethynylenes (OPE), with different chain lengths and different end groups on the main chain to study structure–reactivity relationships through photophysical and antimicrobial properties [41, 42].

Polymers based on *m*-phenyleneethynylenes (mPE) were synthesised to mimic the amphiphilic, cationic and rigid structure of AMP, and were suggested to be good mimics of AMP in terms of their high potency against microbes and low haemolytic activities. Ishitsuka and co-workers [43] designed two mPE (Figure 3.4); the general design consideration for these mPE was to capture the cationic amphiphilic nature of AMP using entirely abiotic backbones. One sample, compound (1), was synthesised via polymerisation techniques and exhibited important MW variation. The acceptable design of these polydisperse structures resulted in non-haemolytic, antibacterial activity [40]. A discrete MW structure, compound (2), was synthesised and found to be more potent and selective than the larger compound (1). In addition to the overall facially amphiphilic structure [44], the discrete oligomer compound (2), had additional similarities to protegrin-1 (PG-1). As shown in Figure 3.4, compound (2) had cationic charges restricted to the ends, which were bridged by a hydrophobic domain in the middle. The structure–property correlation of these two constructions has been studied previously [45].

Wang and co-workers [46] studied the interactions of PPE-based cationic conjugated polyelectrolytes (CPE) and OPE using *Escherichia coli* cells to gain an understanding of the dissimilarities in the killing mechanisms between CPE and OPE in dark conditions. It was shown that the antimicrobial and haemolytic activities of these compounds under dark conditions related to their ability to disrupt bacterial and mammalian cytoplasic membranes. The molecular size, charge density and degree of amphipathicity/hydrophobicity can affect the activities of these compounds; charge density is essential for the initial binding step between antimicrobial compounds and bacteria. It is also clear that the spatial distribution of polar

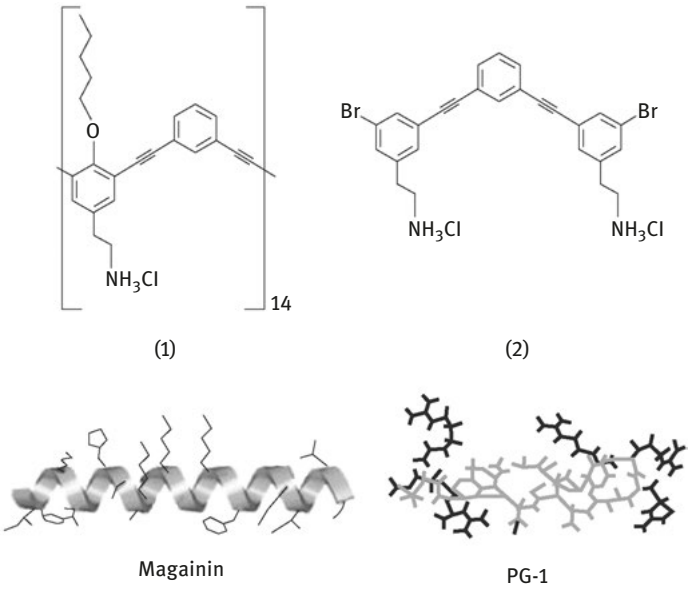


Figure 3.4: Chemical structures of mPE and molecular models of AMP. For magainin (1), residues with hydrophilic (dark grey) and hydrophobic (light grey) side chains are on opposite sides of the helix, giving rise to an amphiphilic structure; for PG-1 (2), the cationic amino acids (dark grey) flank both ends of the peptide. Reproduced with permission from Y. Ishitsuka, L. Arnt, J. Majewski, S. Frey, M. Ratajczek, K. Kjaer, G.N. Tew and K.Y.C. Lee, *Journal of American Chemical Society*, 2006, 128, 13123. ©2006, American Chemical Society [43].

and non-polar groups within a molecule strongly affects its interaction with the phospholipid bilayer and protein [47].

Cationic polyarylene ethylene (CPE) has recently been reported as potent biocidals against *Pseudomonas aeruginosa* in dark conditions due to their high lipophilicity and the presence of accessible quaternary ammonium groups [48]. The layer-by-layer procedure [49] was used to synthesise hybrid antimicrobial and cationic particles from dioctadecyldimethylammonium bromide (DODAB) bilayer fragments (BF), secondary consecutive layers of the anionic polymer carboxymethyl cellulose (CMC) and the cationic polyelectrolyte, poly(diallyldimethylammonium chloride) (PDDA) [50]. Both cationic microbicides, DODAB and PDDA, were combined in a single supramolecular assembly. These assemblies, in the form of small or large particles, were produced from small or large DODAB BF concentrations, respectively. The assemblies DODAB BF/CMC/PDDA exhibited potent antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antimicrobial effect was similar for particles with a mean diameter of 100 or 500 nm and depended only on the amount of positive charges on the particles [50]. These hybrid particles also delivered AmB to *Candida albicans* [51]. The cationic lipid, antibiotic and cationic polyelectrolyte nanostructures in each particle efficiently attacked the fungus.

3.5 Design and synthesis of polynorbonene-based polymers

Functionalised norbornene derivatives have been shown to be outstanding monomers for ring-opening metathesis polymerisation (ROMP) and have been used in the synthesis of a wide range of polymers (Figure 3.5) [52]. Because of the strained nature of the norbornene ring, they are active monomers for living ROMP, resulting in narrow polydispersity polymers along which have large side groups; these amphiphilic polymers exhibit disruption activities of lipid membranes.

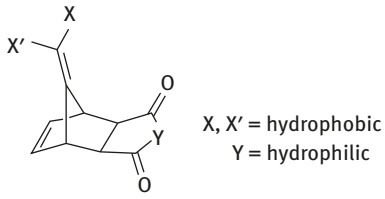


Figure 3.5: General structure of amphiphilic modular norbornene derivatives. Reproduced with permission from M. FiratIlker, Hanna Schule and E. Bryan Coughlin, *Macromolecules*, 2004, 37, 694. ©2004, American Chemical Society [53].

Amphiphilic polymers, based on modular norbornene products (Figure 3.6), exhibited good antibacterial activities and high selectivity for bacteria versus RBC. This category of polymers was synthesised via a ROMP-based simple synthetic procedure that allows better control over monomer composition, MW, polydispersity and amphiphilicity. Small adjustments to the hydrophobic character of the cationic amphiphilic polymer were proved to effectively change the antibacterial and haemolytic activities. Modification of the hydrophobic/hydrophilic balance and MW of these

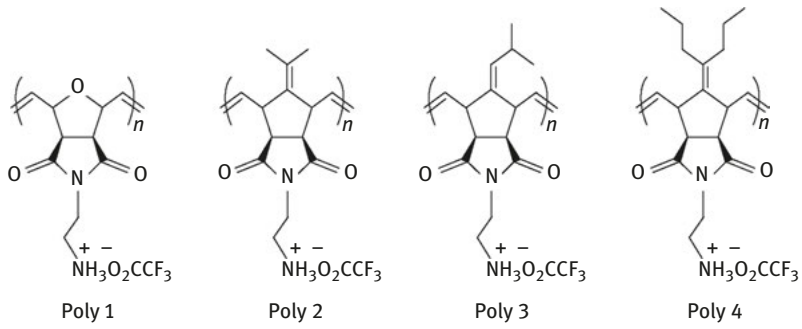


Figure 3.6: Examples of amphiphilic polynorbornene derivatives. 2,3-disubstituted-7-alkylidene norborn-2-ene derivatives, poly 1, poly 2, poly 3 and poly 4. Reproduced with permission from M.F. Ilker, K. Nusslein, G.N. Tew and E.B. Coughlin, *Journal of American Chemical Society*, 2004, 126, 15870. ©2004, American Chemical Society [54].

copolymers allowed synthesis of highly selective, antibacterial non-haemolytic macromolecules. Synthetic high MW polymers that mimic the activities of HDP in the absence of a specific secondary structure have been produced. Polymer-induced fluorescent dye leakage from negatively charged and neutral large unilamellar vesicles was used to assess the disruption to lipid vesicle membranes. As shown in Figure 3.7, poly 2 was inactive against neutral vesicles and exhibited little disruption of negatively charged vesicles at the observed concentrations. Poly(22-*co*-31) exhibited improved activity against negatively charged vesicles while retaining low activity against neutral vesicles, with a selectivity near 6. Poly 3 was highly active against both types of membranes with a lower selectivity of 2. The above-mentioned results demonstrate the membrane activity of these biologically active high MW polymers but miscalculate the degree of selectivity measured for poly(22-*co*-31) during in vitro experiments [54].

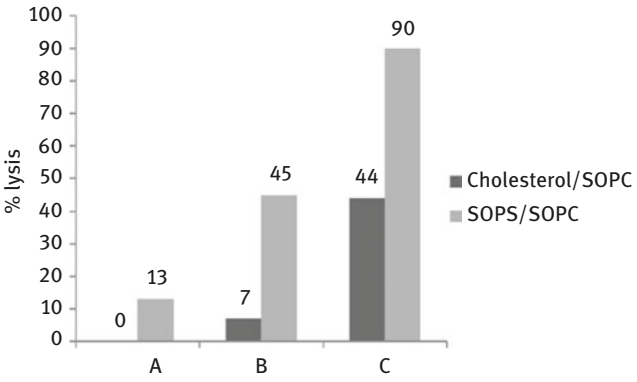


Figure 3.7: Lysis of neutral vesicles (cholesterol/SOPC) and negatively charged vesicles (SOPS/SOPC), at 3 min, caused by 25 μm/mL poly 2. (A) *M*n 9,950 g/mol; poly(22-*co*-31), (B) *M*n 15,300 g/mol and (C) *M*n 10,500 g/mol poly 3. Percent lysis values are given on top of the bars. *M*n: number average molecular weight, SOPC: stearoyl-oleoyl-phosphatidylcholine and SOPS: stearoyl-oleoyl-phosphatidylserine. Reproduced with permission from M.F. Ilker, K. Nusslein, G.N. Tew and E.B. Coughlin, *Journal of American Chemical Society*, 2004, 126, 15870. ©2004, American Chemical Society [54].

Al-Badri and co-workers [55] studied the effect of fine-tuning the cationic parameter of synthetic mimics of antimicrobial peptides (SMAMP) on haemolytic and antibacterial activities. A category of novel norbornene monomers that carry one, two or three Boc-protected amine-functionalities was synthesised (Figure 3.8). ROMP of the monomers, followed by deprotection of the amine groups led to cationic antimicrobial polynorbornenes that carry one, two and three charges per monomer repeat

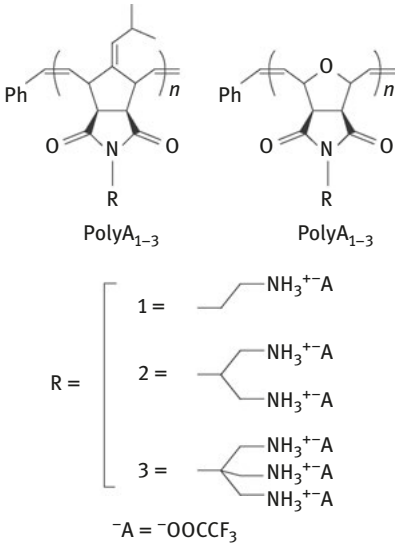


Figure 3.8: Antimicrobial polynorbornene derivatives. Reproduced with permission Z.M. Al Badri, A. Som, S. Lyon, C.F. Nelson, K. Nusslein and G.N. Tew, *Biomacromolecules*, 2008, 9, 2805. ©2008, American Chemical Society [55].

unit. It was observed that enhancing the number of amine groups on the most hydrophobic polymer effectively decreased its haemolytic activity.

Colak and co-workers [56] investigated the hydrophilic adjustments of an amphiphilic polynorbornene, poly 3, via the incorporation of hydrophilic, biocompatible groups, and determined the effects on haemolytic and antibacterial activity. It was shown that increasing the hydrophilicity reduced the antibacterial properties, which could be a result of the overall charge decrease. In addition, the hydrophilicity of the biocompatible groups decreased the haemolytic activities of the new polymers; therefore, enhanced selectivity was achieved. Those polymers were significantly more potent with single μg/mL MIC values.

Al-Ahmad and co-workers [57] studied the potential of poly(oxonorbornene)-based SMAMP, a favourable new class of antimicrobial polymers with cell selectivity and low-resistance improvement potential, for clinical applications. The results showed the specific problems that can occur when testing the antimicrobial activity of amphiphilic cationic polymers, and established the working hypothesis that more hydrophilic SMAMP polymers on the market were 'doubly selective', that is, they are not only selective for bacteria over mammalian cells, but also for Gram-positive over Gram-negative bacteria. Transmission electron studies showed that the cellular envelopes of both *Escherichia coli* and *Staphylococcus aureus* were harshly damaged as a result of SMAMP action on the bacterial membrane, which strengthened the argument that SMAMP closely resemble AMP.

3.5.1 Copolymer synthesis

Monomers were produced via the ring-opening of oxonorbornene anhydride with the respective alcohol. The unreacted acid group was then further esterified. The monomers were mixed in a suitable ratio and polymerised by applying a third-generation Grubbs catalyst. After quenching, living polymerisation with ethylvinyl ether and deprotection with TFA yielded the chosen SMAMP copolymers.

3.6 Design and synthesis of facially amphiphilic arylamide polymers

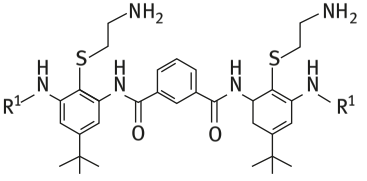
Lately, a number of synthetic peptides, containing designed sequences, have been extended to mimic biologically active structures; in particular, facially amphiphilic peptides made from β-amino acids have been shown to mimic the structures and biological function of natural AMP, such as magainins and cecropins. Nevertheless, these natural peptides, in addition to their β-peptide analogues, are costly to synthesise and hard to produce on a large scale, limiting their potential application to certain pharmaceutical uses. Therefore, Tew and co-workers [58] designed a category of facially amphiphilic arylamide polymers that capture the physical and biological properties of this category of AMP, but are easy to synthesise from low-cost monomers.

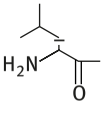
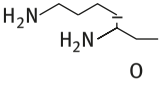
Polymers based on two different repeated units, compound 3, can be synthesised via polycondensation of the diamine with isophthaloyl chloride. The diamine resulted from the chemical 4-*tert*-butyl-phenol, which was prepared from available 2,6-dinitro-4-*tert*-butyl-phenol and other low-cost starting materials in some high-yielding steps (>90%). Oligomers, compounds (4) and (5), containing two or three AB units (two components present in the compound), were also synthesised using solid-phase techniques and purified to homogeneity.

By methodically changing the side chains and possibly the polymeric backbone, it should be feasible to adjust the antimicrobial selectivity and toxicity of the polymers in a method analogous to the production of AMP and β-oligomers [59].

Liu and co-workers [60] designed a series of oligo(aryl amide(s)) that had an amphiphilic secondary structure correlated to compound 1 (Table 3.2) by changing the attaching group so as to modify the general charge, hydrophobicity and hydrophobic moment, resulting in molecules with good activity and selectivity. The oligomers are significantly smaller than comparable antimicrobial oligomers, which might have important benefits in terms of tissue distribution, as well as production cost. Compounds 2–4 (Table 3.2), which have increasingly hydrophobic substituents, display decent activity against both Gram-negative and Gram-positive bacteria with MICs of 6–12 mg/mL for *Escherichia coli* and *Staphylococcus aureus*, respectively (Table 3.2). However, these compounds are also toxic to human

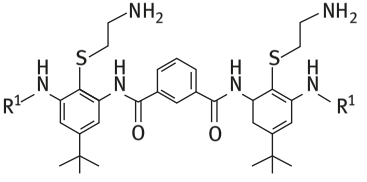
Table 3.2: Antibacterial activity and selectivity (antimicrobial template shown directly below).

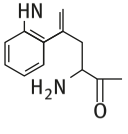
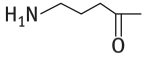
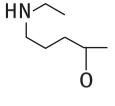


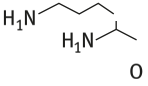
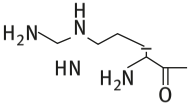
Compound	R^1	MIC (µg/ml)		HC_{50}(µg/mL)	Selectivity (HC_{50}/MIC)		Relative hydrophobicity (log K_{OW})[a]
		Escherichia coli	Staphylococcus aureus		Escherichia coli	Staphylococcus aureus	
1	H	12.5	50	12	0.96	0.24	3.51
2		6.25	12	40	6.4	3.3	3.12
3		6.25	6.25	9	1.4	1.4	3.74

(continued)

Table 3.2 (continued)



Compound	R^1	MIC (µg/ml)		HC_{50}(µg/mL)	Selectivity (HC_{50}/MIC)		Relative hydrophobicity (log K_{OW})[a]
		Escherichia coli	Staphylococcus aureus		Escherichia coli	Staphylococcus aureus	
4		6.25	6.25	7	1.1	1.1	3.86
5		25	50	790	32	16	1.45
6		25	100	1,230[b]	49	12	2.99

7		50		370	7.4		0.33
8		6.25	12.5	715	110	57	−1.71

Angewandte Chemie International Edition, 2004, 43, 1158. ©2004, Wiley [60]

[a]:K_{OW}=*n*-octanol/water partition coefficient and [b]: the HC50 value (concentration required for 50% cell lysis) was achieved by inferring the fitted curve to 50% lysis.

Reproduced with permission from D. Liu, S. Choi, B. Chen, R.J. Doerksen, D J. Clements, J.D. Winkler, M.L. Klein and W.F. DeGrado.

RBC, and the toxicity enhances as a function of the hydrophobicity of the side chain. By contrast, the introduction of more polar substituents to give compounds 5–8 led to oligomers with significantly lower toxicity towards RBC. Compound 8 (Table 3.2), which contains the dibasic Arg substituent, was the most active of this category.

The emergence of drug-resistant bacteria has compromised the usage of many conventional antibiotics, leading to heightened interest in a variety of AMP. While these peptides exhibit good antibiotic properties, their size, stability, tissue distribution and toxicity have hindered efforts to connect these capabilities. To address such issues, Choi and co-workers [61] established small (molecular mass <1,000 Da) arylamide foldamers that mimic AMP.

3.6.1 Synthesis of an arylamide framework

An important property in the design of these acrylamides involves a thioether moiety, which is a useful point of connection for basic groups. The thioether also creates intramolecular hydrogen bonds [62, 63] to neighbouring amides, hence limiting rotation about the N–C torsional angle of the amide nitrogen and the phenyl ring. Nevertheless, the molecule displays important torsional flexibility related to ω, which is the torsional angle connecting the amide carbon and the phenyl group of the isophthalic acid ring. The rigidity of the arylamides scaffold was enhanced by applying a 4,6-dialkoxy-substituted isophthalic acid linker to create intramolecular O–H–N hydrogen bonds (generic structure 2), thus limiting rotation around the aryl–CO bond. The ether substituents are also suitable points from which to attach side chains.

Compounds that mimic the amphiphilic nature of AMP, such as β-amino acid helices and antimicrobial polymers, have been investigated [64, 65]. One class of such compounds is the arylamides foldamers, which consist of an arylamide backbone and several charged and hydrophobic groups resulting in a topographically amphiphilic construction [66]. These compounds exhibit important selectivity and activity against bacteria with decreased toxicity in animal models.

Mensa and co-workers [67] studied the mechanism of action of two acrylamides: polymyxin (PMX) 10070, which has a 2-ethyl guanidinium-charged substitution, and PMX 10072, which has a 2-ethylamine substitution on the arylamides backbone. The amphiphilic topology of these compounds was preserved via intramolecular hydrogen bonding [68]. Coarse-grained molecular dynamics simulations have demonstrated that the equilibrium conformation of these compounds in a hydrated bilayer environment is in the interfacial region of the membrane, perpendicular to the normal bilayer, that is, the axis that is perpendicular to the plane of the lipid bilayer. In these simulations, the charged side groups are localised to the lipid head group region of the bilayer, while the hydrophobic face is implanted into the apolar lipid acyl chain region [69]. The energetic cost for these compounds to adopt a long-lived

membrane-spanning conformation is high, rendering a mechanism, which includes the formation of stable multimeric assemblies in the membrane, unlikely. As an alternative, transient associations involving water, phospholipid head groups and acrylamides improve the permeability of the membrane to solutes and alter the surface tension of the membrane [70], possibly disturbing the strength and conformation of surrounding membrane proteins. These authors proved that the arylamides compounds disturb the absorbency of bacterial membranes. Reporter gene assays coupled with direct observation of cell morphology using scanning electron microscopy proved that adjustment of the arylamides caused important disruption of the outer membrane. This observation was supported by the work of Ivankin and co-workers who showed that the arylamides preferentially bound to the lipopolysaccharide moieties of the cell membrane [70]. Furthermore, exposure to arylamides leads to improved permeability of the inner membrane to small substrates and deficiencies in protein translocation through the membrane. Both arylamides displayed bactericidal activity, with PMX 10070 decreasing the viable cell count by >99.9% at 80 min and PMX 10072 decreasing the count by the same amount within 60 min. In contrast, PMX B sulphate, a readily available and widely investigated cyclic AMP, initiated much faster bactericidal activity, decreasing viability by >99.9% in about 10 min. The effects of different concentrations of arylamide under such conditions highlighted the decreased growth of *Escherichia coli* cultures treated at the early exponential phase (optical density 600 nm = 0.2) at 1×, 2× and 4× MIC of PMX 10070, PMX 10072 and PMX B sulfate. However, the attenuation of growth shows that the compounds are inhibitory, that is, the cells are able to grow, even if at a lower ratio. One reason for this effect is that at high cell densities, larger volumes of the antimicrobials are required to achieve the critical surface concentration, which is vital for bactericidal activity.

3.7 Design and synthesis of massive polymers

3.7.1 Synthesis of nanostructures of biomimetic polymers

Biomimetic nanostructures have a widespread range of applications as a result of their exceptional wetting, adhesive and optical properties [71]. Biodegradable nanostructures can be used to support tissue regeneration as they mimic the properties of the extracellular matrix (ECM). ECM-like collagen nanofibres [72] allow a natural setting for enhancing the attachment, proliferation and compactness of stem cells [73]. Biodegradable polymer nanostructures that mimic the features of ECM may exhibit suitable cell interactions. A polymer/cosolvent structure containing a polymer, a decent solvent and a poor solvent was used to produce new biodegradable nanofibres. The proportion of the suitable solvent to the poor solvent stabilised the

steadiness of the polymer solution; therefore, a metastable polymer solution could be achieved at RT by modifying this fraction. A non-solvent was then applied as a coagulant (i.e., a polymer/cosolvents/coagulant quaternary system). The system may be joined with cooling temperature-induced phase separation (TIPS). Applying these methods, new nanostructures can be synthesised from different types of biodegradable polymers without the requirement of crystallinity and high MW of the polymer, or heating the polymer solution to higher temperatures. Mouse preosteoblasts (MC3T3-E1 cells) and human bone marrow-derived mesenchymal stem cells (hBM-MSC) were used to prove the osteoinductive capability of these nanofibrous matrices. The osteogenic difference of hBM-MSC was calculated in three-dimensional (3D) polylactic acid (PLA) nanofibrous (<100 nm) scaffolds and conventional microporous scaffolds, without osteogenesis induction additives, for a 21-day period; it was concluded that 3D nanofibrous scaffolds had an osteoinductive influence on hBM-MSC [74].

3.7.2 Flexible sequence-random polymers

Synthetic polymers tend to be non-selective in their membrane-disrupting effects [54]. Mowery and co-workers [75] reported a novel category of random copolymers that replicate the selectivity of natural HDP for bacterial membranes. These materials were synthesised via the ROP of β-lactams. Differing the cationic/lipophilic ratio, length and other properties allowed identifying short polymers, which were active against both Gram-positive and Gram-negative bacteria but showed a low affinity for lysis of human RBC (haemolysis). The composition of one of these polymers was an average length of 21 subunits and an average MW of 2,800 (Mn/M_W = 1.4), as well as m:n (the proportion of monomers in the polymer sample) = 2:3 (referred to as 360).

This polymer is capable of binding to negatively charged vesicles, inducing the segregation of anionic lipids. The formation of anionic lipid-rich domains results in the formation of phase-boundary defects through which leakage can occur. Isothermal titration calorimetry indicated that the polymer associated with the negatively charged lipopolysaccharide of Gram-negative bacteria and to the lipoteichoic acid of Gram-positive bacteria.

The polymer efficiently permeabilises anionic vesicles with compositions, which mimic those of bacterial membranes. The polymer binds to anionic phospholipid vesicles but not zwitterionic vesicles, which causes phase separation in anionic phospholipid mixtures, clustering the negative charge. The polymer permeabilises the outer membrane of *Escherichia coli* ML-35p in a biphasic manner; low polymer concentrations permeabilise the inner membrane of *Escherichia coli* ML-35p, whereas high concentrations of the polymer can block the active transport of *ortho*nitrophenyl-*P*-n-galactoside in wild-type *Escherichia coli* K12 [17].

3.7.3 Synthesis of β-peptides

AMP are able to limit/stop the growth of a wide range of pathogenic bacteria and are, hence, a significant part of the intrinsic immune system. Over the last few years, significant attention has been focused on increasing the use of AMP as intravenously controlled antibiotics. The study of alternatives of natural α-peptides has prepared a valuable guide for the design of synthetic antimicrobial β-peptides, which are a category of polyamides that have been shown to adopt a diverse range of helical conformations. Recently, a category of amphiphilic L+2 helical β-peptides were designed, which were proposed to mimic the general physico-chemical features of a category of membrane-active AMP, including magainin and cecropin (Figure 3.9). While these peptides presented potent antimicrobial activity, they also displayed important activity against human RBC; furthermore, in contrast to other peptides, they exhibited minimal activity against mammalian cells [36].

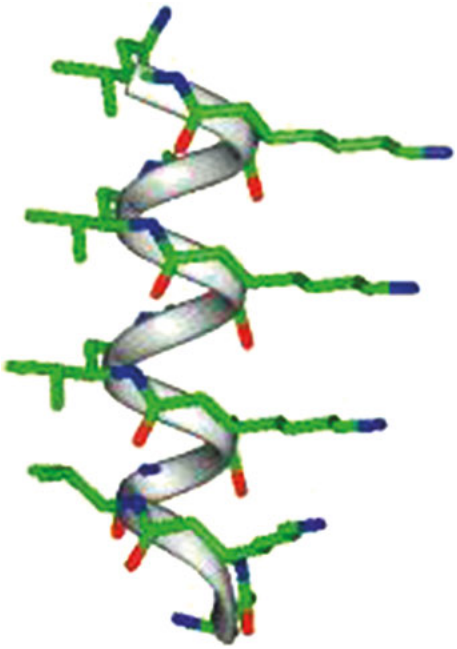


Figure 3.9: Molecular model in an L+2 helical conformation. Reproduced with permission from D.H. Liu and W.F. DeGrado, *Journal of American Chemical Society*, 2001, 123, 7553. ©2001, American Chemical Society [36].

Synthesis of H-(β^3-HAla-β^3-HLys-β^3-HVal)n-NH_2(n)4,5. α-amino acid pentafluorophenyl esters [fluorenylmethyloxycarbonyl (Fmoc)] (peptide amide linker)-polyethylene glycol-polystyrene resin (588 mg, 0.1 mmol) was allowed to swell in dimethylformamide (DMF) (5 ml) for 30 min before the synthesis. The Fmoc was deprotected with 20%

piperidine/DMF (3 × 5 mL × 5 min) and washed with DMF (5 × 5 mL × 2 min). Amino acid couplings were synthesised by adding a 2 mL solution of amino acid (0.25 mmol), 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (95 mg, 0.25 mmol), hydroxybenzotriazole (34 mg, 0.25 mmol) and *N*,*N*-diisopropylethylamine (139 mg, 0.8 mmol) in DMF to the resin, shaking for 4 h, and washing with DMF (5 × 5 mL × 2 min). The peptides were removed from the resin using **trifluoroacetic acid/**triisopropylsilane TFA/TIS (95:5) for 2 h. The solution was concentrated and the peptide was precipitated by the addition of cold ether. The peptides were purified using high-performance liquid chromatography on a reverse phase C4 column, with a linear gradient from 20% to 50% solvent B, which was composed of 90% acetonitrile, 10% water and 0.1% TFA.

DeGrado and co-workers [59] first reported the de novo design of amphipathic, cationic, monosubstituted β-peptides as antibacterial complexes against the Gram-negative bacterium K91 *Escherichia coli*. However, as potent as these β-peptide antibiotics are they exhibited low selectivity for bacteria, as confirmed by extensive mammalian RBC lysis (haemolysis). A mainly stable secondary structure formed by β-peptides is the L+2 helix (also known as a 14-helix or a 31-helix (Figure 3.10).

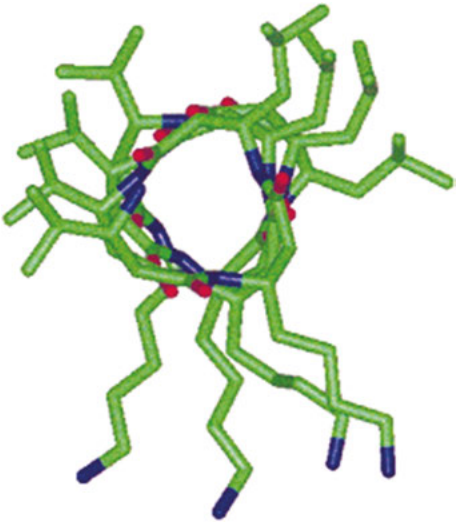


Figure 3.10: Molecular model of the amphiphilic β-peptide H-(β 3-HVal-β 3-HLys-β 3-HLeu)4-OH in an L+2 helical conformation. This axial view shows the segregation of hydrophobic and positively charged residues on opposite sides of the helix. Reproduced with permission Y. Hamuro, J.P. Schneider and W.F. DeGrado, *Journal of American Chemical Society*, 1999, 121, 12200. ©1999, American Chemical Society [59].

They also changed their original 14-helix designs to substitute a valine-like (β-HVal) residue with a less hydrophobic alanine-like (β-HAla) residue. In support of their original theory, this adjustment eliminated haemolytic activity, while maintaining good antibacterial efficiency in both 12- and 15-residue oligomers. Porter and co-workers [76] studied 14-helical antibacterial β-peptides, and the correlation between their folded structure and activity. Such work will enable oligomer design by

preparing a clear relationship between physico-chemical features and the biophysics of the antibacterial mechanism.

3.8 Conclusion

Antibiotic-resistant bacteria cause life-threatening infections in hospitals and society in general. There is a vital requirement to develop new antimicrobial agents, but this task involves extensive scientific trials. This chapter reviewed broad-spectrum polymeric antimicrobials, which are not susceptible to current resistance, and mechanisms of bacteria to mimic the antimicrobial action of natural HDP, which exert their effect by permeabilising the bacterial cytoplasmic membrane. Most cationic antimicrobial polymers appear to work in a manner similar to membrane-active AMP, such as magainin. The design and synthesis of different polymers, such as methacrylate-based copolymers, PPE, polynorbornene by-products, amphiphilcarylamide polymers and large polymers, have been studied as biomimetic polymers in different applications.

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4 Polymer–metal nanocomposites with antimicrobial activity

Abstract: The antibacterial effect of metal nanoparticles (MNP) has been used in a variety of commercially available products and devices for many years. Because of the knowledge acquired within the last decade in the field of nanotechnology, the use of MNPs has been thoroughly investigated. In this concept, the development of nanocomposite materials with antimicrobial activity is important for human health. It has been reported that MNP properties alone do not determine the common features of polymer – metal nanocomposites. The polymer matrix can function as the MNP stabilising media, which stops MNP aggregation and release to the surrounding environment. As an innovative class of materials, polymer–metal nanocomposites have shown many advantages over conventional antimicrobial materials, ensuring there is no negative effect for human health and the environmental.

Keywords: Nanocomposite, antibacterials, polymer–metal matrix, biomedical engineering

4.1 Introduction

Modern industry has an increasing requirement for new materials with specific properties. Over the last few years, many studies have used new technology with the intention of obtaining nanomaterials, which display specific functionality. Antimicrobial nanocomposites are multiphase materials that inhibit microbial development and are desirable for various everyday applications, such as food packaging, water management and medical use. Polymer–metal nanocomposites have specific applications related to their unique properties; this important area of nanoscience offers multidisciplinary methods to provide materials for the fields of physical, chemical and materials science in conjunction with areas such as engineering and topology [1].

Polymer nanocomposites are combinations of polymers containing inorganic or organic fillers of definite geometries (fibres, flakes, spheres, particulates and so on). The use of fillers, which have one dimension on the nanometre scale, enables the production

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of polymer nanocomposites. Functional nanocomposites with specific properties can be custom-made by combining metal nanoparticles (MNP) into the polymer matrix.

Based on their dimensions, which are in the nanometre range, three types of fillers can be distinguished. Isodimensional nanoparticles (NP), such as spherical silica NP have three nanometric dimensions. Nanotubes or whiskers are stretched constructions in which two dimensions are in the nanometre range and the other dimension is larger. When only one dimension is in the nanometre range, the composites are termed polymer-layered crystal nanocomposites, and are obtained by the complete intercalation of the polymer inside the galleries of layered host crystals [2].

There are different approaches to NP synthesis and the major methods include chemical reduction, ultraviolet (UV) and gamma irradiation [3], optical reduction [4], hydrogel method [5], sedimentation method, micelles [6] and biosynthesis method [7]. Among these methods, the chemical reduction process is the best engineering procedure for NP preparation; this technique has the highest production efficiency and can be used in an extensive range of NP and nanocomposite manufacturing methods [8].

The metal and metal oxide nanomaterials usually applied as antimicrobial agents are silver (Ag), gold, zinc oxide, silica, titanium dioxide, alumina and iron oxides. The antimicrobial features of nanozinc oxide and magnesium oxide have recently been revealed. Compared with nanosilver, zinc oxide and titanium dioxide NP are expected to lead to less expensive and safer food packaging solutions in the future [9].

NP of noble metals have been studied with increasing attention, as they exhibit considerably different physical, chemical and biological properties from their bulk counterparts. Metal-based nanoconjugates can be used in different biomedical applications, such as probes for scanning electron microscopy (SEM) to picture cellular components, drug delivery and disease detection, diagnosis and therapy. There is considerable interest in using metal and semiconductor clusters as advanced additives for plastics and important investigations are currently being performed in this novel area of composite science. However, their application has been limited by difficulties related to handling and processing. Actually, due to the fact that they are self-aggregated they have high surface free energy and can be oxidised or become contaminated in air [10]. MNP are so reactive that when their surfaces touch each other, they lose their nanometric size and unique properties. These properties of NP, in part, determined by the conditions of synthesis, create huge problems in their fabrication and use [11]. In particular, the polymer-assisted synthesis of inorganic NP is perhaps one of the most effective and common methods to overcome the stability problem of MNP while maintaining their functionality. MNP prepared by this method show stability over a long period against aggregation and oxidation, whereas NP synthesised in the absence of polymers are prone to quick aggregation and oxidation [11, 12].

The progress of polymer-stabilised MNP via this method is recognised as one of the most brilliant answers to the problem of MNP stability. As a result, the incorporation of MNP into polymeric matrices has recently drawn a great deal of consideration

as polymer–metal nanocomposites have already proved to be uncommon and display excellent properties in many practical applications.

The alteration of commercially available ion exchange resins and the advancement of stable polymeric membranes containing MNP, which display specific functionality, such as biocide or catalytic activity, is the focus of great attention [13]. The key advantage of the polymer–metal nanocomposite is the location of MNP near the surface of the polymer, which substantially improves the efficiency of their biocide and catalytic activity.

4.2 Synthesis of polymer–metal nanocomposites

Polymer-stabilised MNP can be obtained using different methods. In the ex situ preparation, NP are dispersed after their preparation in a solid or liquid environment by applying different mechano-chemical methods. The problem with these approaches is that stabilisation is restricted by the risk of reaggregation of the MNP over time. On the other hand, using in situ preparation, MNP are developed directly in the stabiliser environment, resulting in a material that can be directly applied for a desired application; consequently, in situ methods are the focus of considerable attention due to their technical benefits.

4.3 Mechanisms of antimicrobial polymer–metal nanocomposites

The mechanism of toxicity of antimicrobial materials, obtained from blending an antimicrobial NP and a non-active polymer, is similar to the mechanism of the NP itself [14]. Consequently, in polymer–metal nanocomposites, the main toxic mechanism relates to the NP. There are two possible mechanisms, which depend upon the species considered as the active agent: (a) the MNP or (b) the metal ions released from the particles [15]. Nevertheless, many reports show that ion release is the driving force behind the antimicrobial properties of antibacterial NP [16]. Actually, most investigations regarding antimicrobial MNP are dedicated to the release of the metal ion, which is absorbed by the bacteria [17]. This aspect was driven by the evidence from polymer–metal nanocomposite studies where the antimicrobial effect of these materials was connected with the metal ion release rather than with the leakage of the particle [18]. The existence of a polymer film covering the NP, determined via X-ray photoelectron spectroscopy (XPS) analysis of thermoplastic polymer–copper composites, proved the theory that ion release is the key mechanism in this system. Nevertheless, the exact method of NP action is related to the particular features of the

polymer used, for example, in antimicrobial hydrogels the particle release has been stated to be the main process, which determines its functionality [19].

The mechanisms for nanocomposites based on thermoplastics, or dense polymers, are the most complex from a material point of view. In dense polymers containing embedded MNP, the ion release is the main mechanism behind their biocide activity. XPS analysis showed that these NP are not present on the surface of nanocomposites [18] or at concentrations lower than the polymers in the nanocomposite [20]; hence, the only mechanism for the release of metal ions is corrosion of the particles, existing in the bulk of the polymer, owing to diffusion of water molecules from the bacterial medium onto the surface of the particles [21]. Non-polar matrices such as polyethylene or polypropylene allow the diffusion of water molecules [22]. The polymer–particle interface can improve water diffusion via holes or micron-scale flaws, allowing fast Knudsen diffusion; this mechanism also applies to more polar matrices. When a polyvinyl methyl ketone was applied as the polymer matrix, the lowest depth at which the nanocomposite became hydrated, and ultimately released the soluble copper species, was expected to be around 50 nm, which was about 1/10 of the total film thickness of that item [20]. When water containing dissolved oxygen touches the metal particles in the polymer bulk, the standard corrosion process occurs [18, 21]. Ions resulting from the corrosion or dissolution process can then diffuse out via the polymer matrix and be released. This mechanism has been proven for metal copper NP by comparing the X-ray diffraction analysis of the original composite with the diffraction of the same sample but immersed in water. In the latter case, new diffraction peaks were observed, which correlated with a copper (I) oxide layer formed on the surface of the particle [18]. All these processes, particularly those associated with the dissolution step, can be enhanced by the presence of bacteria close to the composite which, as a result of their organic compounds, cause a pH change and increase the surface affinity [23]. In hydrogels, the release of NP from the matrix causes a much larger free space in the polymer matrix of these polymers compared with dense thermoplastic matrices [24]. Furthermore, the diffusion problems of water into/out of the matrix can be avoided because of the nature of the hydrogel; thus, in hydrogels the mechanisms are much closer to pure MNP.

4.4 Polymer–silver nanocomposites

Infections caused by antibiotic-resistant bacteria expose an important risk to human health around the world. These bacteria are very resistant to traditional antibiotics owing to acquired resistance, inadequate diffusion and intracellular inactivation. Therefore, the development of novel antimicrobial materials with high protection and antibacterial activity, which lack bacterial resistance, is critical.

Cationic polymers that retain a high number of positive charges and exhibit membrane-disrupting activity towards negatively charged microbial surface phospholipids, display potent antimicrobial activity against bacteria, fungi and viruses [25]. To obtain stable antibacterial properties, most cationic polymers are immobilised onto a substrate surface or prepared in hydrogels [26, 27]. Nevertheless, their antimicrobial activities may be decreased as diffusion through the cell membranes into the cytoplasm is limited during immobilisation [28]. Nanomaterials, which are capable of diffusing into the bacterial membrane and disrupt its homogeneity, provide a novel application for the antimicrobial use of cationic polymers. Silver NP, one of the major nanomaterials, show broad-spectrum antimicrobial activity against antibiotic-resistant strains of bacterial and fungal species. Silver NP are applied for the treatment of burns and marketed as a water disinfectant and room spray [29]. The mixture of silver NP with cationic polymers simplifies long-term stability of dispersed silver NP in an aqueous solution. This nanocomposite easily forms core–shell structured NP with a silver NP core and cationic polymer shell, with the alkyl tail orientated towards the surrounding conditions. The formation of NP is estimated to improve the local density of the positive charge and support its adsorption onto negatively charged bacterial membranes via electrostatic interactions, thus improving its antimicrobial efficacy and diffusivity through the bacterial membrane [30]. Furthermore, this NP has two biologically active fragments, which were joined with the goal of merging different modes of synergistic action, is optimal for wound healing. Remarkably, the convergence of two antimicrobial materials into a single one does not seem to induce bacterial resistance.

Various nanocomposite materials containing silver NP have been made into fibres [31], polymeric resins, polymeric membranes [32] and textile fibres, and have been used in various studies as a sterilisation agent. The microbial pollution of drinkable water sources poses a key risk to community health and the rise of microorganisms resistant to several antimicrobial agents has heightened the demand for better disinfection approaches. In some countries, the significance of potable water for the public has highlighted the urgent requirement for the development of new technologies and materials; this type of requirement could be a perfect application for nanomaterials containing silver NP [33]. It is worth noting that ion exchange materials are already extensively applied in different water treatment procedures, essentially to remove undesired or toxic ionic impurities, including ions which cause water hardness, iron, heavy metals and others. The key problem of using ion exchange materials in water treatment is that they are frequently affected by surface contamination by bacteria, fungi and algae.

An ion exchange resin bead, which has fungal and bacterial growth on it after use in a traditional domestic tap water treatment filter, is typically used for the elimination of undesired metal ions (ions causing water hardness, iron, heavy metals and so on). The stabilisation and immobilisation of silver NP in such matrices is therefore a promising method as two complementary water treatment processes

could be achieved with a single material, while at the same time improving the safety of the nanocomposites [13].

The surface alteration of ion exchange materials used in conventional water treatment has been carried out. The difference in cell viability over time of *Escherichia coli* suspensions in contact with a membrane nanocomposite (containing silver NP) or with the same membrane without silver NP has been investigated. To determine the bactericidal features of the newly synthesised nanocomposites and the bare matrix, a 1 cm^2 sample of each material was immersed in 20 mL of bacterial suspension and maintained at 37 °C with gentle agitation (300 rpm). 100 μL of each suspension was collected at different times and bacterial counts were performed using plating and incubation in Petri dishes containing lysogeny broth (LB). The nanocomposite membrane killed 100% of the bacteria in about 2 h, whereas for the unchanged polymer (without silver NP), the cell viability remained almost constant during the first 4 h. After this time, due to the lack of nutrients in the media, the cell viability decreased a little, to the same extent as pure *Escherichia coli* suspensions.

Rhodanine by-products have been used as antiviral, antibacterial, antihistaminic and anticorrosion agents [34, 35]. Additionally, they have also been used to identify metal ions as the rhodanine molecule has metal-binding functional groups, such as thioamide and amide [36, 37]. It is thought that the improved antimicrobial efficacy of the silver/polyrhodaninenanofibres is due to the joint antimicrobial activity of silver and polyrhodaninenanofibres. Silver NP-embedded polyrhodaninenanofibres can be prepared via chemical oxidation polymerisation.

The antimicrobial activity against the Gram-negative bacterium *Escherichia coli* was assessed using the surface of a glass slide [38]; the glass slide was coated with the silver/polyrhodaninenanofibre and an uncoated glass slide was prepared for comparison. An *Escherichia coli* suspension was then sprayed on the glass slides. The bacterial growth on LB agar was determined photographically after overnight incubation. *Escherichia coli* growth was detected on the uncoated glass slide, while the coated glass prevented bacterial growth, as shown in Figure 4.1a. The inhibited growth of *Escherichia coli* on the surface of the coated glass demonstrates the antimicrobial activity of the silver/polyrhodaninenanofibre. Additional antimicrobial studies determined the antimicrobial kinetics against Gram-negative *Escherichia coli*, Gram-positive *Staphylococcus aureus* and yeast (*Candida albicans*) [39–41]. A total of 3 mg of silver/polyrhodaninenanofibre was injected into a 200 μL (106–107 colony forming unit(s) (CFU)/mL) volume of aqueous microbial solution and the percentage decrease of microbial colonies was determined as a function of contact time, as shown in Figure 4.1b. As the contact time period increased, the percentage decrease grew larger and reached an asymptotic value; finally, no microbial growth of any of the three microorganisms used was identified after 60 min This result demonstrates that the silver/polyrhodaninenanofibre has effective antimicrobial efficacy against Gram-negative and Gram-positive bacteria, and yeast. Furthermore, the antimicrobial kinetic test was also performed using pure silver NP and a polyrhodaninehomopolymer, the results showed

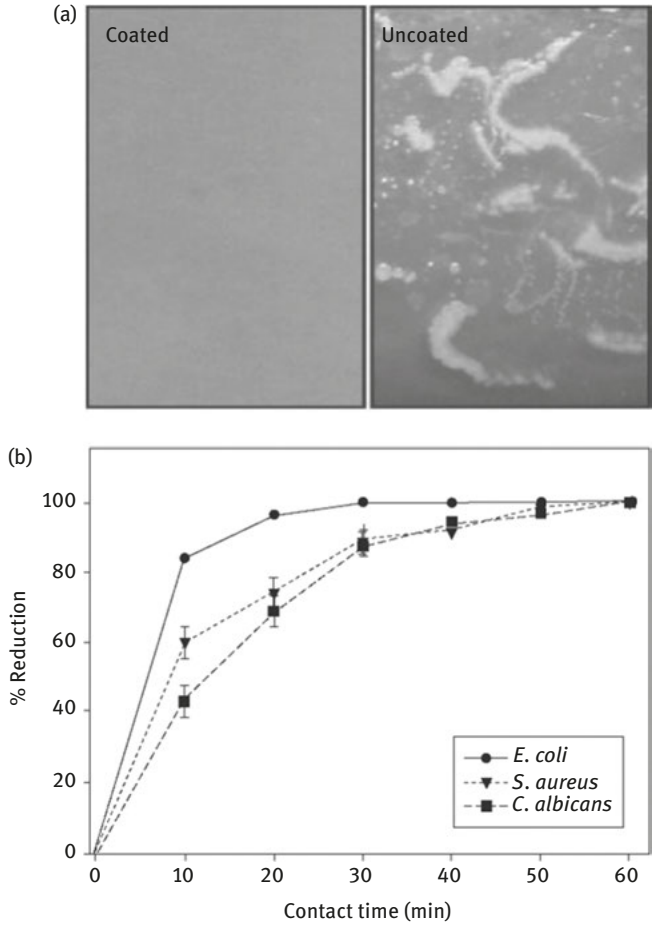

Figure 4.1: (a) Photographs of surviving colonies on the silver/polyrhodanine-coated and uncoated glass slide after spraying the *Escherichia coli* suspension. (b) The plot of percentage reduction of *Escherichia coli, Staphylococcus aureus* and *Candida albicans* versus contact time (min) with the silver/polyrhodaninenanofibre . The percentage reduction was calculated as percentage reduction (A–B)/A × 100 (where A is the number of surviving microbial colonies in the blank solution and B is the number of surviving microbial colonies in the silver/polyrhodaninenanofibre). Reproduced with permission from H. Kong and J. Jang, *Biomacromolecules*, 2008, 9, 2677. ©2008, American Chemical Society [42].

that the silver/polyrhodaninenanofibre enhanced antimicrobial efficacy compared with pure silver and polyrhodaninehomopolymer due to the combined antimicrobial activity of silver and the polyrhodaninenanofibre [42].

In order to minimise the risk of increasing antibiotic resistance associated with the use of antimicrobial devices, transition-metal-containing polyurethanes (PU) have been loaded with ciprofloxacin, which was chosen because it possesses

a different mechanism of action [43]. In this way, the presence of two antibacterial agents in the polymer allows the development of an antimicrobial polymer whose activity is not limited by the increasing occurrence of antibiotic resistance.

Among all the transition-metal-containing PU, only carboxylated polyurethane (PEUA) (one carboxyl group per repetitive unit)-silver NP possesses antibacterial activity against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* for a 16-day period, as demonstrated both by the Kirby–Bauer test and SEM observations of the polymer surfaces, shown in Figure 4.2. In contrast, PEUA-copper and PEUA-iron do not show zones of inhibition (ZIO) of bacterial growth, probably due to the lack of ion elution from these polymers.

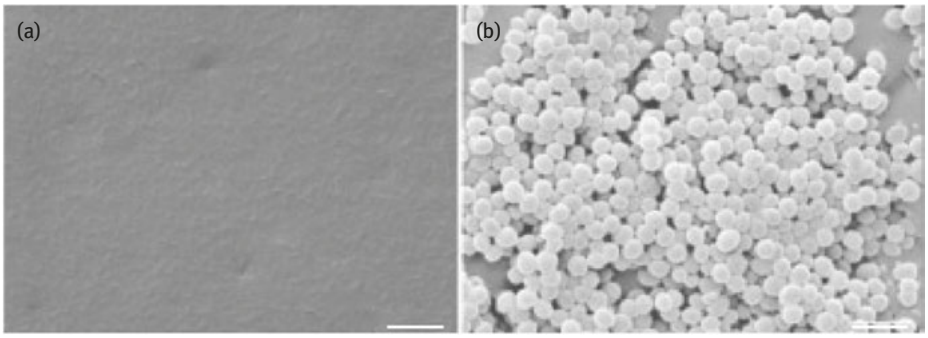

Figure 4.2: SEM micrographs showing the surface of PEUA-silver (a) free from *Staphylococcus epidermidis* colonisation and a mature biofilm on the PEUA surface (b) after 48 h of incubation. Scale bar is 2 μm. Reproduced with permission from I. Francolini, V. Ruggeri, A. Martinelli, L. D'Ilario and A. Piozzi, *Macromolecular Rapid Communications*, 2006, 27, 233. ©2006, Wiley-VCH Verlag GmbH & Co. KGaA [43].

On the other hand, layer-by-layer (LbL) assemblies have demonstrated their importance in a variety of biomedical applications. Polyelectrolyte multilayers have also been prepared using silver nitrate and/or cetrimide as the antimicrobial agents [44]. The substitution of cetrimide for silver dramatically enhances the antimicrobial efficacy of these films, as evidenced by the ZOI data. Furthermore, cetrimide is not susceptible to reduction as in ionic silver, resulting in highly transparent, colourless films.

Table 4.1 summarises the composition and properties of six antimicrobial thin film systems. These six films represent three compositions deposited on untreated or corona-treated polyethyleneimine. In all but one case, the corona-treated substrate produced films with stronger antimicrobial action than the untreated counterpart, as evidenced by the size of the Kirby–Bauer ZOI. Corona treatment oxidises the surface of the polyethylene terephthalate film via an electrical discharge (i.e., corona), creating phenolic modifications that impart a strong negative charge [45]. This treatment is

Table 4.1: Treatment, composition and properties of antimicrobial thin films.

Film	Corona	Cetrimide	Silver	ZOI (mm)		Transmittance
		(No. bilayers)	(No. bilayers)	Staphylococcus aureus	Escherichia coli	(%)
A	Y	16	0	11.3 ± 1.3	3.0	100
B	N	16	0	8.8 ± 0.3	1.9	99
C	Y	8	8	7.0 ± 0.9	2.6	46
D	N	8	8	8.0 ± 0.7	1.3	67
E	Y	0	16	1.2 ± 0.4	2.1	97
F	N	0	16	0.8 ± 0.6	2.0	99

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well-known to enhance the interaction of high surface energy (e.g., water-based) materials with the substrate surface [46]. It is believed that this enhanced surface charge results in greater deposition of the positively charged antimicrobial species, which allows greater killing power per unit surface area. The corona treated silver-only system in Film 'E' in Table 4.1 shows a 1.2 mm ZOI against *Staphylococcus aureus* and 2 mm ZOI against *Escherichia coli*. This result matches ZOI results detected for commercial wound dressings, using sputtered (e.g., Acticoat by Nucryst Pharmaceuticals [Fort Saskatchewan, CA, USA]) or dispersed silver salt (e.g., Arglaes Film by Medline Industries [Mundelein, IL, USA]), when tested under identical conditions. Despite having a smaller overall amount of active ingredient, the efficacy of this multilayer is likely to be due to more rapid delivery of the ionic silver. The improvement in antimicrobial activity by replacing silver with cetrimide is much more dramatic than the effects of the corona treatment.

The non-cytotoxicity of the nanocomposite system was also evaluated in the form of an alginate-chitlac silver NP hydrogel (AC-nAg) [47]. As reported in Figure 4.3, AC-nAg hydrogels did not exert any cytotoxic effect on the cell lines used. In fact, there was no important change in the release of lactate dehydrogenase (LDH) between the AC-nAg treated cells and control groups after 24 and 72 h. These above-mentioned results show that the AC-nAg hydrogels, besides enabling the efficient stabilisation of the silver NP against aggregation, exhibit antibacterial activity without being harmful to mammalian cells. In the AC-nAg gels, NP associated with chitlac are firmly grafted and immobilised in the gel matrix, and thus do not diffuse into the surrounding environment, as proved by the inductively coupled plasma-mass spectrometry analysis.

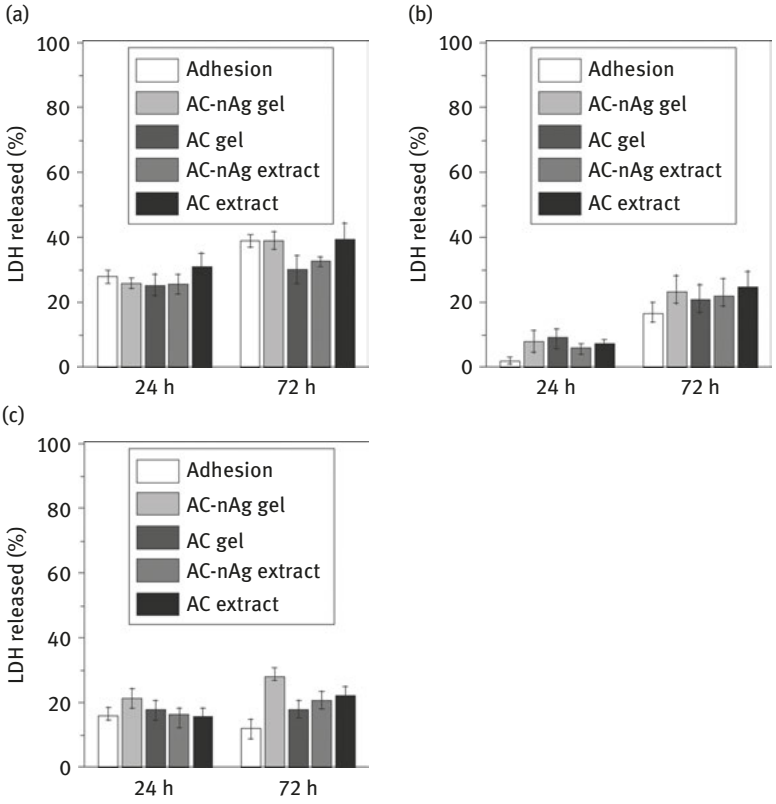

Figure 4.3: Effect of AC-nAg gels on LDH leakage from (a) mouse fibroblast (NIH-3T3); (b) human hepatocarcinoma (HepG2) and (c) human osteosarcoma (MG63) cell lines. The cytotoxicity test was performed with both an extract from the gel material (AC-nAg extract) and with the gel material itself by direct contact with the cell layer (AC-nAg gel). Adhered control cells were cultured in complete Dulbecco's modified Eagle's medium, and cells treated with alginate (ALG)-chitlac gels lacking silver NP (ALG-chitlac gel and ALG-chitlac extract) were run in parallel to AC-nAg gel treated groups. The percentage of LDH release was calculated by dividing the amount of activity in the medium by the total activity (medium and cell lysate) after subtraction of the control. The data are expressed as mean +/– standard deviation of four independent experiments. Reproduced with permission from A. Travan, C. Pelillo, I. Donati, E. Marsich, M. Benincasa, T. Scarpa, S. Semeraro, G. Turco, R. Gennaro and S. Paoletti, *Biomacromolecules*, 2009, 10, 1429. ©2009, American Chemical Society [47].

4.4.1 Swelling performance

Study of the antimicrobial performance of silver-containing nanofibrous webs necessitates their contact with water. The water-swelling performance of nanofibrous webs, comparative to traditional films of the same material, is of primary significance. In principle, the degree of hydrogel swelling is determined by a balance

between the driving force of an exothermic mixing enthalpy ($\Delta H_{swell} < 0$), caused by potential interactions between hydrophilic groups and water molecules, and a loss of polymer chain conformation entropy ($\Delta S_{swell} < 0$) also resulting from the swelling. This performance is defined by the Flory–Rehner theory [48].

For instance, polyethylene glycol (PEG)-based multiblock thermoplastic polyurethanes (TPU), including polyhedral oligosilsesquioxane (POSS) moieties were co-dissolved with silver nitrate and subsequently electrospun to prepare strong hydrogel webs capable of controlled silver ion release for effective antimicrobial behaviour [49]. Because of important thermodynamic incompatibility between POSS moieties and ethylene oxide units, POSS nanocrystals, obtained from microphase separation, serve as physical crosslinking points within an inorganic–organic hybrid network, in turn, enabling new hybrid organic–inorganic hydrogels in the water-swollen state. Water molecules hydrated the hydrophilic PEG blocks leading to mass gain and volumetric expansion. Nevertheless, complete dissolution was inhibited by the existence of hydrophobic POSS-rich domains, serving as physical crosslinks. Figure 4.4a displays photographs of the swelling performance of a cast film enclosing silver nitrate before (i) and after (ii) swelling to equilibrium. The electrospunnanofibrous mats do not expand during immersion in water, but instead shrink slightly, as shown in Figure 4.4b. The reason may be due to the slight contraction of directed relaxation of the fibre upon swelling, axially contracting the fibres to the point of interfibre impingement. Further swelling may be inhibited by 'memory' of the nanofibrous internal microstructure, which may feature highly anisotropic swelling properties, coupled with the interfibre impingement constraint. This poorly understood phenomenon justifies future intensive work; however, the mass gain during dimensional contraction was reproducibly detected in these specimens, as summarised in Table 4.2.

4.4.2 Polymer–gold nanocomposites

BiMNP showed remarkable antibacterial activity against both Gram-positive and Gram-negative bacteria at micromolar concentrations [50]. Biomaterials containing noble metal nanoclusters have been used to reduce infection, in many cases with controversial clinical results. Cosputtering of silver and gold in combination with an organic polytetrafluorethylene (PTFE) component is a technique for manufacturing antibacterial polymer–metal nanocomposite coatings [51]. A comparable amount of silver ions (~7×10^{15} cm^{-2} day^{-1}), which were released into the water from silver-impregnated silicon discs, was determined [52]. The coatings steadily supply silver ions to the surface and should provide continuous antimicrobial properties as long as the coatings remain intact. For example, a 100 nm thick nanocomposite coating containing a 15% metal volume filling factor of silver (~0.15 g m^{-2}) may provide silver ions for more than 300 days according to data in Table 4.3.

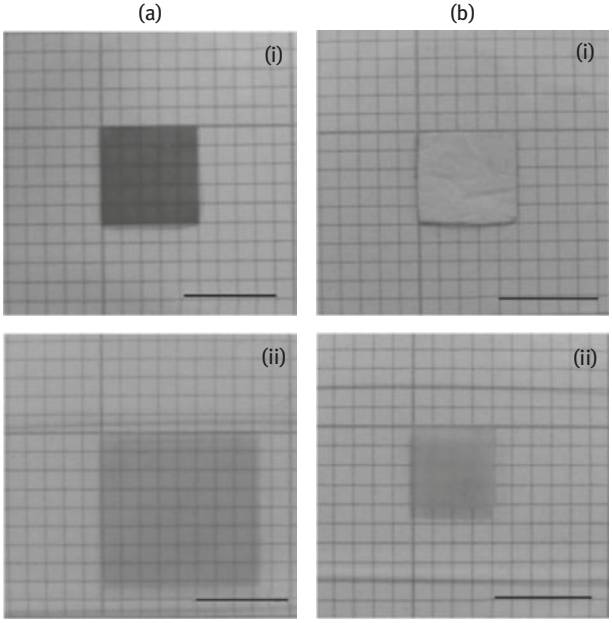

Figure 4.4: Digital images of POSS-PEG TPU with 1.0 wt% silver nitrate loading: (a) cast film and (b) an e-spun fibrous mat made from 20 wt% dimethylformamide, which varied (i) before and (ii) after hydration. The scale bar represents 1 cm. Reproduced with permission from J. Wu, S. Hou, D. Ren and P.T. Mather, *Biomacromolecules*, 2009, 10, 2686. ©2009, American Chemical Society [49].

Table 4.2: Swelling behaviour of POSS-PEG TPU.

	Cast film w/ 1.0 wt% silver nitrate	Electrospun mat w/ 1.0 wt% silver nitrate
Water uptake (%)	474 ± 25	517 ± 36
Swelling ratio (%)	66.1 ± 3.3	−17.4 ± 1.0

Reproduced with permission from J. Wu, S. Hou, D. Ren and P.T. Mather, *Biomacromolecules*, 2009, 10, 2686. ©2009, American Chemical Society [49].

Under ambient conditions, that is, if the model is not in direct contact with water, the lifetime should be even higher; this means that much thinner coatings can be applied. A large increase of the silver ions released, in the order of one magnitude, was detected when a trace amount of gold (less than 1 mg m^{-2} of coating) was additionally deposited on the silver/PTFE composite surface, as shown in Table 4.3. This can be explained by the formation of galvanically coupled silver and gold NP. The gold NP lead to improved silver ion formation as the galvanically paired silver is more active than gold. A similar effect has been detected for silver/platinum

Table 4.3: Comparison of the silver ions released from 100 nm nanocomposite coatings with different silver fillings.

Sample of silver/PTFE in aqueous solution	Silver ions released from 1 cm^{-2} coating per day ($\times 10^{15}$ cm^{-2} day^{-1})	Reference
Silver (15% aqueous solution)	5	51
Silver (30% aqueous solution)	4	51
Silver (15% aqueous solution) + gold (1% aqueous solution)	50	51

Reproduced with permission from V. Zaporojtchenko, R. Podschun, U. Schurmann, A. Kulkarni and F. Faupel, *Nanotechnology*, 2006, 17, 4904. ©2006, IOP Science [51].

coatings [59]. One would expect the above-mentioned effects to be reflected in the antimicrobial properties of the composite coatings.

When silver is dispersed as NP it decreases material usage and provides a very large effective surface for metal ion release. The antibacterial efficiency of the coatings was proved at an extremely small noble metal use: gold: ~1 mg m^{-2} and silver: ~0.1 g m^{-2}. *Staphylococcus aureus* and *Staphylococcus epidermidis* were used as test bacteria as these species usually cause infections related to medical polymer devices. Silver/PTFE and silver–gold/PTFE showed antimicrobial properties against different bacteria. It was found that the release of silver ions correlates with the antibacterial effectiveness of silver composite coatings and is determined by the coating thickness, volume fraction of silver and composition of NP. Polymer/silver–gold nanocomposites exhibit maximum antibacterial effect, followed by silver polymer nanocomposites. A drastic reduction of bacterial growth, by more than five orders of magnitude, was detected on the nanocomposite surface.

4.5 Polymer–platinum nanocomposites

Scientists have developed a simple one-step technique for preparing platinum NP embedded free-standing polydimethylsiloxane (PDMS) composite films [54]. The process involves preparing a homogenous mixture of chloroplatinic acid, silicone elastomer and the curing agent, which is followed by curing. During the curing process, the hardener crosslinks the elastomer and simultaneously reduces the metal salt to form NP. This in situ method avoids the use of any external reducing agent/stabilising agent and leads to a uniform distribution of NP in the PDMS matrix. NP

act as a filler and enhance the mechanical properties of PDMS. The Young's modulus of film containing NP is 3× higher than that of the pure polymer film. The transmission electron microscopy (TEM) images are displayed in Figure 4.5. The NP are irregular and polydispersed in nature with an average particle size of 50 nm. The diffraction of platinum NP (inset of Figure 4.5b) indicates their face-centred cubic crystalline structure. Platinum-containing PDMS films could be used for enzyme immobilisation, optical applications and catalytic activity.

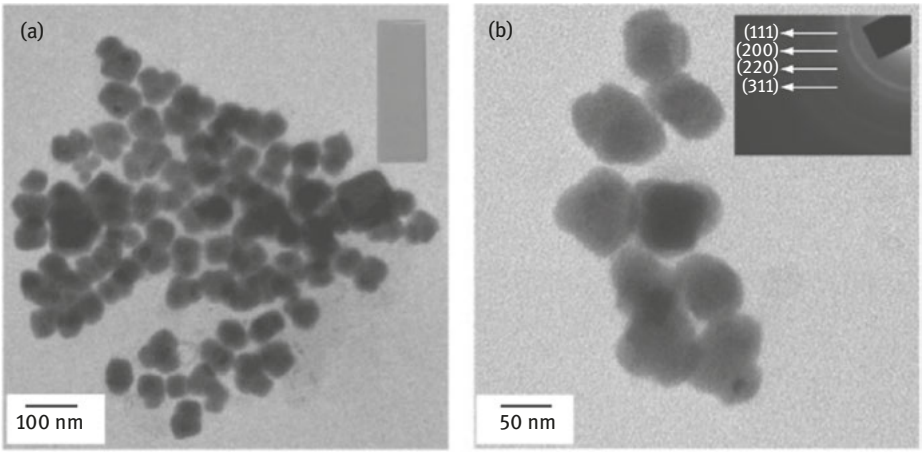

Figure 4.5: TEM images of the platinum NP: (a) TEM image at 100 nm and (b) TEM image at 50 nm. Inset is the photograph of the platinum–PDMS film and 111, 200, 220 and 311 are the diffraction pattern of platinum NP. Reproduced with permission from A. Goyal, A. Kumar, P.K. Patra, S. Mahendra, S. Tabatabaei, P.J.J. Alvarez, G. John and P.M. Ajayan, *Macromolecular Rapid Communications*, 2009, 30, 1116. ©2009, Wiley-VCH Verlag GmbH & Co. KGaA [54].

4.6 Polymer–copper nanocomposites

The use of silver is based on the strong toxic action this metal exerts against prokaryotes (i.e., all types of bacteria), although it is much less toxic against eukaryotes. Fungi can also be effective pathogenic agents and copper has been used for a long time as an effective fungicidal agent. Copper has been used for the discovery of other materials, which are efficient antifungal coatings, although the main concern in this strategy is to minimise their toxicity towards humans [20]. The germicidal activity of copper, both as free [55] or as complexed species [56–59], is also well-known and has been documented for many years. Some exciting work has been published on the biocidal features of technologically appealing materials, such as coordinated polymers containing copper salts [60–62]

and paints [63, 64]. The dispersion of copper or copper oxide particles into organic matrices has been employed by the paint industry to create antifouling coatings, mostly for maritime applications. Such paints (generally known as ablative copper) after immersion in seawater undergo slow rate dissolution processes, eventually resulting in the continuous and massive release of toxic species, such as copper, tin or organometallic compounds, into the marine environment. An international effort to remove or decrease the use of such large-scale coatings is being promoted [65]. The controlled release of inorganic chemical species from nanocomposites is also a hot topic [66, 67]. Some work has been undertaken involving the applications of copper–polymer nanocomposites as a bioactive coating against fungi [68].

Characterisation of the bioactivity of the three nanocomposites was performed in vitro against the yeast, *Saccharomyces cerevisiae*. The nanocomposites were deposited by spin-coating and exposed (in a Petri plate) to the culture broth, which contained a fixed aliquot of living cells. The striking results are reported in Figure 4.6, where the microscope pictures of all the Petri plates employed in a single test (except that of the control experiment) are reported. In the top right of each panel of Figure 4.6 is the total number of CFU per plate. It is apparent that the number of CFU per plate is comparable in the plates shown in panels Figure 4.6a**–f**. In fact, the CFU count on the plates containing the pure polymers and the polymers containing TBAP are comparable, within experimental error, to the control test, showing that neither the simple polymers nor the alkylammonium salt exert any biostatic activity on the growth of the microorganisms. On the other hand, the strong antifungal activity of the nanocomposite is obvious as no CFU are observed on the copper NP-PVMK plate (Figure 4.6g), and a strong reduction in the number of CFU is observed on the copper NP-PVC and copper NP-PVDF plates (Figure 4.6h and i). Although a slight run-to-run variation was detected in the number of CFU determined, the nanocomposites continuously exerted strong biostatic activity on yeast cell growth. Moreover, it was analytically detected that the copper NP-PVMK films showed the strongest biostatic result, while the copper NP-PVDF films exhibited the weakest effectiveness. This correlates well with the lower copper loading achieved in the PVDF matrix, as assessed by XPS analysis.

Comparable and striking biostatic activity was also exerted on other microorganisms and the results are reported in Table 4.4.

The application of low-copper loading materials in the food packaging area is also currently under evaluation. Furthermore, the improvement of a controlled release system could find applications in cases where an adjustable, controlled and perhaps very low ion release via a suitable stabilising shell, could answer the need for oligo-element dispensing on the micrometre scale.

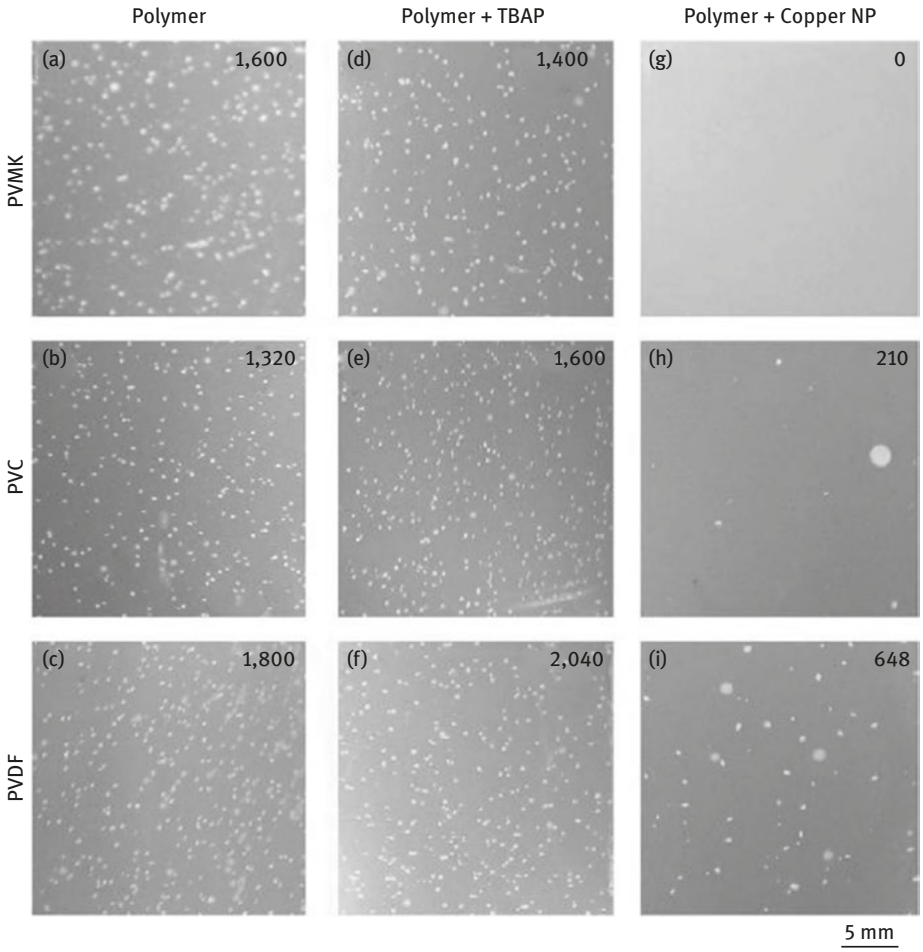

Figure 4.6: Optical microscope pictures, (a) to (i), of the Petri plates after 4 h of yeast incubation. The bottom of each plate was either coated with the bare polyvinylmethyl ketone (PVMK), polyvinyl chloride (PVC) and polyvinylidene fluoride (PVDF) polymers or by polymer–tetrabutylammonium perchlorate (TBAP) films or by the copper NP-PVMK, copper NP-PVC and copper NP-PVDF nanocomposites. Reproduced with permission from N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M. D'Alessio, P.G. Zambonin, and E. Traversa, *Chemistry of Materials*, 2005, 17, 5255. ©2005, American Chemical Society [20].

4.7 Polymer–titania nanocomposites

Different potential materials currently being explored include titania, which can be introduced as a possible candidate for polymer adjustment with important benefits. Titania acts under UV light excitation at energy above the related band gap (3.2 ev)

Table 4.4: Normalised CFU% of different microorganisms after incubation with copper NP-PVMK composite films[a].

	Escherichia coli	Staphylococcus aureus	Listeria monocytogenes	Moulds
Normalised CFU%	0.01	0.03	0	5.7

[a]: The values were obtained by taking the CFU of the control plates as 100%.
Reproduced with permission from N. Cioffi, L. Torsi, N. Ditaranto, G. Tantillo, L. Ghibelli, L. Sabbatini, T. Bleve-Zacheo, M. D'Alessio, P.G. Zambonin and E. Traversa, *Chemistry of Materials*, 2005, 17, 5255. ©2005, American Chemical Society [20].

resulting in energy-rich electron-hole pairs. Subsequently, at the surface of the material, these charge transporters are capable of interacting with microorganisms as they exhibit biocidal properties, which are related to the composition of the polymer-based nanocomposite films. A point of significance involves controlling the degree of titania polymorphism, which is responsible for the existence of the anatase phase containing the biocidal activity, in addition to controlling the main particle size in the nanometre range, to limit scattering. New hybrid or nanocomposite organo–inorganic materials, which combine the interesting abilities of oxides and polymer constituents, are not physical mixtures, although they are known as complex materials that have well-mixed organic and inorganic components. The scale of mixing or, in other words, the degree of homogeneity affects or even governs the properties of the nanocomposite solid material when mixing of the components is effectively achieved, usually at the nanometre scale. In particular, the optimisation of constituent contact has been shown to be critical to obtain titania-containing polymer nanocomposites with better biocidal properties. Another significant point to increase the performance of titania-containing nanocomposite structures concerns optimisation of the light absorption and satisfactory handling of subsequent charge (electron-hole) pair creation and destruction processes. This aim is usually attempted by controlling the morphological–structural defect features of the oxide and/or by extending the absorption power of the oxide into the visible spectra via the doping method [69]. Metal doping has long been recognised as one of the operational methods, which can change the basic band structure of titania, and therefore, increase its visible light sensitivity, in addition to increasing its photocatalytic activity under UV irradiation.

4.8 Polymer–zinc oxide nanocomposites

Several research groups have investigated the biological properties of zinc oxide. Polymer nanocomposites of zinc oxide have been applied many times to inhibit

microbial growth in food and drinks. Film grade low-density polyethylene (LDPE) resin pellets have been mixed with antimicrobial agents containing P105 powder and zinc oxide NP powder with a mean individual particle diameter of about 70 nm; the mixture was fed into a twin-screw extruder device to be cut into master batch nanogranules. Suitable volumes of master batch resins were then added to pure LDPE resin pellets in a single-screw blowing device to synthesis the ultimate nanocomposite film (50 μm thick) containing the desired nanomaterial concentrations (0.25 and 1% for nanozinc oxide; and 1.5 and 5% for P105) [70]. Orange juice was used in these studies as it is one of the most globally consumed fruit products. The demand for natural, high-quality orange juice, in terms of nutritional value, physico-chemical features and sensory properties, while using minimal or no heat treatment, has increased significantly [71]. Natural orange juice, even when refrigerated, has a short shelf life due to microbial spoilage [70]. Thus, the properties of zinc oxide and silver NP-filled LDPE nanocomposite packaging is a new way to protect and extend the shelf life of orange juice; the antimicrobial nanocomposite (0.25 and 1% for nanozinc oxide; and 1.5 and 5% for P105) and pure LDPE films used, 15 × 10 cm in size, were similar to Doypack packaging, which is usually used for the packaging of fruit juice. The packaged fruit juice was instantly covered in aluminium foil and sterilised at 95 °C for 2 min. After cooling and under a sterile laboratory hood, 175 mL of fresh orange juice was poured into each package and wrapped using the heat sealer. Packages enclosing orange juice were kept in a dark and cool environment (4 °C). The samples were characterised in duplicate for their microbiological and sensory properties directly after packaging and after 7, 28 and 56 days of storage.

The colour quality scores of the packages after 28 days of cold storage showed that there was no significant variation in colour ($p < 0.05$). The odour can be seriously affected by microbial growth and can result in fermentation of the orange juice during storage. After 28 days of storage, an important change in the odour was detected in orange juice packed in the test packages and in the pure package, except for the packaging containing 1% nanozinc oxide.

No change in the taste of the packed orange juice during the 28 days of storage is another positive attribute of nanoantimicrobial packaging; it is clear that there is an important change between LDPE + 0.25% nanozinc oxide packaging and other nanosilver packaging materials, the lowest score being related to LDPE + 1% nanozinc oxide and pure LDPE. The sensory panelists rated LDPE + 0.25% nanozinc oxide film followed by LDPE + 5% P105 and LDPE + 1.5% P105 as the best packaging material in terms of total packaging performance. It is important to note that any variation in orange juice flavour during storage may not only be caused by microbial growth but may also be due to heating, storage time and the usual chemical interactions that occur in stored juices [72]. The lower storage temperature of unpasteurised orange juice increases sensory approval, compared with the higher

temperatures, for 72 h [73]. The sensorial shelf life of orange juice is equal to 1/2 of its microbial shelf life and 2/3 of its chemical shelf life [74].

4.9 Conclusion

The development of new nanocomposite materials, which exhibit high protection and antimicrobial activity, and without bacterial resistance, is important as infections resulting from antibiotic-resistant bacteria highlight an important risk to human health. MNP properties alone do not determine the common features of polymer– metal nanocomposites. The formation of MNP within the polymer matrices may considerably alter the polymer morphology, for example, resulting in the development of nanoporosity in gel-type polymers, which improves the speed of mass transfer inside the nanocomposites, and could also alter some other fundamental parameters of great significance in their practical use. The polymer matrix also functions as the MNP stabilising media, which stops MNP aggregation and release to the environment during treatment. The properties of MNP immobilised inside a matrix typically dictate the functional features of the nanocomposites. Preparation of polymer–metal nanocomposites has to focus not only on the desired material properties, which are dictated by its intended application, but also on ensuring the material does not negatively impact upon human health or the environmental.

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5 Biocidal activity of biodegradable polymers

Abstract: Biopolymers are produced from natural materials such as starch, sugar, cellulose and so on. Unlike synthetic biopolymers, natural biopolymers are biodegradable, antibacterial in some cases, safe for both environmental and health point-of-view, easily synthesised and disposed with no by-product toxicity. Biopolymers canbe degraded by naturally occurring enzymes in microorganisms, such as bacteria, fungi and algae. The biodegradation rate in these biopolymers varied in few weeks to several months, depending on their molecular structure. Biocidal activity of natural biodegradable biopolymers has been widely studied and reported in the literatures. The hydrophilic backbone chains, amorphous nature, small size and high porosity of the biodegradable biopolymers endow broad-spectrum biocidal property to these biopolymers. In this chapter, molecular structure of various biodegradable biopolymers, as well as their potential biocidal activity is discussed.

Keywords: Biodegradable biopolymers; biocidal; biodegradation; biomaterials; biomedicine; tissue engineering

5.1 Introduction

Interest in the production of eco-friendly consumer products will continue to increase as more focus is placed on decreasing the rate at which global warming, pollution and landfill are affecting the world. Polymers are considered to be the most vital materials available to science and technology in the 21st century [1], and are the building blocks of all living systems, such as plants, animals and microorganisms. The biological world is made up of a number of polymers such as proteins, polysaccharides, polynucleotides, polyamides, cellulose, starch, polylactic acid (PLA), *cis*-polyisoprene, lignin and so on. Thus, biopolymers belong to a group of macromolecules formed under natural conditions during the growth cycles of all organisms and exhibit both

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biocompatible and biodegradable properties. Biopolymers are regarded as a green source of energy as they are unlimited, self-sustaining natural resources, which are in harmony with nature, and are gaining a huge market in the modern world due to their degradable and cost-effective nature. The diverse properties and versatility of these materials have made them an integral part of our daily life, and as a result, they play a vital role in industry and the economy. They also play important roles in maintaining cell viability by conserving genetic information, storing carbon-based macromolecules, producing either energy or reducing power and protect organisms from hazardous environmental factors.

Polymers are broadly classified as synthetic and natural polymers. Synthetic polymers have become significant since the 1940s and continue to replace glass, wood, constructional materials and metals in many industrial, domestic and environmental applications [2–5]. Synthetic polymers are made from hydrocarbons derived from petroleum. Some of these polymers, such as nylon, polyethylene, polyurethane and so on, are an indispensable part of our daily lives. Because of their stability and durability they offer good mechanical and thermal properties [6], making them suitable for a variety of applications, for example, in automobiles, cosmetics, medicines, biosensors, data storage devices and so on; thus, polymers pervade every aspect of modern society. The disadvantages of synthetic polymers are due to the fact that they are not biodegradable and are difficult to dispose of; in addition, synthesis involves the use of toxic compounds or the release of toxic by-products. Therefore, these polymers have raised concerns from both an environmental and health point-of-view; hence, the discovery of safe and environmental friendly alternatives is crucial.

Over the past two decades, there has been growing scientific interest regarding the use and development of biopolymer materials, which must retain the desired chemical and physical properties of conventional synthetic plastics, as a viable alternative; thus offering a solution to the serious problem of plastic waste disposal [4, 7–9]. Modern approaches, such as biotechnology and genetic manipulation, have enabled the production of these biopolymers on an industrial scale using optimised parameters for their production. These biopolymers have good chemical, thermal and mechanical properties, in addition, they are biodegradable and eco-friendly and can be used as adhesives, adsorbents, cosmetics, drug-delivery vehicles, high strength structural materials and so on.

Biopolymers are a renewable material as they are produced from natural materials, which can be replenished on an annual basis; they can be produced from natural raw materials such as starch, sugar, cellulose and so on. Biopolymers are thus possible alternatives to the traditional, non-biodegradable petrochemical-derived polymers and offer a positive attribute in terms of green chemistry. Biopolymers are degradable polymers, which can be broken down by the action of naturally occurring microorganisms, such as bacteria, fungi and algae. Polymer degradation can be defined as a change in the properties of the biopolymer, such as tensile strength, colour, shape and so on,

under the influence of one or more environmental factors, such as heat, light or chemicals. The biodegradability of the biopolymer is determined by the molecular structure; hence, some biopolymers degrade in a few weeks, while others take several months. The end products formed as a result of biopolymer degradation are stable and can be recycled or reused, hence reducing environmental pollution by polymer waste. The advancement of technology has enabled the production of sufficient quantities of biopolymers from renewable and easily available resources, such as microbes (Table 5.1).

Table 5.1: Microbially derived biodegradable biopolymers and their applications.

Serial No.	Biopolymer	Source	Applications
1	Xanthan	*Xanthomonascampestris*	Salad dressings, emulsions, pharmaceutical combinations, textiles, agricultural products
2	Dextran	*Leuconostocmesentecosides, Leuconostocdextranicum*	Gelling agent in confectionary, crystallisation inhibitor, blood transfusions as a plasma volume extender, antithrombolytic agent
3	Curdlan	*Alcaligenesfaecalis* var. *myxogenes, Rhizobium, Cellulomonasflavigena*	Pharmaceutical industries, food industries, construction fields, drug delivery and so on
4	Pullulan	*Aureobasidiumpullulans*	Food industry, cosmetics, drug and gene delivery, tissue engineering, wound healing
5	Gelrite	*Pseudomonas* species	Thickening or adhesive agent in foods, soil protection
6	Cellulose	*Gluconacetobacter, Agrobacterium, Aerobacter, Achromobacter, Azotobacter, Rhizobium*	Diet foods, speaker diaphragms, medical pads, artificial skin
7	Chitin and CS	Shells of crabs, lobsters, shrimps and insects	Cosmetics, manufacture of artificial skin and absorbable sutures, synthesis of water-soluble prodrugs
8	Polyhydroxyal-kanoate	*Pseudomonas Bacillus, Ralstonia, AeromonasRhodobacter*	Packaging materials, pressure sensors for keyboards, stretch and acceleration measuring instruments, in agriculture as a coating for urea fertilisers and so on

Table 5.1 (continued)

Serial No.	Biopolymer	Source	Applications
9	Polyglycolic acid	*Lactobacillus delbrueckii, Lactobacillus amylophilus, Lactobacillus bulgaricus, Lactobacillus leichmanii*	Fibres and fabrics, packaging materials, interference screws in ankle, knee and hand, tacks and pins for ligament attachment, rods and pins in bone and plates and so on, and surgical sutures, implants and drug-delivery systems
10	Hyaluronan	*Streptococcus equi, Streptococcus equisimilis, Streptococcus pyogenes, Streptococcus uberis*	Tissue engineering, intradermal, injection, cosmetics in ophthalmology and in wound healing, topical delivery of drugs

Biodegradable polymers accelerate the degradation of bacteria or pathogens due to the hydrophilic backbone chain of polymers, which contain atoms, such as O, N, S, in the polymer chain. The amorphous nature, small size and high porosity of biodegradable polymers are the factors, which disrupt the outer bacterial membrane.

5.2 Biodegradable chitin and chitosan polymer material

Chitin and chitosan(s) (CS) are biopolymers that have received considerable attention due to their numerous applications in agriculture, food, textile, the paper industry, the food industry and biomedicine and so on.

Chitin is the most abundant biopolymer on earth except for cellulose. This polymer was an unused natural resource for a long time, but interest has increased in recent years due to its physiological inertness, biodegradability, hydrophilicity and biocompatibility and so on [10, 11]. Chitin is a polysaccharide made of *N*-acetyl-D-glucosamine units connected by β(1→4)-linkages. Chitin is found in fungi and in the shells of crustaceans and molluscs, in the backbone of squids and in the cuticle of insects [12]. Long chitin molecules are linked by covalent bonds and together they form a complex structural network.

Chitin and CS have similar chemical structures (Figures 5.1 and 5.2). Chitin is made up of a linear chain of acetyl glucosamine groups, while CS is obtained by removing enough acetyl groups (CH_3–CO) for the molecule to be soluble in most dilute acids, this process is called deacetylation; hence, the actual difference between chitin and CS is the acetyl content of the polymer. The most useful derivative of chitin is CS containing free amino groups.

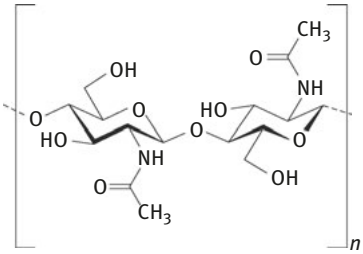

Figure 5.1: Structure of chitin.

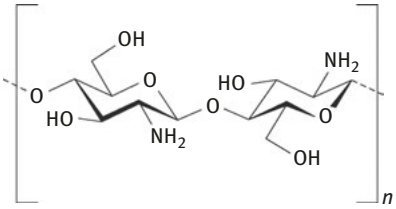

Figure 5.2: Structure of CS.

Industrial chitin is obtained from marine food production waste, that is, crustacean shells from shrimp, crab or krill [13, 14]. The processing of shrimps for human consumption generates 40–50% of the total mass of marine food production waste, which is considered to be one of the main pollutants in coastal areas, as it is dumped into the sea [15]. A small part of the waste is dried and used as chicken feed [14].The major components (on dry mass basis) of shrimp waste are chitin, minerals, carotenoids and proteins; thus, the utilisation of this shell food waste as an alternative source to produce chitin may help solve environmental problems related to waste generation.

In recent years, the production of chitin and CS from fungal sources has been the centre of attention due to potential advantages over other sources, such as crustaceans and shrimps. Crustacean waste is seasonal and a limited amount is available from fishing industry locations. An excessive amount of inorganic materials present in the crustacean waste means that a demineralisation treatment is required prior to use. In the case of fungal sources, the fungal mycelium can be obtained via simple fermentation regardless of geographical location or season [16], and it contains lower levels of inorganic materials compared with crustacean shells, thus reducing the demineralisation process [17]. Chitin is produced by many fungi occurring in *Basidiomycetes, Ascomycetes* and *Phycomycetes* where it is a component of the cell walls and structural membranes of mycelia, stalks and spores [18]. The chitin content of the cell walls is generally higher in the genus *Zygomycetes*, which include species of *Mucor, Rhizopus* and so on [19]. Kishore and co-workers [20] examined the synthesis of CS from the mycelia of *Absidiacoerulea, Mucorrouxii, Gongronellabutieri, Phycomycesblakesleeanus* and

Absidiablakesleeana. Wang and co-workers [21] determined the physical properties of fungal CS from *Absidiacoerulea*, *Mucorrouxii* and *Rhizopusoryzae*. *Aspergillus niger* was also reported to produce CS effectively [22]. The pileus and stipes of fungal fruiting bodies such as *Agaricusbisporus*, *Pleurotusostreatus* and *Lentinulaedodes* produce chitin and it was reported that *Agaricusbisporus* had a higher chitin level than *Pleurotusostreatus* and *Lentinulaedodes* [23]. The production of chitin from waste material generated from stalks and mushrooms with irregular dimensions, which are not suitable for commercial use, have also been reported. The waste generated from *Agaricusbisporus* culture farms was used for the cost-effective manufacturing of commercial chitosans [24, 25]. *Rhizopusoryzae* was reported to produce chitin when potato peel was used as the substrate [26]. A chitin and glucan complex was isolated from the biomass of *Armillariamellea* and the yellow morel *Morchellaesculenta* by Ivshina and co-workers [27].

To obtain the maximum yield of chitin and CS, fungi were harvested at their late exponential growth phase after culturing in media such as yeast peptone glucose broth, potato dextrose broth or molasses salt medium [28]. There are a variety of means for the detection of chitin from fungal samples; however, the best quantitative detection is via the enzymatic method, which involves the hydrolysis of chitin to its oligomers via chitinase. The oligomers are further hydrolysed to monomeric *N*-acetylglucosamine by *N*-acetyl-glucosaminidase. This method is very selective as only chitin is degraded even in the presence of other polysaccharides. The final products, that is, glucosamine units, can be used to estimate the amount of chitin or CS in the material under investigation [29].

CS is insoluble at neutral and alkaline pH, but is soluble in organic and inorganic acids, including acetic, formic, hydrochloric, glutamic and lactic. CS exhibits a variety of physico-chemical and biological properties, which can be used in various fields including the edible film industry, as additives to enhance the nutritional quality of foods, for the recovery of solid materials from food processing waste and in the purification of water [30]. These properties make CS commercially vital as do the properties of biodegradability and biocompatibility in both plant and animal tissues. These materials have the ability to be transformed into gels, beads, fibres, colloids, films, flakes, powders and capsules [30–33]. Additional exclusive characteristics of CS are its non-digestibility and bland taste, which make it an excellent choice as a food additive component, predominantly in the preparation of low-calorie foods [34].

Over 150,000 tonnes of chitin is currently harvested by utilising a by-product of the seafood industry, making it available throughout the year. Chitin and CS are currently in the spotlight due to their numerous applications in biomedicine, waste water treatment, food, cosmetics and the fibre industry [35–39]. The high nitrogen content (6.89%) and other excellent properties, such as biodegradability, non-toxicity, biocompatibility and adsorptive abilities, make chitin and CS more commercially important than other biopolymers [40, 41].

CS is an exciting and promising material in tissue regeneration applications as these biopolymers can be easily constructed into various forms and their derivatives are biodegradable by lysozomal enzymes.

Chitin and CS are basic in nature. CS in solution at pH values below 6.5 carries a positive charge, which makes it readily react with a variety of negatively charged materials or polyanions. The high content of primary amino groups with a pKa of 6.3 is responsible for most of these characteristic properties. The amino groups are also responsible for enabling several chemical modifications of CS, which is a crucial factor in its ongoing development for many applications. The soluble–insoluble transition of CS occurs around pH 6.0–6.5 at the pKa of its primary amino groups. At low pH, the positive charge on the amino groups converts CS to a water-soluble cationic polyelectrolyte and when the pH increases above 6.0 the positive charge on the amino groups is lost and CS becomes insoluble [41]. The degree of *N*-acetylation is dependent on the pKa; hence, the solubility of CS is dependent upon the degree of deacetylation and the method of *N*-deacetylation. The chemical structure of chitin allows easy specific modifications at the C-2 positions, making it a suitable candidate for further development in comparison to other polysaccharides such as cellulose, starch, agar and so on. CS bioplastics fully degrade within two weeks and release nutrients that support plant growth, making it eco-friendly in nature.

CS forms water-soluble salts with inorganic and organic acids including glyoxylate, pyruvate, tartarate, malate, malonate, citrate, acetate, lactate, glycolate and ascorbate. Natural CS showed more solubility in organic acids when the pH of the solution was less than 6.5. Facile water-miscible salts of CS are formed by neutralisation with acids such as lactic, hydrochloric, acetic or formic.

These reactive CS functional groups can be readily subjected to chemical modifications, which alter the physico-mechanical properties of CS [42]. CS tripolyphosphate has the capacity to form a chelated complex with transition metal ions such as silver (Ag^{+}), copper (Cu^{2+}), zinc (Zn^{2+}), manganese (Mn^{2+}) and iron (Fe^{2+}) [43].

The applications of CS are dependent upon the degree of acetylation and its molecular weight (MW) [44]. The degree of deacetylation is an extrinsic property, which can be increased by increasing the temperature or strength of the alkaline solution. The viscosity of CS also influences its biological properties, such as wound-healing properties, as well as biodegradation by lysozyme.

5.2.1 Antimicrobial activity of chitin

The antimicrobial activity of CS is associated with its MW, degree of acetylation, concentration of CS and bacterial inoculum size, as described by Chen and Fernades [45–47]. It was reported that lower MW CS is strongly effective against Gram-negative bacteria, whereas high MW CS is effective against Gram-positive bacteria. CS has several advantages over other types of disinfectants as it exhibits

higher antibacterial activity, a broader spectrum of activity and a lower toxicity to mammalian cells [48].

The antimicrobial activity of CS increases with decreasing pH [49–52], which is due to the fact that the amino groups of CS become ionised at pH values below 6 and carry a positive charge. Unmodified CS does not exhibit antimicrobial activity at pH 7, as it does not dissolve and it does not contain any positive charge on the amino groups [53, 54]. The antimicrobial activity of CS is enhanced upon increasing the degree of deacetylation, due to increasing the number of ionisable amino groups [55].

Several studies report the use of CS as an antimicrobial agent [58, 56]. The antimicrobial activities of CS against foodborne pathogens have been extensively investigated in the food industry [28, 57–60]. Nguyen and co-workers [50] reported that a CS solution can inhibit the growth and development of food-contaminating microbes such as *Aspergillus niger, Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Wu and co-workers [24] examined the bioactivity of chitin and CS obtained from *Aspergillus niger* and *Mucorrouxii* against the foodborne pathogen *Salmonella typhimurium* and plant pathogens *Botrytis cinerea* and *Penicillium expansum*. The biological properties were compared with commercial CS obtained from crustacean shells. The antimicrobial activity of the isolated chitin and CS was similar and comparable with that obtained from commercially purified crustacean CS. The isolated CS exhibited strong antimicrobial activity against both Gram-negative and Gram-positive bacteria such as *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogenes*, respectively [28, 51, 52]. Gram-positive bacteria appeared to be more susceptible to CS compared with Gram-negative species [28, 51]. It has been suggested that the interaction between positively charged CS molecules and negatively charged microbial surfaces results in disruption of the cell membranes, leakage of intracellular constituents and ultimately microbial cell death [61]. CS oligomers are believed to penetrate into prokaryotic cells and interfere with the transcription of ribonucleic acid and protein synthesis [62]. CS also exhibit a potent plaque-reducing action, as well as in vitro antibacterial activity, against several oral pathogens such as *Actinobacillus actinomycetemcomitans, Streptococcus mutans* and *Porphyromonas gingivalis*, which are implicated in plaque formation and periodontitis [63, 64].

It was found that antimicrobial activity of CS could be tremendously enhanced by the introduction of quaternary ammonium functional groups. Guo and co-workers [65] reported the enhanced antifungal activity of quarternised CS derivatives against plant pathogenic fungi *Botrytis cinerea* and *Colletotrichum lagenarium*. Jia and Xu [66] reported that CS derivatives, such as *N,N,N*-trimethyl CS, *N*-propyl and *N-N*-dimethyl CS, exhibited stronger activity against *Escherichia coli* compared with that of unmodified chitin. Zivanovic and co-workers [67] reported that CS polysaccharides exhibited stronger bactericidal effects toward *Listeria monocytogenes* and *Salmonella enterica* serovar

Typhimurium strains, compared with the CS oligosaccharide. Coma and co-workers [32] reported that CS films completely inhibited *Listeria monocytogenes* growth for at least 8 days.

5.2.2 Antioxidant properties of chitosan

Oxidative stress occurs in biological systems if the balance between oxidant formation and the endogenous (internal) antioxidant defence mechanism is disturbed [68]. During aerobic metabolism, reactive oxygen species and free radicals are generated naturally in the body, which cause the oxidation of lipids, proteins, sugars, sterols and nucleic acids. During the ageing process, the antioxidant defence system weakens, resulting in the accumulation of reactive oxygen species and free radicals. The formation of these free radicals are toxic as these cause cellular damage leading to many pathological conditions such as arthritis, cancer, stroke, atherosclerosis, retinal damage, diabetes and heart attack [69, 70].

Chitin and its derivatives have received considerable attention as they are non-toxic and can be easily delivered into living systems and used in food technology. The antioxidant properties of chitin and CS depend on the degree of deacetylation and MW (Table 5.2); CS with the lowest degree of deacetylation exhibited the best scavenging activity [71–73]. In order to improve the antioxidant properties of CS, various modifications were applied to CS molecules to overcome its solubility limitation. Sulfation represents a very important family of CS derivatives with enhanced biological activities, especially antioxidant properties. It has been shown that sulfated CS and sulfanilamide CS have significantly better free-radical scavenging activities than unmodified CS [72, 74].

An oligosaccharide prepared from CS containing a 10% degree of deacetylation displayed free-radical scavenging activity [75]. Xie and co-workers [76] reported that the free-amino groups in chito-oligosaccharides react with free radicals forming a stable macromolecule radical, which results in antioxidant activity. Low MW CS are reported to be more effective antioxidants than high MW CS.

5.3 Facile synthesis and importance of Biopol [Poly (3-hydroxybutyrate-co-3-hydroxyvalerate]

Poly(3-hydroxy butyric acid) and poly(3-hydroxyl valeric acid) copolymers are formed by the fermentation of glucose with the aid of *Alcaligeneseutrophus* species. As a result of using copolymers, PHBV is synthesised via polymerisation (Figure 5.3).

Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV) is a soft, malleable and thermosoftening biodegradable polymer material. It can be easily converted

Table 5.2: Modified derivatives of chitin/CS.

Serial No.	CS derivatives	Preparation method	Advantage of modified chitin/ CS	Applications
1	*O* and *N* carboxy methyl CS	Reductive alkylation. Direct alkylation	Formation of an amphoteric polymer, which extends the range of pH, enhancing CS solubility in different solvents	Modified drug delivery pH responsive drug delivery DNA delivery. Targeted drug delivery. Permeation enhancer. Cosmetics
2	CS 6-O-sulfate	Esterification using inorganic acids	Polyampholite soluble in water	Anticoagulant Haemagglutination inhibition activities. Antisclerotic Antiviral Antihuman immunodeficiency virus. Antibacterial Antioxidant Enzyme inhibition activities
3	*N*-methylene phosphonic CS	Phosphorylation of amino groups using phosphoric acid and formaldehyde	Amphoteric, good cationcomplexing efficiency for cations such as Ca^{2+} and transition metals (copper (II), cadium (II), zinc (II) and so on)	Development of prodrugs (used for the corrosion of iron-oxide-based prodrugs). Protects metal surfaces
4	Trimethylchitosan ammonium	Quaternisation of CS with methyl iodide in sodium hydroxide under controlled conditions	Water-soluble over all the practical pH range	Used in the paper industry due to good flocculating properties
5	Quaternised CS and *N*-alkyl CS	Alkylation of CS followed by quaternisation	Higher aqueous solubility in a much broader pH and concentration range	DNA delivery
6	Hydroxyalkyl CS	Reacting CS with epoxide	Marked surface activity and foam-enhancing properties of CS	Antimicrobial, temperature sensitive injectable carrier for cells

Table 5.2 (continued)

Serial No.	CS derivatives	Preparation method	Advantage of modified chitin/ CS	Applications
7	Sugar-modified CS	Reductive *N*-alkylation using sodium cyanoborohydride and unmodified sugar or sugar-aldehyde derivative	Enhance water solubility	Drug targeting
8	Cyclodextrin-linked CS	Reductive amination using formylmethylene, crosslinking by glutaraldehyde and so on	Form non-covalent inclusion complexes with a number of molecules altering their physico-chemical properties	Drug delivery
9	*N*-acyl CS	Acylation using acyl chlorides and anhydrides	New physico-chemical properties such as the formation of polymeric assemblies, including gel, membranes and fibres and so on	DNA delivery
10	CS-grafted copolymers	Copolymerisation reactions	Modifying the chemical and physical properties of chitin and CS to widen their practical use	Tissue engineering, antibacterial and superoxide scavenging (antioxidant) activity

DNA: Deoxyribonucleic acid

into films, which are applicable for packaging, carry bags, disposable bottles and so on. The hydrophilic backbone chain of the polymer, which contains atoms of oxygen in the polymer chain, accelerates the degradation of bacteria and pathogens.

PLA is widely used in medical and packaging industries and has received considerable attention due to its mechanical and physical properties. PLA/ nisin is used as an antibacterial food packaging material and this combination shows positive results against Gram-positive bacteria such as *Clostridium bacillus, Staphylococcus* and so on. PLA/silver films were effective against *Escherichia coli* (Gram-negative bacteria) and *Staphylococcus aureus* and *Vibrio parahaemolyticus* [77, 78].

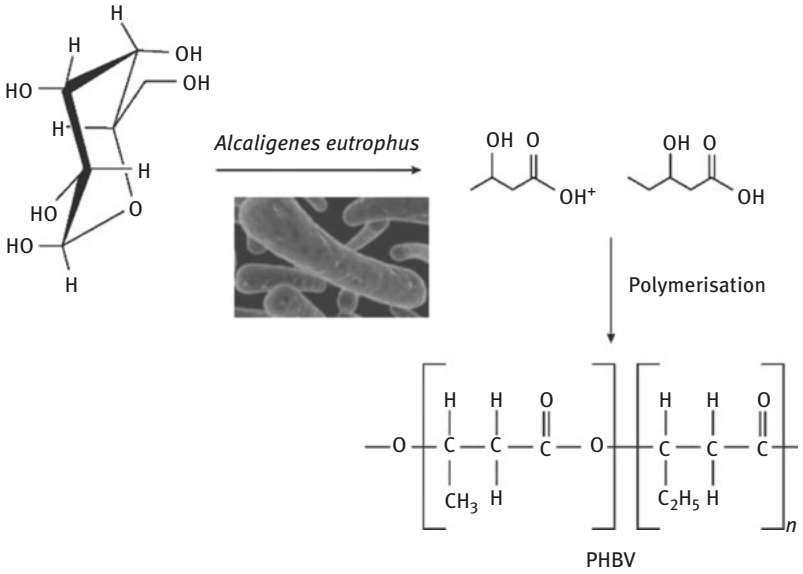

Figure 5.3: Greener route synthesis of the PHBV (Biopol) polymer.

5.4 Antibacterial importance of a biodegradable polypyrrole/dextrin conductive nanocomposite

The polypyrrole (Ppy)/dextrin nanocomposite is synthesised via in situ polymerisation and the preparation of this nanocomposite is shown in Figure 5.4. The backbone chain of this nanocomposite polymer contains hydrophobic side chains, which disrupt the microbial cell membrane leading to leakage of the cytoplasm in bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. This material can be implemented in the fields of biomedicine, biosensors and food packaging due to the biodegradable property of dextrin, as well as the antibacterial properties of the Ppy [79].

5.5 Antibacterial biodegradable polymer–nanocomposite

Pande and co-workers reported biodegradable polymer–nanocomposites that exhibit antibacterial activity, for example, a gum tragacanth-zinc oxide nanocomposite which showed antibacterial activity against *Escherichia coli* [80]. Gum

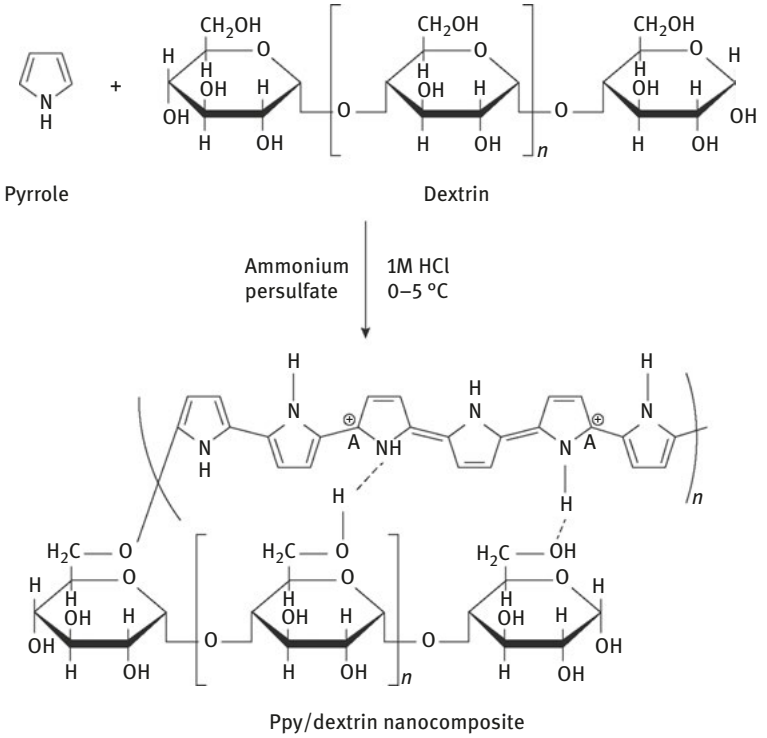

Figure 5.4: Reaction of Ppy/dextrine nanocomposite.

tragacanth exhibits high solubility in water and is a novel route to prepare zinc oxide nanoparticles using economical natural polysaccharide binders. This polysaccharide chain contains D-galactameric acid, L-fucose, D-xylose and D-galactose , and has an O atom in the polymer chain along with zinc oxide NP, which helps to penetrate the outer membrane of *Escherichia coli*. In addition, an *Abelmoschusesculentus*-silver nanocomposite has shown antibacterial activity against *Escherichia coli* (Gram-negative), *Staphylococcus aureus* (Gram-positive) and *Candida* (fungi) [81]. An easily synthesised *Abelmoschusesculentus*-iron nanocomposite has shown antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* [82], as a result of the folic-acid-containing N and O atoms, which help to disrupt the outer membrane of bacteria. Pectin–GeO_2 nanocomposite has been synthesised by hydrothermal method as a promising candidate for biomedical and environmental applications [83].

5.6 Conclusion

Chitin is a natural polymer with unique structural and multidimensional properties with wide-ranging applications in the biomedical and pharmaceutical fields. The chemical structure of chitin and CS allows specific modifications at the C-2 position, which reflects its advantages over other polysaccharides such as cellulose, starch, galactomannans and so on. The chemical structure of chitin and CS is also responsible for their solubility in aqueous or organic solvents, which further enhances their biocidal activities, potential biomedical applications and simple synthesis. Chitin is biodegradable, renewable and exhibits antimicrobial properties; in addition, it has biomedical applications and thickening properties as discussed in this chapter. The huge market potential of these polymers, due to their unique properties and wide-ranging applications in various sectors, suggests that research work should be intensified on exploring the availability of raw materials that could be a source of chitin and CS. Biodegradable polymer–nanocomposites are the focus of research into developing eco-friendly medicinal applications.

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6 Polylactic acid and polyethylene glycol as antimicrobial agents

Abstract: There have been several efforts to induce antibacterial properties in different materials for a variety of applications. Antimicrobial agents can be incorporated or induced in different polymeric matrixes. Polylactic acid (PLA) is an aliphatic polyester derived from renewable resources, such as corn starch, tapioca roots, chips or sugarcane. Polyethylene glycol (PEG) is also a polyether compound with many applications, ranging from industrial manufacturing to medicine. These two polymers are capable of delivering antibacterial effects to targeted places. This chapter describes the development and advancements regarding the use of PLA and PEG polymers for antimicrobial purposes.

Keywords: Antimicrobial agents, polylactic acid, polyethylene glycol

6.1 Introduction

The poor wettability of many antimicrobial agents has presented a challenge for antimicrobial activity as it is difficult for these compounds to interact with microorganisms in aqueous media [1]. Furthermore, these compounds have frequently been incorporated into food systems after nanoencapsulation, which detrimentally affects antimicrobial activity [2]. Embedding these compounds into biodegradable polymers has been shown to be a promising approach in the food industry for the controlled delivery of antimicrobial agents to avoid the onset of food-related illnesses due to microbial growth [3]. Among the different polymers used for nanoencapsulation, poly(d,l-lactide-*co*-glycolide) (PLGA) has received considerable attention, due to approval for use by the US Food and Drug Administration (FDA), and is widely used in many biomedical applications such as the targeted delivery of active genes, proteins, vitamins and pharmaceutical drugs [4, 5].

Polylactic acid (PLA) has caused less environmental concern as an alternative membrane-like material due to its non-toxic effect; however, they are hydrophobic

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polymers and easily release polluting molecules [6]. Another interesting polymer in this category is polyethylene glycol (PEG), which is well-known to cause a low degree of pollution, with adequate biocompatibility, high hydrophilicity, non-ionic charge and high void volume, which leads to its high resistance to protein. Since it is a water-soluble polymer, it is not usually employed for polymer blending. It has been shown that nanoparticles (NP), based on the PEG–PLA copolymer, could be considered as a potential alternative to glioblastoma chemotherapy; in addition, these NP have been widely investigated as drug carriers [7–9].

PLA is a non-toxic and biodegradable material that is extensively used in coating membranes [10] and as carrier compounds [11–18]. The application of PLA in controlled drug-delivery systems is often limited due to its poor wettability. It has been reported that polymeric micelles of the diblock copolymer, PEG-*b*-PLA, can prevent the function of P-glycoprotein (Pgp) ensuring no multidrug resistance could be acquired and hence could be used for the treatment of cancer.

Aliphatic polyesters (such as PLA, polyglycolic acid and their copolymers) are the most important class of biocompatible polymers used in biomedical applications. This class of polymers has shown superior properties over conventional polymers, such as excellent biocompatibility, biodegradation and thermal, physical and mechanical properties, which make them suitable for applications in drug delivery and tissue engineering [19–21].

6.2 Antimicrobial activity of polylactic acid and polyethylene glycol

6.2.1 Poly(d,l-lactide-co-glycolide)

It has frequently been reported that the antimicrobial activities of many naturally occurring phenolic compounds in fruits play an important role in their protection against pathogenic microorganisms. It has been suggested that the by-products of tropical fruit production contain high levels of essential healthy compounds, which can be extracted and used for many therapeutic applications; of these by-products, phenolic compounds have shown both antimicrobial and antioxidant activities.

As an alternative to the use of harmful synthetic additives in food preservation, a novel approach has involved the use of bioactive extracts. Among the biodegradable and biocompatible nanoencapsulants, which are available, PLGA is widely used in pharmaceutical applications such as gene therapy, targeted drug delivery and the delivery of active agents. Recent studies have investigated the effect of different ratios of PLGA on the general characteristics of PLGA-loaded tropical fruit juices and their antimicrobial activity against foodborne bacteria [22].

Ritger and Peppas proposed an equation for the release of fruit extract by-products from PLGA NP. This model could be seen as a generalised form of the Fickian diffusion in eq. 6.1 [23]:

$$M(t)/M_0 = b_1{*}\exp(-k_1 t) + b_2{*}\exp(-k_2 t) \tag{6.1}$$

where M(t) is the fruit extract content in the NP (mg) at time t (hours), and Mo is the initial fruit extract content (mg), b_1, b_2 (dimensionless), and k_1 and k_2 (1/h) are constants. The values k_1 and k_2 are the fruit extract release rate constants [24].

The release behaviour of tropical fruit extracts from PLGA NP was examined over different time periods. The release behaviour for all tropical fruit extracts, containing 50:50 and 65:35 PLGA NP, was an immediate biphasic release in the first hour followed by a similar pattern of release over the time period. There are several factors, which influence the release of active molecules, such as penetration of the release medium into the NP and diffusion of the active molecules through the NP, or swelling and degradation of the NP matrix. The fast release of the active compound has been attributed to the surface properties of PLGA NP. Eventually the release rate slows down as the active compound has to travel a longer distance from the polymer chains, which are deeper within the PLGA matrix. Generally, the rate of PLGA biodegradation is slow; hence, release of the active compound is highly dependent upon its diffusion and PLGA swelling.

Biodegradable polymers, such as poly(L-lactic acid) (PLLA) and PLGA are suitable for drug-delivery applications due to their non-toxicity and adjustable biodegradability [25, 26]. PLGA and PLLA films have been used for the treatment of periodontal disease [27], glaucoma [28] and cancer [29], and as a component of biodegradable stents [30, 31].

6.2.2 Polylactic acid

PLA is a non-toxic and biodegradabale polymer, which is classified as safe by the FDA, and is suitable for all food packaging applications [32, 33]; PLA is suitable for products, which are not sensitive to oxygen permeability. The important advantage of PLA production, compared with other degradable polymers, is the reduction of carbon dioxide emission. Carbon dioxide is known to be the leading contributor to global warming. As carbon dioxide is absorbed from the air when corn is grown, specifically for the production of PLA, this could aid in the decreased emission of greenhouse gases, in comparison with the production of hydrocarbon-based polymers. Using wind energy, researchers determined that −0.7 kg of carbon dioxide gas was utilised for PLA generation; hence, PLA has a negative carbon footprint and could act as a greenhouse gas sink via the employment of novel process technology.

Active packaging is defined as a smart system that includes interactions between the packaging components and food [34]. Active packaging is a new approach for expanding the shelf life or sensory properties through preserving food quality for

longer. Traditional food packaging is limited in its ability to prolong the shelf life of food products. The important properties of active packaging include: oxygen and ethylene scavenging ability, carbon dioxide emission, moisture regulation, antimicrobial properties, antioxidant release and release or adsorption of odour [35].

The most attractive applications of PLA and its copolymers (PLGA) in the human body are as a carrier for releasing various drugs in pharmaceutical and medical applications [36–38]. Some studies have investigated the suitability of PLA as an active packaging material. Antioxidants have been added to food packaging material with the aim of allowing their migratation into the food, due to the fact that prooxidants, which can be present in high concentrations and lead to food quality deterioration, are readily reduced by antioxidants.

6.2.3 Poly(d,l-lactide)-polyethylene glycol-poly(d,l-lactide)

Bone is a natural composite comprising type I collagen and calcium phosphate minerals, of which nanocrystalline apatite is the main component [39, 40]. Certain osteoconductivebioceramics exert an effect on bone cell attachment and growth factor binding or release, and can accelerate the treatment of bone defects [41–43]. Polymer composite scaffolds can be produced, via electrospinning, which contain a specific amount of electrical charge to form non-woven fibrous meshes with fibre dimensions in the nano- to microscale [44–46].

6.2.4 Polyethylene glycol-poly(d,l-lactide)

According to several studies [47–51], stimuli–responsive materials, that is, magnetic NP, such as hollow magnetic structured particles, have captured considerable attention as versatile carriers for therapeutic applications due to their large inner volume, low density, high specific surface area, controllable structure and outstanding magnetic properties. PEG–PLA, a type of biodegradable amphiphilic natural polymer, has traditionally been selected as the therapeutic delivery support due to its outstanding advantages, including good biocompatibility, non-toxicity, miscibility with many solvents and good compatibility with organic ingredients [52–54]. PLA exhibits many favourable physico-chemical characteristics, such as safety, stability, biodegradability and lack of intrinsic immunogenicity, and is a suitable candidate for delivery application. Using the phase separation method, Ren and co-workers [55] prepared magnetite/PLA-*b*-PEG copolymer composite particles; however, they observed that the process was slow and included multiple complex steps. Magnetite/silicon dioxide hollow microspheres (HMS), which were surface coated by PEG–PLA, have been synthesised via precipitation polymerisation [56].

HMS have been chemically modified using (3-aminopropyl)triethoxysilane (APTES). A solution (30 mL) of HMS (0.1 g) and APTES (2 mL) was prepared in toluene. In fact, APTES, an aminosilane, can be used in the silanisation process to functionalise the HMS surfaces with alkoxysilane molecules. APTES can also be used to covalently bond thermoplastics to polydimethylsiloxane (PDMS). APTES-functionalised HMS surfaces have been shown to be non-toxic to embryonic rat cardiomyocytes in vitro. In this study, the resulting magnetite/silicon particles were washed with ethanol and then dried under vacuum at 60 °C for 24 h [57]. The obtained amine-functionalised HMS (HMS–NH_2) were dispersed by ultrasound in toluene (30 mL). Another modification involved d,l-lactide (0.4 g) and PEG (0.1 g), which were placed into a three-neck round-bottom flask containing a magnetic stirrer. The catalyst, $Sn(Oct)_2$, was added to the flask at a concentration of 0.1% of the solution; the flask was put into an oil bath to react for 24 h at 110 °C in nitrogen under ambient conditions [58].

To remove PLA–PEG, the resulting product was first dissolved in dichloromethane and petroleum ether was then used to rinse off the catalyst. The final product was then placed under vacuum at room temperature (RT) and dried for 48 h. However, a little cytotoxicity was observed for HMS at a concentration higher than 800 μg/mL and HMS@PEG–PLA at a concentration higher than 400 μg/mL. In addition, the cell viability decreased due to the reduced survival capacity of the cells.

Overall, HMS@PEG–PLA displayed no obvious cytotoxicity, which could adversely affect humans and should be considered as a candidate for biological applications and drug-delivery systems.

Hydrogels can absorb a significant amount of water and exhibit good flexibility, comparable to natural tissue, and these properties have led to many applications in the biotechnological and medical fields [59, 60]. To achieve the sustained release of hydrophobic compounds, Murakami and co-workers developed a tissue-adhesive hydrogel covalently bonded to a reactive polymeric micelle formed from aldehyde-terminated CHO-PEG-*b*-PLA copolymers [61, 62]. Recently, several approaches have been proposed to fabricate materials in sheet form to maximise the surface area, for example, a layer-by-layer sheet of polyelectrolytes [63–65], a self-assembled monolayer sheet [66] and an NP-fused sheet [67–69].

A methoxy-terminated PEG-*b*-PLA copolymer (CH_3O-PEG-*b*-PLA) was synthesised using the anionic ring-opening polymerisation (ROP) of both ethylene oxide and d,l-lactide in anhydrous tetrahydrofuran (THF). A typical experimental procedure for the synthesis of CH_3O-PEG-*b*-PLA is described as follows.

2-methoxyethanol (2 mmol) and potassium naphthalene (2 mmol) are first mixed in anhydrous THF for 1 h. The purified ethylene oxide (182 mmol) is then added to the obtained potassium 2-methoxyethoxide solution (total volume: 25 mL) at 80 ºC. After the mixture has been stirred for 48 h, purified D, L-lactide (42 mmol) is added to the solution. Polymerisation is carried out and the resulting block copolymer is precipitated by ice-cold 2-propanol, which had been stored in a freezer overnight, followed by centrifugation at 10,500 rpm.

Among the various nanodrug-delivery systems that are available, core–shell polymeric micelles with a nanosized structure have received considerable research interest due to their good biocompatibility, long circulation time and ability to solubilise hydrophobic drugs and selectively target tumours [70, 71]. According to Kabanov and co-workers [72], polymeric micelles can function as biological response modifiers and inert carriers for hydrophobic anticancer agents, including paclitaxel and doxorubicin.

Considering that uptake was inhibited by cholesterol-depleting reagent, methyl-β-cyclodextrin, the inhibition of Pgp function by PEG-*b*-PLA micelles could be due to the interaction between micelles and the cell membrane, and subsequent lipid raft-mediated endocytosis [73].

It has been reported that NP internalised via clathrin-dependent endocytosis were generally entrapped within the endosomes and then fused with lysosomes, resulting in lysosomal degradation of the load [74]. On the other hand, NP internalised by caveolin-mediated endocytosis could normally bypass lysosomal degradation [75]. It was also shown that polymeric micelles incorporating nile red (M-NR) were capable of colocalisation with tubulin, an important protein that shapes the microtubule network of cells; this colocalisation might be advantageous for M-NR uptake via dynamin and caveolin-mediated endocytosis [76, 77].

The physico-chemical properties of NP, such as surface charge, size and morphology, can influence the cellular uptake mechanisms of NP and their intracellular fates [78]. C6 cells (cloned from a rat brain glial tumour, which was induced by *N*-nitrosomethyl-urea) and HepG2 cells (a perpetual cell line consisting of human liver carcinoma cells) were transfected with GFP-tagged wild-type caveolin-1 to confirm that caveolin-1 was involved in the endocytic pathway of PEG-*b*-PLA polymeric micelles.

Caveolin-1 is an established marker of caveolin-dependent endocytosis and can colocalise with M-NR, which has been confirmed by the use of confocal microscopy. Wild-type caveolin-1 significantly amplified the fluorescence power of internalised M-NR in HepG2 cells expressing low levels of caveolin-1, whereas the negative mutant, caveolin-1 Y14F, significantly reduced the fluorescence power of internalised M-NR in C6 cells expressing high levels of caveolin-1 [79].

Flow cytometry was used to further investigate the effects of wild-type caveolin-1 and the caveolin-1 Y14F mutant on the cellular uptake in HepG2 and C6 cells. It has been reported that wild-type caveolin-1 in C6 cells and caveolin-1 Y14F in HepG2 cells did not affect M-NR uptake [80]; this could be attributed to the expression of caveolin-1, which was abundant in C6 cells and lower in HepG2 cells.

Interestingly, none of the dominant negative mutants of either caveolin-1 or dynamin-2 alone inhibited M-NR internalisation; this is perhaps due to these dominant negative mutants not completely inhibiting the function of caveolin-1 or dynamin-2 GTPase. Another reason could be the presence of other internalisation mechanisms, which were not eliminated during M-NR uptake. Confocal microscopy

was performed to track the intracellular fate of M-NR and to demonstrate the colocalisation of M-NR within cell compartments. M-NR did not colocalise with the cell membrane marker, DiO, and entered the cytoplasm after 4 h of incubation. Even though internalised M-NR was mostly localised in the lysosome, a small amount was detected colocalised with the endoplasmic reticulum. As a result, the internalised PEG-*b*-PLA micelles were mainly delivered to the lysosome, where the load was released into the cytoplasm and became active. To gain better insight of the therapeutic effectiveness of PEG-*b*-PLA micelles as a drug-delivery system, the effects of the physico-chemical properties of PEG-*b*-PLA micelles on cellular uptake mechanisms and intracellular trafficking need to be further investigated.

Inflammation, proliferation and maturation are the steps involved in the complex process of healing wounds on the surface of biological tissues. In all of these steps, proteins (such as blood coagulation factors and growth factors) play an important role. Growth factors are proteins that control the growth, differentiation and metabolism of cells to regulate the process of tissue repair. In addition, haemostasis, which is induced by blood coagulation factors, can prevent further blood loss at the beginning of the inflammation phase. Macrophages are involved in enhancing the secretion of growth factors.

Wound-dressing materials in sheet form are used to cover wounded areas and promote wound healing. Bandages and gauze have generally served as wound-dressing materials; however, these conventional materials absorb macrophages and growth factors, which could prevent wound healing.

Chitosan (CS), a biomacromolecule obtained by deacetylating chitin, is used in many biomedical and pharmaceutical applications owing to its properties, which include biodegradability, non-toxicity and antimicrobial activity.

Acetal-terminated PEG is a modifier of CS and can be synthesised via ROP. In a recent study, 3,3-diethoxypropanol and potassium naphthalene were mixed in THF for 1 h. The purified ethylene oxide was then added to the obtained potassium 3,3-diethoxypropioxide solution and polymerisation was carried out for 48 h at 25 °C. The precipitated material was filtered and then lyophilised in benzene [81].

An acetal-terminated PEG-*b*-PLA copolymer, a micelle-forming polymer, was also synthesised via ROP. Firstly, 3,3-diethoxypropanol and potassium naphthalene were mixed in THF for 1 h. The purified ethylene oxide was then added to the obtained potassium 3,3-diethoxypropioxide solution and polymerisation was carried out for 48 h at 25 °C. After polymerisation, purified d,l-lactide was added to the solution. The resulting polymer was precipitated, stored in a freezer, centrifuged and then lyophilised in benzene [82].

Disuccinimidyl (DS)–PEG, a crosslinker of hydrogel, has been synthesised by the reaction between *N*,*N*′-disuccinimidyl carbonate (DSC) and the hydroxyl groups at both ends of PEG-diol. Firstly, DSC and triethylamine were added to anhydrous acetonitrile containing PEG-diol. The synthesised polymer was precipitated, filtered and then lyophilised in benzene. The molecular weight of the obtained polymers

was then determined using gel permeation chromatography and ^{1}H-nuclear magnetic resonance [83].

In a recent study, CS was dissolved in an acetic buffer solution. A solution of acetal-PEG in acetic buffer was then added to the CS solution (with different weight ratios) and stirred for 3 h at RT [84]. In a separate container, sodium cyanoborohydride ($NaCNBH_3$) was then added to the reaction solution and stirred for 18 h at RT. To remove the unreacted PEG, the final solution was freeze-dried, redispersed in acetone and then centrifuged. The precipitated material was again dissolved in acetic buffer solution, dialysed against water and then freeze-dried to achieve a white powder of PEG-*g*-CS.

The aldehyde groups in acetal-PEG, which is a modifier of CS, enable binding to the amino groups present on the CS backbone. Acetal-PEG-*b*-PLA can serve as a micelle-forming polymer to create aldehyde-terminated polymeric micelles, which act as crosslinkers. These micelles and DS–PEG were then used as crosslinkers due to the fact that both aldehyde groups on the surface of the polymeric micelle and the succinimidyl groups at the termini of DS–PEG can bind with the amino groups of the backbone chain of CS.

Tuberculosis (TB), one of the most deadly and contagious diseases in the world, is a chronic granulomatous bacterial infection, which is still an active infectious disease, especially in India. Multidrug-resistant TB is becoming an epidemic disease and is most prevalent in developing countries, which also has elevated rates of human immunodeficiency virus infection [85]. According to a study [86], a limited number of antibiotics, such as rifampicin (RIF), isoniazid and ethambutol, are the essential choices for clinical disease management. Among the available treatments, RIF is an important drug in the therapy of TB [87] and a number of drug-delivery systems have been developed to release RIF for the treatment of TB [88].

Using the method reported in [89], CS–PLA NP were prepared at RT. To achieve this, PLA was dissolved in dichloromethane to form a fine dispersion. This solution was quickly poured into an acetic acid solution containing CS and polyethylene oxide (PEO). The mixture was then sonicated to form an emulsion and stirred until the organic solvent fully evaporated. NP were precipitated by the addition of water and then cooling; the suspension was finally evaporated using a rotary evaporator.

The water-in-oil-in-water emulsion technique has been used to encapsulate a hydrophobic drug. An aqueous drug solution was first poured into a polymer solution containing PLA and dichloromethane to form a water-in-oil (w/o) emulsion. This emulsion was then rapidly poured into an acetic acid solution containing CS and PEO. The mixture was sonicated to form an emulsion and then continuously stirred until the organic solvent fully evaporated. NP were precipitated upon the addition of water and then cooled. Finally, NP were evaporated using a rotary evaporator. The above-mentioned procedure was repeated and PEG and gelatin were added to an appropriate portion of acetic acid solution containing CS and PEO,

followed by continuous stirring until the organic solvent evaporated. The prepared NP were: CS–PLA–RIF, CS–PLA–RIF–PEG and CS–PLA–RIF–PEG–gelatin.

Smaller NP particle sizes ensure a lower level of uptake by the reticuloendothelial system, which improves the effectiveness of the drug and reduces the side effects. The zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid closely involved with the distributed NP particle. The zeta potential is known to be affected by the electrical charge contained within the region bounded by the slipping plane; thus, it is widely used for the quantification of the charge concentration. In the case of CS–PLA–RIF, a nanorange particle size possessed a zeta potential value of approximately 21 ± 2.2 mV; upon increasing the amount of RIF coating from 10 to 50%, the particle size and zeta potential values both increased. This increase could be due to the interaction between RIF and the CS–PLA surface. At the concentration of 10% RIF, the particle size slightly increased to 192 and 197 nm upon the addition of PEG and gelatin, respectively.

As the RIF concentration increased up to 50%, a significant difference in the particle size was observed; however, as the RIF concentration got closer to 50%, the zeta potential remained constant. This could be attributed to the saturation status at the concentration of 50% RIF. In fact, an excessive amount of RIF in the solution would not change the coating strength or zeta potential of CS–PLA, CS–PLA–PEG and CS–PLA–PEG–gelatin NP.

A zeta potential value of less than −30 mV or higher than +30 mV indicates stability of the NP suspensions. In CS–PLA, CS–PLA–PEG and CS–PLA–PEG–gelatin NP, this value was above +30, demonstrating stability. The positive values obtained for zeta potential indicated that the NP surface was positively charged.

To further characterise the interaction between the CS–PLA–PEG–gelatin NP particles and RIF, samples were analysed using Fourier-Transform infrared (FTIR) spectroscopy. The FTIR spectra of CS–PLA, CS–PLA–PEG and CS–PLA–PEG–gelatin complexes at 10% RIF were determined.

CS is an amino glucose, which is characterised by a small proportion of amide groups linked to acetic acid via amide. The FTIR of CS–PLA–RIF, CS–PLA–RIF–PEG and CS–PLA–RIF–PEG–gelatin exhibited peaks at around 946 and 1,068 cm^{-1} corresponding to the saccharine structure, a strong peak at 1,635 cm^{-1} corresponding to amide characteristics, as well as a characteristic peak at around 1,539 cm^{-1} corresponding to protonated amines. The amine band shift to 1,539 cm^{-1} in the FTIR spectrum indicates a change in environment of the amine group through its interaction with gelatin. The FTIR spectra of CS–PLA–PEG–gelatin were determined at ROF concentrations of 10% and 50%. Changing the concentration of the RIF-coated polymer matrix influenced the FTIR spectra, resulting in a variation of peak intensity. The complexes were stabilised via the electrostatic interaction between the positively charged CS (NH_3^+) and negatively charged pectin (COO^-). An increasing intensity of the NH_3^+ group band was captured in the spectra of the CS–PLA–PEG–gelatin complex [90];

however, the smaller size of the peak could be due to the smaller amount of polymer entrapped in the polymer matrix.

Resveratrol (*trans*-3,40,5-trihydroxystilbene) (RSV) is an edible polyphenolic-phytoalexine, which is present in grapes, peanuts, red wine and a variety of food sources, and can help prevent numerous age-associated diseases, including cardiovascular disease, Alzheimer's and cancer [91–93]. Despite its beneficial properties, the use of RSV in therapeutic applications has been limited by its instability, poor solubility, inefficient systemic delivery and low bioavailability [94]. It has been reported that NP have been regarded as a potential alternative to glioblastoma chemotherapy and NP based on the PEG–PLA copolymer have been extensively investigated as possible drug carriers for many years [7, 8]. The blood–brain barrier (BBB) restricts the penetration of agents into the glioma if given systemically [95]. Receptors expressed on the BBB, such as the transferrin (Tf) receptor and insulin receptor, have been used for brain-targeting drug-delivery research [96]. The receptor is of particular interest because its expression is restricted to the brain capillaries, which allows the use of zeta potential to target the glioma [97–99].

Over the last decade, PEG–PLA NP have been extensively investigated for their potential as controlled and targeted drug carriers [100]. Siddigui reported that PEG– PLA NP-mediated delivery could enhance the bioavailability of chemopreventive agents for cancer control in vitro and in vivo, whilst also limiting any toxicity [101]. This study demonstrated that PEG–PLA successfully encapsulated RSV and improved the physico-chemical properties of RSV to enhance the dissolution percentage, which contributed to the particle size of NP–RSV or Tf–NP–RSV being smaller than that of free RSV.

Triblock polycarbonate (PC) polymers consisting of three critical components, including antifouling PEG, antimicrobial cationic PC and a tethering or adhesive functional block have been developed. The individual blocks were designed to have comparable lengths, which were subsequently grafted onto a prefunctionalised catheter surface via covalent bonding under mild conditions. The anchoring/adhesive functional moiety, based on a maleimide functional carbonate, was positioned at either the centre or end of the polymer block and subsequently tethered to the surface via Michael addition chemistry. The placement of the adhesive block was investigated in terms of its effect on antimicrobial and antifouling properties. The surface coated with the polymer containing the centre-positioned tethering block (2.4 k-V) was unable to prevent bacterial fouling, even though it demonstrated a higher bacteria killing efficacy in solution compared with the surface coated with the polymer containing the end-positioned tethering block (2.4 k-S). The 2.4 k-S coating effectively resisted the fouling of both Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* under conditions that simulated the device lifetime (1 week). The 2.4 k-S coating was also able to resist protein fouling and platelet adhesion and did not cause significant haemolysis; therefore, this polymer coating

shows potential for the prevention of bacterial fouling, biofilm formation and catheter-associated bloodstream infections.

Antifouling activity is one of the most important properties that catheters should ideally exhibit to prevent catheter-associated infections. To quantitatively investigate bacterial fouling on polymer-coated silicone rubber surfaces, the number of viable bacterial cells on the surfaces was measured [102]. After 1 day of incubation, there was a high number of viable *Staphylococcus aureus* and *Escherichia coli* cells on both pristine and thiol-functionalised surfaces (*Staphylococcus aureus*: 8.8 and 8.6 log colony forming units (CFU), respectively; *Escherichia coli*: 8.5 and 8.2 log CFU, respectively) (Figure 6.1).

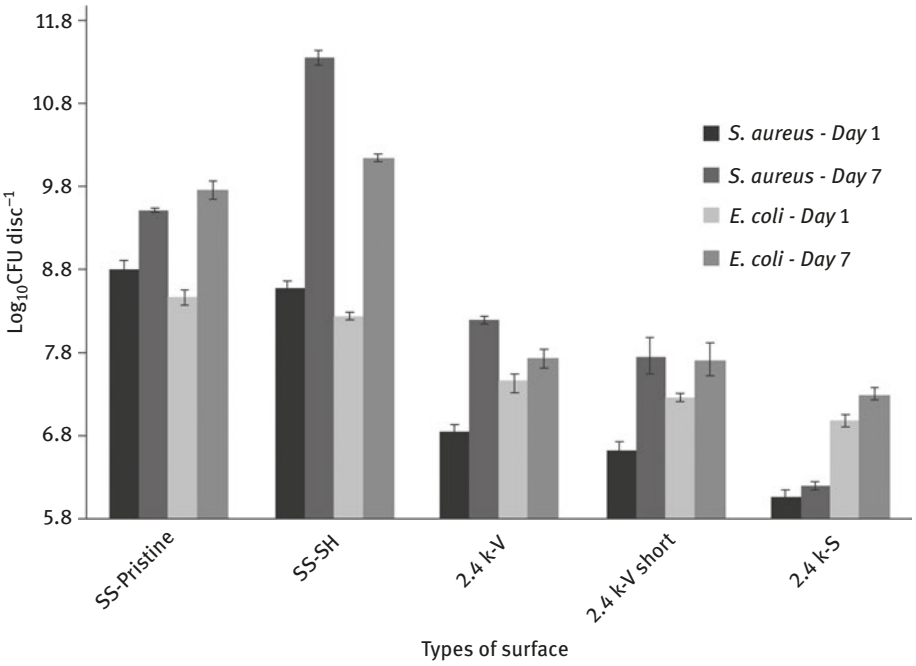

Figure 6.1: Analysis of viable surface CFU of *Staphylococcus aureus* and *Escherichia coli* at 1 and 7 days. Reproduced with permission from Z.X. Voo, M. Khan, K. Narayanan, D. Seah, J.L. Hedrick and Y.Y. Yang, *Macromolecule*s, 2015, 48, 1055. ©2015, American Chemical Society [103].

The CFU values increased significantly, especially on the thiol-functionalised surface after 7 days. The polymer-coated surfaces showed significant antifouling activity on both day 1 and day 7, with 2.4 k-S being more effective. The higher antifouling efficacy offered by the 2.4 k-S coating indicates that positioning the antifouling PEG block as the outermost layer is more effective at preventing bacteria from adhering onto the surface.

Pristine PDMS silicone and thiol-functionalised surfaces, and surfaces coated with the two polymers, were tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. With the pristine surface serving as the control, the killing efficiency of the thiol-functionalised surface and surfaces coated with the two copolymers was studied. The number of *Staphylococcus aureus* in solution decreased slightly when the solution was incubated with the thiol-functionalised PDMS surface for 24 h compared with the pristine surface, while the surfaces coated with the cationic polymers 2.4 k-V and 2.4 k-S killed 98.5 and 89.4% of the bacterial cells, respectively. In addition, there was significant reduction in the viable colonies of *Escherichia coli* in solution when incubated with the 2.4 k-V- and 2.4 k-S-coated surfaces, with killing efficacies of 93.9 and 82.5%, respectively. The 2.4 k-V polymer coating exhibited a greater killing effect against both *Staphylococcus aureus* and *Escherichia coli* in solution than the 2.4 k-S polymer coating. Unlike 2.4 k-S, where PEG shields the cationic antibacterial block, the configuration of 2.4 k-V offered easier access of the cationic block to bacteria in solution, leading to more effective eradication of the bacteria. 2.4 k-V short, containing a shorter cationic block, displayed comparable antibacterial activity in solution to 2.4 k-V (99.4 versus 98.5% against *Staphylococcus aureus*; 96.9 versus 93.9% against *Escherichia coli*), which was also more effective than 2.4 k-S. As Gram-negative *Escherichia coli* possess an additional lipopolysaccharide-containing outer membrane, which Gram-positive *Staphylococcus aureus* do not, the polymer coatings exhibited slightly lower killing efficiency against *Escherichia coli* than *Staphylococcus aureus*.

The use of antimicrobial peptides (AMP) has many advantages over the use of conventional antibiotics due to their broad spectrum of activity, selectivity and minimal development of bacterial resistance [104, 105]. The combination of a stabilised AMP with a PEG network has been developed. PEG hydrogels are intrinsically resistant to protein adsorption and cell adhesion, and thus serve as an excellent starting point in designing antimicrobial biomaterial surfaces [106, 107]. The versatility and adjustability of PEG hydrogels make them suitable for use in various biomedical applications [108]. The incorporation of AMP throughout the entire hydrogel, and not only on its surface, permits (small) unavoidable damage during, for example, surgical handling, thereby preserving its membrane-disrupting bactericidal activity.

To evaluate the bactericidal activity of the hydrogel coatings (20×20 mm^2) against *Staphylococcus aureus* (ATCC® 49230™), a slightly modified Japanese Industrial Standard (JIS) JIS Z 2801:2000 apparatus was used [109]. All samples were washed to remove any non-bound peptide by shaking in 15 mL of water for at least 16 h at 150 rpm at RT, and were subsequently sterilised in 70% ethanol and dried in a sterile environment for at least 30 min prior to bacterial inoculation. An overnight culture was diluted 100-fold with tryptic soy broth (TSB) and incubated for another 3 h to yield a logarithmically growing test bacteria (*Staphylococcus aureus* ATCC® 49230™), which was used to prepare a suspension with a concentration of 2×10^5 CFU per mL in 10 mM phosphate buffer at pH 7.0, containing 1% (v/v) TSB-peptic-tryptic (PT). Samples of hydrogel-coated polyethylene terephthalate (PET) were inoculated with 32 µL of

bacterial suspension and diluted with 32 µL of PT; sterilised parafilm (18 × 18 mm^2) (i.e., slightly smaller than that of the coated surface) was placed on top of the inoculated hydrogel coatings. As a positive control, 32 µL of 1.2 mM Ac-HHC10 in PT replaced PT. All hydrogel-coated samples containing bacteria were covered with parafilm and placed individually in wells of a 96-well plate and incubated at 37 °C for 24 h. After incubation, 1.6 mL of 0.1% Tween® 80 in phosphate buffered saline (PBS) was added to each well followed by sonication of the plate for 30 s and gentle shaking for 2 min. This procedure does not affect bacterial viability. Seven tenfold serial dilutions in PT were made in a 96-well plate. Subsequently, duplicate 10 µL aliquots of the undiluted suspension and the seven dilutions were pipetted onto blood agar plates (Oxoid, Basingstoke, UK). The blood agar plates were incubated overnight at 37 °C and the numbers of colonies were counted the following day. A similar procedure was used for testing against *Staphylococcus epidermidis* ATCC® 35984™.

Increasing the concentration of peptide in the hydrogel to 4 wt% led to increased antibacterial activity [109]. Complete killing of the bacteria was observed for 6, 8 and 10 wt% hydrogels. These results indicated a minimal peptide concentration of 6 wt% is needed for bactericidal surface activity. The activity of the bactericidal hydrogel containing 10 wt% peptide was further evaluated against *Staphylococcus epidermidis* ATCC® 32940™ over shorter incubation times. For this, a known amount of bacteria was placed on the hydrogel surface and incubated for 0, 1, 2 and 4 h (Figure 6.2).

A PET sheet was coated with a prepolymer mixture containing 0, 6 and 10 wt% *inverso*-CysHHC10. In contrast to the samples for antimicrobial testing, both sides of the PET sheet were coated to increase the total amount of hydrogel per square centimetre. Test samples (2 × 2 cm^2) were weighed and subsequently swollen for 24 h at 37 °C and weighed once more to assess the equilibrium swollen weight. These swollen samples were then transferred to three buffered solutions (pH 5.4, 7.4 and 9.4 using sodium acetate buffer, PBS and sodium carbonate buffer, respectively). Their weight was recorded after incubating for 0, 1, 2, 7 and 30 days at 37 °C. As a control to determine the remaining peptide in the hydrogel, samples were cut into two pieces and incubated in Coomassie brilliant blue for 60 min, followed by washing with destaining solution [5 mL, 8% acetic acid in ethyl alcohol (25% aqueous), 3 × 15 min]. The stained hydrogels were then decolourised using 1 mL of 3% SDS in isopropyl alcohol (50% aq) for 16 h at 37 °C. The blue-coloured solution was then diluted 10× and the absorbance was measured at 596 nm.

It was shown that the bactericidal activity of the 10 wt% hydrogel is achieved within 1 h against both strains associated with the biomaterial, whereas the bacteria concentration in contact with the blank hydrogels increased within 4 h. These results showed that AMP immobilised in a soft, hydrogel network have a sufficient degree of freedom to retain their fast-acting bactericidal properties. The covalent attachment of AMP in a hydrogel should hypothetically ensure bactericidal activity over a prolonged period of time. To verify the hydrolytic stability in vitro, three different hydrogels

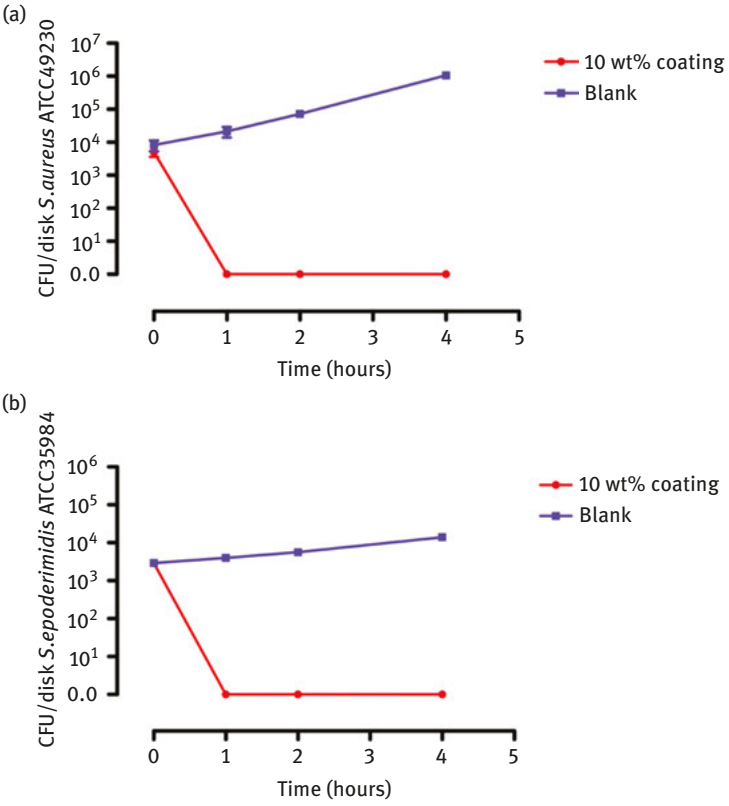

Figure 6.2: Bactericidal activity of AMP hydrogel (10 wt% and blank) against (a) *Staphylococcus aureus* ATCC® 49230™ and (b) *Staphylococcus epidermidis* ATCC® 39584™ at t = 0, 1, 2 and 4 h. Reproduced with permission from R.T.C. Cleophas, J. Sjollema, H.J. Busscher, J.A.W. Kruijtzer and R.M.J. Liskamp, *Biomacromolecules*, 2014, 15, 3390. ©2014, American Chemical Society [109].

containing 0, 6 and 10 wt% of *inverso*-CysHHC10 were incubated in buffered solutions. Under both acidic and physiological conditions, no degradation was observed after 30 days at 37 °C for all hydrogels. However, susceptibility of the ester linkage to hydrolytic degradation was expected. To verify this event, an accelerated degradation assay was carried out in a basic (pH 9.4) buffered solution at 37 °C for 30 days. In contrast to the previous acidic and physiological conditions, complete loss of integrity was observed for all hydrogels in a basic environment (Figure 6.3).

6.3 Conclusion

PLGA have been shown to decrease ($P < 0.05$) the concentration of fruit extracts required to inhibit pathogens and can be successfully used for nanoencapsulation to

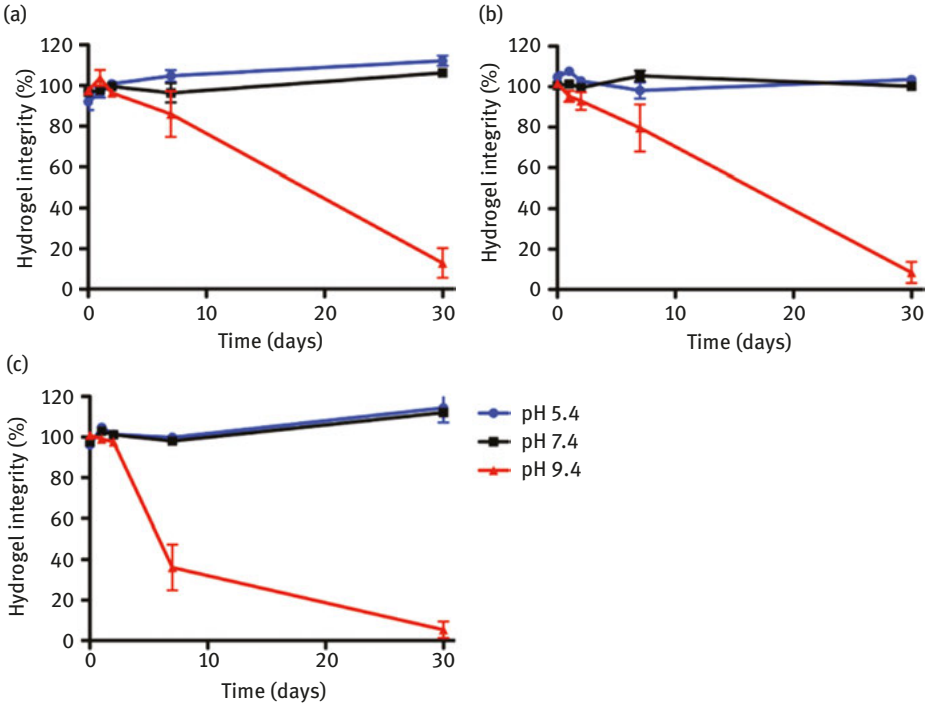

Figure 6.3: Effect of pH buffered solutions (5.4, 7.4 and 9.4) on the integrity of blank (a), 6 wt% (b) and 10 wt% (c) hydrogel over 30 days. Reproduced with permission from R.T.C. Cleophas, J. Sjollema, H.J. Busscher, J.A.W. Kruijtzer and R.M.J. Liskamp, *Biomacromolecules*, 2014, 15, 3390. ©2014, American Chemical Society [109].

deliver these natural antimicrobials to pathogens in food systems. Nanoencapsulation of fruit by-product extracts with PLGA provides an opportunity to expand the fundamental exploitation of fruit by-products, which are rich in phytochemicals, and enhance their antimicrobial activity by enabling quick aqueous dispersion and improved physico-chemical properties (controlled release and reduced particle size). Lactic acid showed antimicrobial activity against the primary bacterial vaginosis (BV) pathogen *Gardnerellavaginalis* with an MIC of 3.6 mg/mL, in addition, PEG nanocarrier-based hydrogels are used for the vaginal administration of lactic acid to prevent and treat BV. Many petrochemical-based polymers have recently been substituted by PLA for almost all pharmaceutical and direct food contact packaging materials because of its safety, biodegradability and ability to be custom modified. Sheet-formed materials have a large contact area relative to the drug-targeted site, leading to advantages over conventional particle-shaped drug carriers found in biomedical applications. CH_3O-PEG-*b*-PLA can self-assemble and form stable micelle-like w/o emulsions with a hydrophilic (not hydrophobic) inner core in organic solvents. Elucidation of the

endocytosis and intracellular trafficking of PEG-*b*-PLA micelles is likely to provide a clue for understanding the diverse functions of this copolymer to develop more effective drug-delivery systems. Tf-modified PEG–PLA is used as the carrier of RSV and this polymeric delivery-system is used to target brain tumours. Tf–NP–RSV have the potential to treat glioblastoma due to two reasons; the main reason is that Tf conjugated to NP results in the transport of more therapeutic agents to the glioma site; in addition, maintaining the NP size at around 150 nm is essential for improving the pharmacokinetics and is advantageous for endocytosis by brain capillary endothelial cells, as demonstrated elsewhere.

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7 Conducting polymers with antimicrobial activity

Abstract: Conducting polymers (CPs) are known for their diverse applications in many fields, including biomedical applications such as drug delivery and antimicrobial polymers. The most commonly known CPs is polyaniline (PANI), polypyrrole (Ppy), polythiophene (PTh) and polyacetylene. PANI is used in biomedical applications because of its insolubility in common laboratory solvents, thereby it is difficult to degrade. f-PANI-based polymers are potentially well suited for use as biocompatible scaffolds for tissue engineering and as antimicrobial wound dressings, with the advantage of being able to kill microbes without the use of antiseptics such as iodine or silver. In this chapter, we have discussed about antimicrobial properties associated with PANI, Ppy and Pth.

Keywords: Conducting polymers, polyaniline, polypyrrole, polythiophene, antimicrobial activity

7.1 Introduction

The discovery of conducting polymers (CP) has given a new dimension to the present era of polymer applications. Polymers, to date, are known as a class of heat sensitive [1], flexible and electrically insulating amorphous materials [2]; the presence of covalent bonds in saturated carbon compounds is responsible for their insulating property [3]. As desirable properties for a wide range of applications can be conveniently attained by tailoring the polymer structure and by incorporating additives, they are the focus of considerable research to determine the possibility of transforming insulating polymers into conducting or semiconducting materials, which display special characteristics, such as low density, ease of fabrication, flexibility of design and low energy and labour requirement for fabrication and processing [4]. In addition, the use of polymeric antimicrobial agents have several advantages, including they are non-volatile, chemically stable, do not easily permeate through the skin of man or animal and may enhance the efficacy of some existing antimicrobial agents whilst minimising the environmental problems accompanying the residual toxicity of these agents, in addition to prolonging their lifetime [5, 6]. Therefore, they can reduce manufacturing losses associated with volatilisation, photolytic decomposition and transportation. In the field of biomedical polymers, infections

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associated with biomaterials represent a significant challenge to the more widespread application of medical implants [7–9]. In particular, interest has been focused on functional polymers and CP due to their diverse applications in many fields, for example, biomedical applications including drug delivery and antimicrobial polymers. Functional CP have potential advantages over smaller analogous molecules and their usefulness is related to both the functional groups and polymeric nature; the characteristic properties of these polymers depend on the extraordinarily large size of their molecules [10–13].

In the mid-1970s, polyacetylene was reported by Shirakawa to be the first polymer capable of conducting electricity [5]. Since the late 1970s, a large number of polymers have been added to the list of CP such as polypyrrole (Ppy) [14], polythiophene (PTh) [15], polyacetylene [16], polyaniline(s) (PANI) [17] and so on.

7.2 Antimicrobial activity of conducting polymers

7.2.1 Polyaniline

PANI is a typical phenylene-based polymer, which has a chemically flexible –NH-group in its polymer chain flanked on either side by phenylene rings. The protonation, deprotonation and various other physico-chemical properties of PANI are due to the presence of this –NH-group.

PANI is different from other CP in three important aspects: (i) the charge conjugation in PANI is not symmetrical, that is, the Fermi level and band gap are not formed in the centre of the band, and hence the valence band and conjugation bands are quite asymmetric, (ii) carbon rings and nitrogen atoms are both within the conjugation path; this conjugation differs from Ppy as the hetero atoms contribute significantly to the π-bond formation and (iii) the electronic state of PANI can be changed through variation of either the number of electrons or number of protons. When an electroactive polymer is oxidised or reduced, it displays a significant change in intrinsic electrical conductivity, for example, PANI and PTh; these are also known as electroconductive polymer/conducting electroactive polymers. The earliest reported application of CP was the use of free-standing polymers as chemical sensor devices, which are analytical devices that convert the chemical potential energy of a targeted analyte into a proportionate measurable signal, usually electrical or optical.

There have been a number of reviews of CP with regard to their biomedical applications, for example, the commercially available soluble CP PANI grafted to lignin, and PANI sulfonic acid and Ppy are effective scavengers of the stable 1,1-diphenyl -2-picrylhydrazyl (DPPH) free radical. This property may be particularly beneficial in tissues suffering from oxidative stress, where the ability to lower the excessive levels

of reactive radical species is desirable. This free-radical reducing ability of CP was revealed by the efficient scavenging of DPPH radicals, with two or four radicals scavenged per aniline or pyrrole monomer unit during the 30 min test period of the DPPH assay, indicating the potential for CP to be effective antioxidants when present in a biological medium. The various vitamins and polyphenol free-radical scavenging antioxidants present in beverages, fruits and vegetables are currently of great interest, as these antioxidants may offer protection against various diseases, such as cardiovascular diseases and cancer [18].

The emeraldine salt forms of copolymers appear to be more antimicrobially effective than the emeraldine base forms of the same copolymer. It seems that the presence of an acidic functional group (–COOH) in the polymer chain improves the antibacterial efficacy of the copolymer. This could, in theory, be due to the acidic dopants on the molecular chains of the copolymers reacting with the bacteria (or other relevant microbial organism), which result in microbial death. Alternatively, it could be due to the electrostatic adherence between copolymer molecules and the bacteria, which carry charges of different polarity, the bacteria walls may break and the bacterial contents become exposed or leak out, which cause the bacteria to die [19].

The antimicrobial properties of conductive functionalised-polyaniline (f-PANI) have been investigated by exploring their interaction with different bacteria. It was observed that low concentrations of PANI strongly inhibited the growth of wild-type *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, as well as several antibiotic-resistant clinical pathogens [20].

Nanofibres of PANI combined with fluconazole have been prepared via a simple and cost-effective sol-gel method using d-10-camphorsulfonic acid as a dopant and surfactant, and ammonium persulfate as the oxidant. The synthesised nanostructured material was dissolved in dimethylsulfoxide (DMSO) at different concentrations and tested for its antifungal properties against *Candida albicans*, *Candida tropicalis* and *Candida krusei*. The results showed that, compared with nanofibre structured conducting PANI, PANI-doped with fluconazole displayed higher antifungal activity against all the species tested. It is very evident that PANI-doped fluconazole exhibited considerably enhanced antifungal activity; *Candida tropicalis* is more susceptible than *Candida albicans* and *Candida krusei* [21].

PANI-zirconium (IV) sulfosalicylate has also been tested against various bacterial (*Escherichia coli*, *Bacillus thuringiensis* and *Pseudomonas aeruginosa*) and fungal strains (*Aspergillus niger*, *Fusarium oxysporum* and *Penicillium chrysogenum*); relatively higher activities were observed compared with well-known antibiotics [22].

PANI-*g*-chitosan (CS) has been screened for its antimicrobial activity against *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Escherichia coli*, *Candida albicans*, *Candida tropicalis* and *Candida krusei*. The results of the antimicrobial activity of PANI and PANI-*g*-CS were assessed based on the average diameter of zones of inhibition (ZOI). The results confirmed that PANI-

g-CS has an enhanced antimicrobial activity compared with PANI. PANI and PANI-*g*-CS also show greater antifungal activity than antimicrobial activity [23].

The antibacterial properties of silver/polymethyl methacrylate (PMMA) nanofibres against both Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria were evaluated using the modified Kirby–Bauer method to assess the minimum inhibitory concentration (MIC), and a kinetic test. The MIC test demonstrated that the silver/PMMA nanofibres had enhanced antimicrobial efficacy compared with that of silver sulfadiazine and silver nitrate at the same silver concentration [24].

Boomi and co-workers [25] studied pristine PANI, PANI–silver, PANI–gold and PANI–silver–gold nanocomposites, which were successfully synthesised via the chemical oxidative polymerisation method using aniline as the monomer, ammonium persulfate as the oxidant and metal (silver, gold and silver–gold) colloids. Pristine PANI, PANI–silver, PANI–gold and PANI–silver–gold nanocomposites were tested for antibacterial activity using the agar well diffusion method. The PANI–silver–gold nanocomposite exhibited higher antibacterial activity against both Gram-positive bacteria (*Streptococcus* sp. [MTCC 890], *Staphylococcus* sp.) and Gram-negative bacteria (*Escherichia coli* [MTCC 1671] and *Klebsiella* sp. [MTCC 7407]) than the PANI–silver nanocomposite, PANI–gold nanocomposite and pristine PANI. The novelty of this study is the polymer–bimetal synthesis and its antibacterial potential.

Hussaini and Eldars [26] reported an investigation into the copolymers of aniline and *o*-phenylenediamine (*o*-PDA)/hydrophilic bentonite nanocomposites, which were synthesised by 5:1 M ratios of the respective monomers with different percentages of nanoclay via modified in situ chemical copolymerisation. The antibacterial activities of PANI–*o*-PDA/bentonite nanocomposites against both Gram-positive and Gram-negative bacteria were assessed.

Lashkenari and Eisazadeh [27] reported a study, which described the preparation of a colloidal PANI/polyvinyl alcohol (PVA) nanocomposite via the chemical polymerisation of aniline in the presence of ammonium peroxydisulfate as an oxidant and PVA as a stabiliser. The prepared polymer was then tested for antibacterial properties against the Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and Gram-positive bacteria, *Staphylococcus aureus*. The antibacterial properties were assessed using the disc diffusion, MIC, minimum bactericidal concentrations (MBC) and bactericidal effect methods. The MIC results clearly showed that the colloidal PANI/PVA nanocomposite strongly inhibited the growth of wild-type *Escherichia coli* (19 ± 0.5 mm) followed by *Pseudomonas aeruginosa* (17 ± 0.5 mm) and *Staphylococcus aureus* (17.5 ± 0.5 mm) bacteria. *Staphylococcus aureus* was completely killed after exposure for only 15 min, whereas *Escherichia coli* was completely killed after exposure for 25 min.

Ma and co-workers [28] reported the synthesis of graphene/zinc ferrite/PANI composites, which are photocatalytic inorganic antibacterial agents that show great potential and are prepared via in situ polymerisation and characterised using modern testing technology. The antibacterial properties of the samples were

investigated against *Staphylococcus aureus, Escherichia coli* and *Candida albicans* under daylight conditions. The results showed that the antibacterial activity of the samples was influenced by the strain and the mass ratio of PANI to graphene/zinc ferrite ($m_{P/GZ}$) in the graphene/zinc ferrite/PANI composite. The sample with an $m_{P/GZ}$ value of 0.6 exhibited the best antibacterial activity with an MIC of 12.5 μg/mL for *Candida albicans*; moreover, the antibacterial mechanisms were discussed.

Boomi and co-workers [29] reported the synthesis of a platinum colloid and platinum– palladium colloid, pristine PANI, PANI/platinum nanocomposite and PANI/platinum– palladium nanocomposite, which were synthesised using a simple chemical method. The synthesised materials were evaluated for antibacterial activity using MIC and MBC. The results indicated that the nanocomposites exhibited improved antibacterial activity in comparison to pristine PANI and individual metal colloids. This is the first report of the chemical synthesis of a PANI/platinum–palladium nanocomposite, which exhibits antibacterial activity at micromolar concentration levels.

Pathania and co-workers [30] synthesised a PANI zirconium (IV) silicophosphate (ZSP) nanocomposite ion exchanger using the sol-gel method by mixing PANI gel into the inorganic precipitates of ZSP. The PANI–ZSP nanocomposite ion exchanger was utilised as a photocatalyst for the removal of methylene blue dye from water; a degradation efficiency of 82% was achieved after 2 h of exposure. The nanocomposite ion exchanger has been successfully used as an antibacterial agent against *Escherichia coli*.

Developing an efficient nanocomposite possessing antimicrobial efficacy against a broad spectrum of microbes, including bacteria, fungi and an algal consortium, which pose serious challenges to human survival, remains a challenge. Looking at this aspect of nanomaterials, Pramanik and co-workers [31] reported the fabrication of biobased hyperbranched polyester amide/PANI nanofibre modified montmorillonite (MMT) nanocomposites via an ex situ polymerisation technique at varied weight percentages (1.0, 2.5, 5.0 wt%) of the modified MMT (nanohybrid). Antimicrobial activity of the nanocomposites against a spectrum of bacterial and fungal strains, and a consortium of algal species, along with other desired performance characteristics confirmed them as potent antimicrobial materials with potential applications in the health and biomedical industries.

Farias and co-workers [32] reported the synthesis of thin hybrid films made of PANI and a ceramic used in the technological industry, titanium dioxide, which were prepared via the layer-by-layer self-assembly technique. Aiming to improve the dispersion of the ceramic in the polymer matrix, the commercial surfactant, cetyltrimethylammonium bromide (CTAB), was used during the formation of the films. The best conditions for deposition resulted in synergic interactions between the conjugated materials. The antibacterial activity of the PANI (titanium dioxide)/CTAB films was studied and the obtained results suggest their use as antimicrobial coatings.

The use of PANI in biomedical applications is often limited due to its insolubility in common laboratory solvents, thereby making it difficult to process [33]. It has been reported that the insolubility of PANI in most common solvents can be circumvented, to some extent, by copolymerising aniline with substituted anilines, which impart solubility to the resulting f-PANI-based polymers [34–39]. More importantly for current purposes, however, even though f-PANI typically have lower conductivities than PANI, they are moderately more soluble (depending on the functional group) in common solvents, including *N*-methyl-2 pyrrolidone, dimethylformamide, DMSO, tetrahydrofuran, pyridine and in some cases, water, thereby increasing the flexibility of PANI for use in biological applications.

Gizdavic-Nikolaidis and co-workers [40] recently demonstrated that poly(aniline-*co*-3-aminobenzoic acid) could be electrospun from solution blends containing polylactic acid to form nanofibrous mats. In turn, these mats allowed attachment and proliferation of mammalian cells while maintaining their antibacterial potency. These results also prompted the authors to suggest that these novel conductive f-PANI-based materials are potentially well suited for use as biocompatible scaffolds for tissue engineering and as antimicrobial wound dressings, with the advantage of being able to kill microbes without the use of an antiseptic such as iodine or silver. The molecular structures of PANI and f-PANI change after interaction with *Escherichia coli* and *Staphylococcus aureus* bacteria. The antibacterial effects of aniline-based CP were explained via the electrostatic adherence between the polymer molecules and bacteria, which carry opposite charges, hence the walls of the bacteria break down and the intracellular fluid leaks out, causing death. Poly(aniline-*co*-3-aminobenzoic acid) emeraldine salt was used to explore the bactericidal activities of f-PANI, as it proved to be one of the more effective aniline copolymers in terms of inhibiting microbial growth. The bacterial strains exhibited different susceptibilities, with *Pseudomonas aeruginosa* being completely killed after exposure for only 15 min, whereas *Staphylococcus aureus* and *Escherichia coli* were completely killed after exposure for 45 and 180 min, respectively. It is worth noting that the suggested mode of action of cell death by CP is via disruption of the microbial cell walls and membranes as a result of electrostatic contact [41] . Along this line, CP, such as f-PANI, appear to share a similar mode of killing with other more potent antimicrobial compounds, in particular, cationic antimicrobial peptides that appear to act by disruption of and insertion into anionic bacterial membranes, thereby compromising membrane integrity and cell division, leading to cell lysis and death [42, 43]. It has been observed that growth inhibition zones are typically visible when *Escherichia coli* and *Pseudomonas aeruginosa* are allowed to grow overnight in the presence of f-PANI.

Recently, for antibacterial activity of polyaniline and polyaniline/Ag nanocomposites has been synthesised by in situ polymerization. In antibacterial analysis, it has been observed that *E. coli, K. pneumoniae, P. aeruginosa* and *S. aureus* bacteria does not form colonies after 24, 48 and 72 h with above synthesised conducting

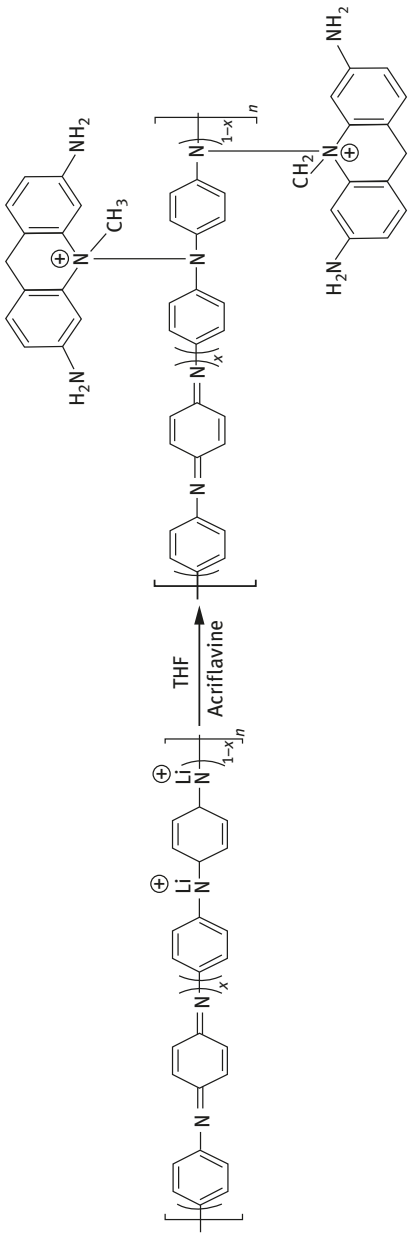
polymers. Similarly, film sample of polyaniline/Ag does not form inhibition zones with *E. coli* and *K. pneumonia* while *S. aureus* and *P. aeruginosa* form inhibition zones with 4wt% Ag content film. As a result it has been found that film sample having 4wt% Ag content and showed good antibacterial activity as compared to PANI [44].

PANI and poly(3-aminobenzoic acid) (P3ABA) are novel antimicrobial agents as non-leaching additives to provide contamination resistant surfaces. The activity of PANI and P3ABA has been investigated in suspension and as part of absorbent and non-absorbent surfaces. The effect of inoculum size and the presence of organic matter on surface activity have been determined. PANI and P3ABA both demonstrated bactericidal activity against *E. coli* and *S. aureus* in suspension and as part of an absorbent surface and P3ABA shows greater activity as compared to PANI after a 24 h treatment. As a result, it has been conclude that P3ABA used to create contamination-resistant surfaces [45].

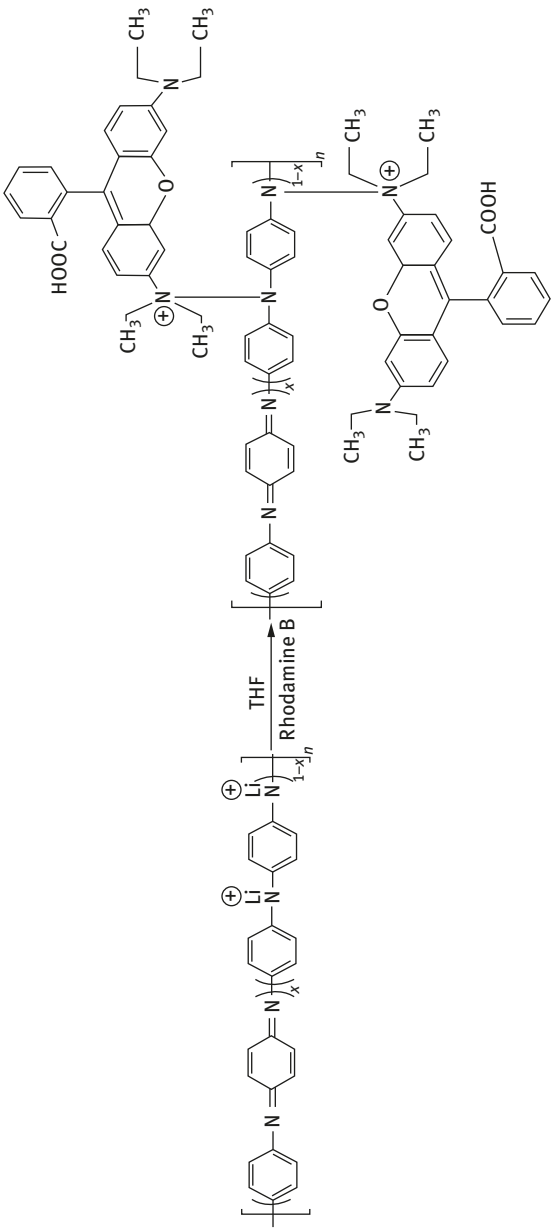
Azo dyes are known for their medicinal importance and are widely recognised for their use in several therapeutic areas, including antineoplastics [46], antidiabetics [47], antiseptics [48], antibacterial [49] and antitumour. They are known to be involved in a number of biological reactions such as the inhibition of deoxyribonucleic acid (DNA), ribonucleic acid, protein synthesis, carcinogenesis and nitrogen fixation. Azo dyes are important compounds in the medical and pharmaceutical fields and it has been suggested that the azoimine linkage might be responsible for the biological activities displayed by some Schiff bases [50]. In addition, evans blue and congo red azo dyes have been studied for their role in inhibiting the viral replication of the human immunodeficiency virus. This effect is believed to be caused by azo dyes binding to both protease and reverse transcriptases of this virus [51]. The existence of an azo moiety in different types of compounds is the source of their antibacterial and antifungal activities. In recent times, the exploration of azo dyes as antimicrobial agents has received considerable attention [52].

Dye-substituted PANI were synthesised via the oxidative polymerisation of aniline [53, 54]. It was observed that the dye-substituted PANI showed reasonably good antibacterial and antifungal activity compared with different individually used acid-doped PANI and dyes (Schemes 7.1–7.3).

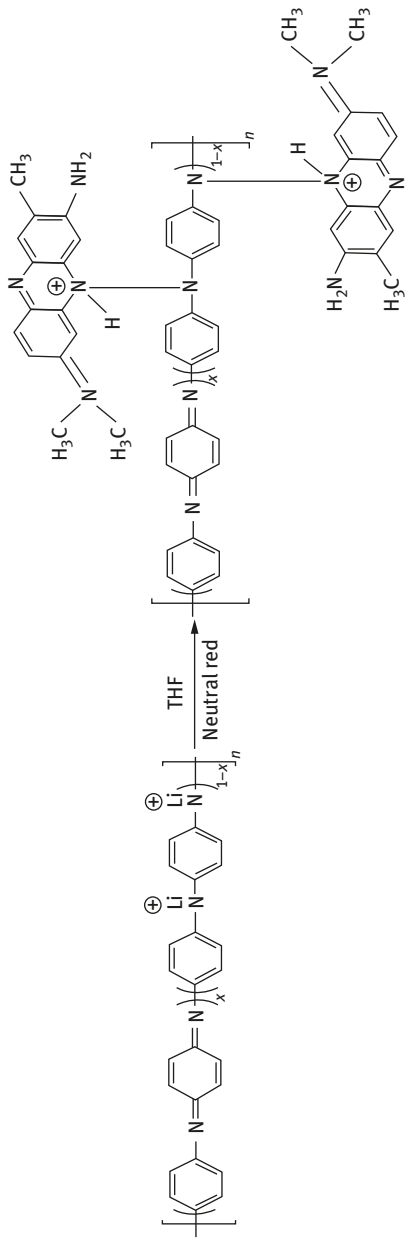
The antimicrobial activity of different PANI were studied in different concentrations (5, 25, 50, 100 μg/mL) of four pathogenic bacterial strains (*Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenus*) and three fungal strains (*Candida albicans, Aspergillus niger* and *Aspergillus clavatus*). The antibacterial and antifungal activity of orthophosphoric-doped PANI, acriflavine-substituted PANI, rhodamine-B-substituted PANI and neutral-red-substituted PANI were assessed in terms of MIC, which is the lowest level of antibiotic in a culture media that will prevent microbial growth while ZOI is the area around an antibiotic disc that has no bacterial growth. To find the MIC, incubate bacteria in decreasing concentrations of antibiotic, determine the lowest concentration of



Scheme 7.1: Synthesis of acriflavine-substituted PANI.



Scheme 7.2: Synthesis of rhodamine-B-substituted PANI.



Scheme 7.3: Synthesis of neutral-red-substituted PANI.

antibiotic that exhibits microbial growth and the dilution just before the MIC. Dye-substituted PANI showed very good antimicrobial activity compared with ortho-phosphoric-acid-doped PANI. The abstraction of hydrogen atoms from the benzenoid ring by butyl lithium and further attachment of dye moieties enhances antimicrobial activity, which may be attributed to the differing extent of charge delocalisation and alteration in chemical structure and conductivity. The presence of quaternary ammonium cations further affects antimicrobial activity. It is clear from the above-mentioned results that the possible causes for the enhanced antimicrobial activity (Figures 7.1 and 7.2), compared with acid-doped PANI chains, are the delocalisation of electron density in the side groups and presence of quaternary ammonium ions.

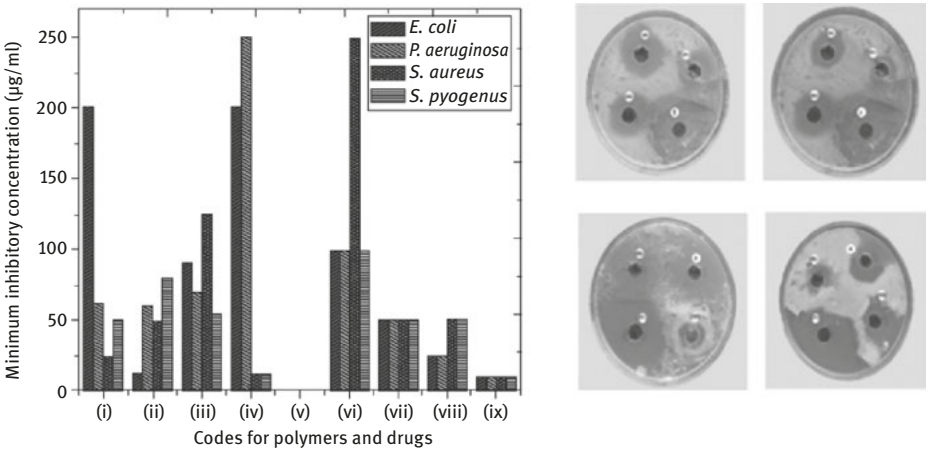


Figure 7.1: Antibacterial activity against (1) *Escherichia coli*, (2) *Pseudomonas aeruginosa*, (3) *Staphylococcus aureus* and (4) *Streptococcus pyogenes*. Codes for polymer–drug combinations used: (i) orthophosphoric-acid-doped PANI, (ii) acriflavine-substituted PANI, (iii) rhodamine B-substituted PANI, (iv) natural-red-substituted PANI, (v) gentamycin, (vi) ampicillin, (vii) chloramphenicol, (viii) ciprofloxacin and (ix) norfloxacin. Reproduced with permission from N.K. Jangid, N.P.S. Chauhan and P.B. Punjabi, *Polymer Bulletin*, 2014, 71, 2611. ©2014 Springer [53].

7.2.2 Polypyrrole

Seshadri and Bhat were the first to report the antibacterial activity of π-conjugated polymers (i.e., Ppy and PANI) [55]. Conjugated polymers, also called intrinsically conducting polymers (ICP), are employed in the textile field due to their electrical properties [56]. ICP can be produced via chemical oxidative polymerisation from water solutions of the monomers. Solid surfaces placed in the polymerisation bath

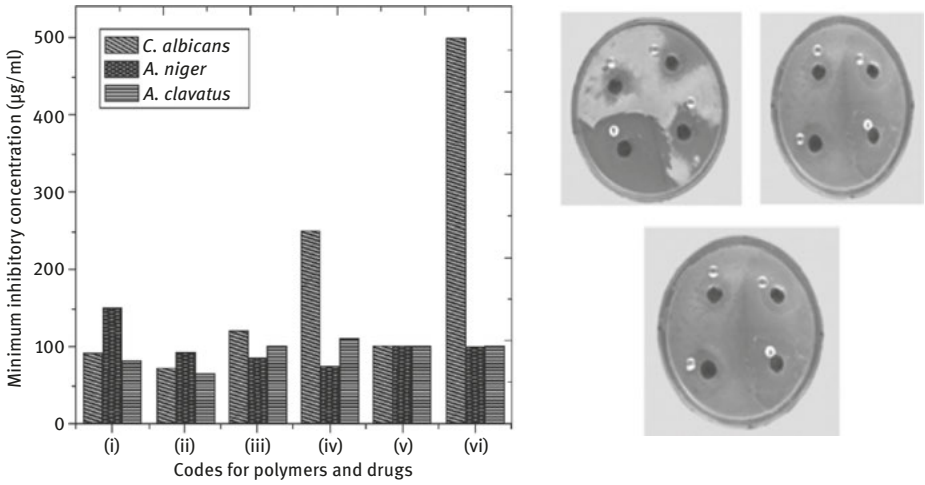


Figure 7.2: Antifungal activity against (1) *Candida albicans*, (2) *Aspergillus niger* and (3) *Aspergillus clavatus*. Codes for polymer–drug combinations used: (i) orthophosphoric-acid-doped PANI, (ii) acriflavine-substituted PANI, (iii) rhodamine-B-substituted PANI, (iv) natural-red-substituted PANI, (v) nystatin and (vi) greseofulvin. Reproduced with permission from N.K. Jangid, N.P.S. Chauhan and P.B. Punjabi, *Polymer Bulletin*, 2014, 71, 2611. ©2014 Springer [53].

are spontaneously coated with a film-like dense layer of ICP. Synthesis via oxidative polymerisation produces positive charges along the backbone chain of Ppy. In Ppy, one positive charge is formed for each three to five repeat units. Positive charges are counterbalanced by counterions (also called dopants), namely anions present in the polymerisation solution that are embedded in the polymer matrix [57]. The positive charges appear to be responsible for the antibacterial activity of these types of polymers.

The antibacterial efficacy of Ppy deposited on cotton fabrics was quantitatively evaluated under dynamic contact conditions according to the American Society for Testing and Materials (ASTM), ASTM E2149-01. Moreover, the bactericidal mechanism of Ppy on *Escherichia coli* bacteria was preliminarily investigated [58].

Prior to washing, fabrics coated with Ppy(Cl) and Ppy dicyclohexyl sulfosuccinate (DSS) ions showed 100% reduction of bacteria, while untreated cotton fabric had practically no antibacterial activity. After dry-cleaning, Ppy(Cl) and Ppy(DSS + Cl) fabrics showed high bacterial reductions at 99 and 98%, respectively.

Antibacterial efficacy decreased after non-ionic and anionic laundering of the fabric. In particular, the antibacterial activity of Ppy(Cl) samples was severely degraded by anionic laundering. The antibacterial activity of Ppy has been investigated against *Streptococcus pneumoniae* (RCMB 010010), *Enterococcus faecalis* (RCMB 010068) and *Staphylococcus aureus* (RCMB 010028), that is, Gram-positive bacteria. The agar disc diffusion method was used to determine the antibacterial

activity and ampicillin was used as a reference drug against Gram-positive bacteria. Ppy showed in vitro antibacterial activity against the tested bacteria [59] . The antibacterial activity results of the fabricated polymer, using the inhibition zone method, are listed in Table 7.1.

Table 7.1: Antimicrobial activity of Ppy against test microorganisms using the inhibition zone method.

Gram-positive bacteria	Ppy (mm)	Standard (mm)
		Ampicillin
Streptococcus pneumoniae	15.8 ± 0.58	23.8 ± 0.2
Staphylococcus aureus	15.0 ± 1.22	28.3 ± 0.1
Enterococcus faecalis	14.9 ± 0.63	20.3 ± 0.3

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As shown in this table, the prepared polymer showed better antibacterial activity due to the presence of the –NH group and aromatic ring, thus increasing its partially cationic character [60, 61], that is, the positive charge was strengthened, leading to better antibacterial activity [62]. Ppy showed higher antibacterial activity against the Gram-positive bacteria and caused inhibition zone diameters of 15.8 ± 0.58, 14.9 ± 0.63 and 15.0 ± 1.22 mm for *Streptococcus pneumoniae, Enterococcus faecalis* and *Staphylococcus aureus*, respectively. This may be attributed to the cell wall of Gram-positive bacteria being completely composed of peptide polyglycogen. The peptidoglycan layer is composed of networks with many pores, which allow easy access of foreign molecules into the cell whilst also allowing more rapid absorption of ions into the cell.

Additional evidence for the activity of Ppy against Gram-positive bacteria comes from their MIC values. MIC is defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. The MIC values of Ppy against *Streptococcus pneumoniae, Enterococcus faecalis* and *Staphylococcus aureus* were 62.5, 125 and 125 mg/mL, respectively, and are presented in Table 7.2.

MIC values confirmed the noticeable antibacterial activity of the fabricated polymer by the response of *Enterococcus faecalis, Streptococcus pneumoniae* and *Staphylococcus aureus*. The antimicrobial properties of pure nanofibrillated cellulose (NFC) coated with Ppy–silver composite (Scheme 7.4) films were tested on yeast (*Candida albicans*), a Gram-negative bacterium (*Salmonella* serovar Infantis) and a Gram-positive bacteria (*Listeria monocytogenes* and *Staphylococcus aureus*) (Table 7.3) [63].

The degree of antimicrobial activity against the representative yeast and bacterial strains was determined by checking the inhibition of growth zones around the sample

Table 7.2: Antimicrobial activity as MIC (μg/mL) against test microorganisms.

Gram-positive bacteria	Ppy	Standard
		Ampicillin
Streptococcus pneumonia	62.5	0.24
Staphylococcus aureus	125	0.03
Enterococcus faecalis	125	3.9

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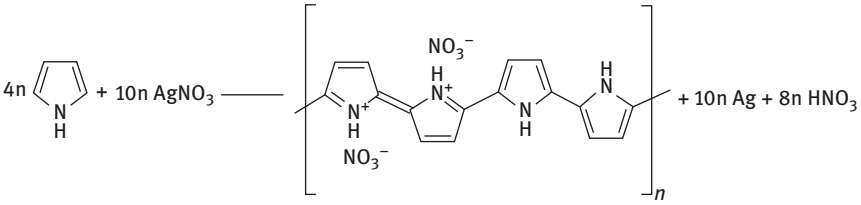

Scheme 7.4: Oxidation of pyrrole with silver nitrate to electrically conducting Ppy–nitrate.

Table 7.3: Antimicrobial effects of composite films against *Candida albicans, Listeria monocytogenes, Staphylococcus aureus* and *Salmonella serovar* Infantis.

Sample	*Candida albicans*	*Listeria monocytogenes*	*Staphylococcus aureus*	*Salmonella serovar* Infantis
NFC	–	–	–	–
NFC/Ppy	–	±	–	–
NFC/Ppy–silver (20)	–	+	+	–
NFC/Ppy–silver (80)	–	++	+	–

–: No zone of inhibition around the sample;
±: Just discernible growth inhibition;
+: A distinct zone of inhibition of approximately 2 mm around the sample; and
++: An inhibition zone of ≥3 mm around the sample.
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edges. The NFC film did not show any inhibition against the tested microorganisms. The NFC/Ppy composite film exhibited antimicrobial activity against *Listeria monocytogenes*, but no inhibition of *Candida albicans*, *Staphylococcus aureus* or *Salmonella* serovar Infantis. The incorporation of silver nanoparticles (NP) into the NFC/Ppy

composite, induced clear antimicrobial activity (inhibition zone of approximately 2 mm) against the Gram-positive bacteria *Listeria monocytogenes* and *Staphylococcus aureus*, even for the NFC/Ppy–silver NP film, which showed little antimicrobial activity. Increasing the concentration of silver NP increased the inhibition level further against *Listeria monocytogenes* (inhibition zone ≥3 mm). Ppy–silver nanocomposites on cotton fabrics [64] and individual fibres of cellulose [65] have also shown substantial antimicrobial properties against *Staphylococcus aureus*, a common cutaneous bacterium and pathogen. The antimicrobial effect of NFC/Ppy–silver composites against the growth of *Staphylococcus aureus* makes the NFC/Ppy–silver composite a potential candidate for wound-healing applications.

The effects of nickel oxide/Ppy/silver nanocomposites on the growth of four different bacteria, that is, *Pseudomonas aeruginosa*, *Bacillus circulans*, *Escherichia coli* and *Staphylococcus aureus* have been determined. Antimicrobial activity was confirmed in the test nanocomposites containing 2% and 3% silver NP, which showed a very large spectrum of antimicrobial activity against all the tested bacterial strains even though the concentration of silver NP was very small, that is, 0.02 g and 0.03 g/mL in the respective suspensions. All the test nanocomposites exhibited high antimicrobial activity against the four different bacteria used; however, the test nanocomposite containing 3% silver showed better antimicrobial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. The antimicrobial activity test carried out against these different kinds of bacterial strains is for qualitative and comparative purposes only. Hatchert and Henry [66], and Basu and co-workers [67] reported that cell damage may be caused by the interaction of silver NP with phosphorous- and sulfur-containing compounds, such as DNA in the cell; silver tends to have a high affinity for such compounds. Silver+ ions strongly interact with the available –SH groups of the cellular biomolecules [68] and disturb its essential cellular functions such as permeability and respiration. Such interactions in the cell membrane would prevent DNA replication [69], which would lead to bacterial death [70]. The binding of the NP particles to bacteria depends on the size of the NP particle and surface area available for the interaction; in this experiment, the synthesised silver NP were small; hence, they could easily reach the nuclear content of the bacteria resulting in a larger bactericidal effect than bigger particles. It has been reported that all the Gram-positive and Gram-negative bacteria were very sensitive to silver-encapsulated nickel (II) oxide/ Ppy/silver nanocomposite particles. These polymer-supported metal and metal oxide nanocomposites exhibit properties, which are applicable to filtration processes as *Escherichia coli* and *Staphylococcus aureus* are mainly found in contaminated water and this core–shell nanocomposite exhibited effective antimicrobial activity against these microbes. Moreover, the catalytic oxidation mechanism of silver was responsible for inactivating *Escherichia coli* and *Staphylococcus aureus*. The results, confirmed by antibacterial testing, provide evidence to suggest that the silver NP adsorbed to the surface of the nickel (II) oxide/Ppy core–shell and were active against bacteria; hence, this core–shell

nanocomposite can provide an environmental friendly and cost-effective alternative to water filtration processes [71].

The antibacterial activity of the Ppy-*g*-CS copolymer was found to increase upon increasing its concentration and showed stronger activity against bacteria than penicillin (10 mg), rifampicin (RIF) (5 mg) and trimethoprim (25 mg), whereas it showed equipotent activity with the antibiotics amikacin (30 mg) and erythromycin (15 mg). To compare the antibacterial activity of Ppy-*g*-CS with CS and Ppy, the samples were tested against *Klebsiella pneumoniae* (RSKK 574), *Escherichia coli* (ATCC® 11229™), *Bacillus megaterium* (DSM 32), *Enterococcus faecalis* (ATCC® 7966™) and *Staphylococcus aureus* (ATCC® 25923™). It is well known that CS itself exhibits antimicrobial activity due to the presence of its amino groups. It was observed that non-conductive CS chains exhibited very low antibacterial activity when compared with Ppy, Ppy-*g*-CS and reference antibiotics. The inhibition zones caused by Ppy were shown to change in the following order: *Enterococcus faecalis* (17 mm) >*Escherichia coli* (15 mm) = *Klebsiella pneumoniae* (15 mm) >*Bacillus megaterium* (14 mm) = *Staphylococcus aureus* (14 mm). According to these values, Ppy showed good antibacterial activity as a result of the positive charges in the doped structure, which interact with bacterial cell walls. Recently, the antibacterial activity of cotton fabrics against *Escherichia coli* was reported to improve after coating with Ppy, the increase in antibacterial activity was attributed to the interactions of the positive charges on the Ppy chains with the bacterial cell walls. It is worth noting that CP are reported to cause cell death by disrupting microbial cell walls via electrostatic contact. The conducting biodegradable Ppy-*g*-CS copolymer exhibited greater antibacterial activity than CS and Ppy. While the inhibition zone diameter of CS was the lowest against the test bacteria, it increased up to 18–24 mm after grafting with conducting Ppy chains. This may be attributed to the synergistic effect of the protonated amino groups (in acidic medium) of the CS backbone and the positively charged Ppy chains, which assist in the sustained release of ions into the nutrient media prolonging antibacterial activity. In the presence of the Ppy-*g*-CS copolymer, the ZOI of *Bacillus megaterium* (23 mm) and *Enterococcus faecalis* (24 mm) were bigger than those formed by *Klebsiella pneumoniae* (22 mm), *Escherichia coli*(22 mm) and *Staphylococcus aureus* (20 mm). The Ppy-*g*-CS copolymer caused the biggest ZOI against *Enterococcus faecalis*, 24 mm, when compared with CS and Ppy. The enhanced antibacterial effects of the Ppy-*g*-CS copolymer could be explained by electrostatic interactions between the polycationic copolymer chains and all the tested bacteria, which possess opposite charges. The ions released from the more positively charged copolymer chains may attach to the negatively charged bacterial cell walls, causing the walls of the bacteria to break down, which compromises cell integrity leading to leakage of the intracellular fluid, cell lyses and eventual death of the bacteria. Similar antibacterial effects were reported in the literature for biodegradable Ppy/dextrin nanocomposites, which demonstrated up to 74.5% biodegradation in the presence of natural soil microorganisms;

therefore, this conducting Ppy-*g*-CS copolymer was classified as a biodegradable material. The degree of antibacterial activity of the copolymers changed upon increasing the concentration of Ppy/dextrin. Approximately 1–2 mm increases in ZOI were observed when the Ppy/dextrin concentrations were increased from 50 to 75 and then 100 μL. The antibacterial activities of Ppy and Ppy-*g*-CS were also compared with reference antibiotics. Ppy showed stronger activity than penicillin (10 mg) and trimethoprim (25 mg); equipotent activity with RIF (5 mg) and lower activity than amikacin (30 mg) and erythromycin (15 mg). On the other hand, the Ppy-*g*-CS copolymer exhibited a significant enhancement in antibacterial activity compared with reference antibiotics. It was observed that the Ppy-*g*-CS copolymer showed good activity in comparison with penicillin (10 mg), RIF (5 mg) and trimethoprim (25 mg), while it showed equipotent activity with amikacin (30 mg) and erythromycin (15 mg). Ppy, Ppy-*g*-CS and reference antibiotics were screened for antifungal activity against *Candida albicans* and *Saccharomyces cerevisiae*. According to the results obtained, no antifungal activity was observed for both of the materials or reference antibiotics, which can be attributed to the physical and chemical characteristics of the fungi [72].

7.2.3 Polythiophene

The antimicrobial activity of a gold NP–PTh nanocomposite against common pathogenic bacteria has been studied. The gold NP–PTh nanocomposite was readily soluble in water [73] and electrostatic interactions, mediated by the protonated form of the nanocomposite in conjunction with weak van der Waals forces, promoted by the binding of the gold NP–PTh nanocomposite with negatively charged bacterial cell walls belonging to both Gram-positive and Gram-negative strains. The degree of antibacterial activity of the various concentrations of gold NP–PTh nanocomposite against common Gram-positive and Gram-negative pathogenic bacteria is shown in Figure 7.3. Essentially, a dose-dependent growth inhibition was unequivocally evident for all pathogens, indicating broad-spectrum bactericidal activity of the gold NP–PTh nanocomposite. At the highest gold NP–PTh nanocomposite concentration of 150 μM, there was a remarkable inhibitory effect, which resulted in the virtual elimination of the bacterial pathogens.

Amongst the tested target pathogens, it is worth mentioning that the nanocomposite inhibited the growth of *Staphylococcus aureus*, which was earlier tested to be a presumptive methicillin-resistant *Staphylococcus aureus* strain [74]. To obtain a true measure of the bactericidal potency of the gold NP–PTh nanocomposite, it is imperative to ascertain the loss of viability of target cells in a nutrient-free medium, where the outcome of the assay is not likely to be influenced by the ability of bacterial strains to proliferate. To achieve this goal, cells of *Escherichia coli* and *Listeria monocytogenes* Scott A (10^6 colony forming units [CFU]/mL each) were suspended in sterile phosphate

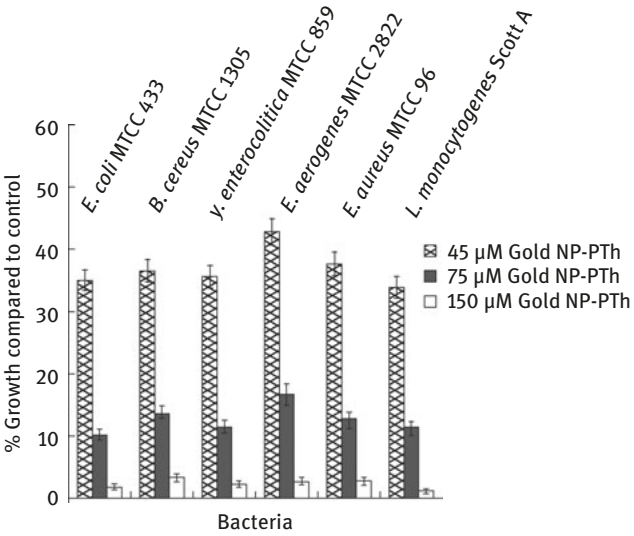

Figure 7.3: Antibacterial activity of the gold NP–PTh nanocomposite against common pathogenic bacteria. Reproduced with permission from M.D. Adhikari, S. Goswami, B.R. Panda, A. Chattopadhyay and A. Ramesh, *Advanced Healthcare Materials*, 2013, 2, 599. ©2013, Wiley-VCH Verlag GmbH & Co. KGaA [75].

buffered saline (PBS) and treated with varying concentrations of the gold NP–PTh nanocomposite. It was observed that an increase in nanocomposite concentration and interaction time resulted in a corresponding decline in the viability of the target pathogens. At high gold NP–PTh nanocomposite concentrations of 112 and 150 µM, the reduction in cell viability was rapid and following 12 h of interaction, the target cell populations were eradicated as the viable cell counts were found to be below 1.0 $\log_{10}$ CFU. It should be mentioned that the pH of the PBS solution varied from 4.8–3.7 with increasing concentration of gold NP–PTh (15–150 µM). Retention of cell viability of the target pathogens in pH-adjusted PBS solution clearly indicated that the loss of target pathogen viability upon exposure to the nanocomposite was not influenced by pH. The MBC of the gold NP–PTh nanocomposite for both *Escherichia coli* and *Listeria monocytogenes* was determined to be 112 µM. In the context of the bactericidal activity of the gold NP–PTh nanocomposite, the particle size of the nanocomposite is likely to have a considerable impact. In an earlier study we showed, using dynamic light scattering experiments, that the gold NP–PTh composite particles are essentially submicrometre in size, with the majority of the particles displaying a size distribution ranging from 100–300 nm, and the smaller particle sized nanocomposites demonstrated an increased propensity to interact with bacterial cells. The smaller sized particles in the composite displayed a strong interaction with bacterial cells owing to a larger surface area for effective contact with the cells. Because of the superior interaction with bacterial cells, the smaller-sized nanocomposite particles conceivably play

a significant role in facilitating the bactericidal activity of the gold NP–PTh nanocomposite. The binding of the gold NP–PTh nanocomposite onto bacterial cells should be further investigated as the nanocomposite was inherently fluorescent and the emission intensity of the composite was concentration dependent. A dose-dependent binding of the nanocomposite to the bacterial cells was evident and a Scatchard plot obtained from the gold NP–PTh fluorescence measurements revealed that the binding affinity of the composite for *Escherichia coli* MTCC 433 and *Listeria monocytogenes* Scott A were comparable, with dissociation constant values of 12×10^{-3} and 8.09×10^{-3} M, respectively. This comparable affinity may account for the generality of the gold NP–PTh nanocomposite and bacteria interaction, and probably explains the broad-spectrum bactericidal activity of the composite [75].

7.2.4 Polyacetylene

Polyacetylenic lipids and their derivatives have been isolated from a wide variety of microbial species and are known for an array of interesting properties. Polyacetylenic compounds containing three conjugated unsaturated moieties, such as yne–ene–yne, yne–yne–ene and yne–yne–yne, display a diverse range of biological activities, including cytotoxicity, antifungal, antimicrobial, herbicidal and antibacterial [76–79]; hence, polyacetylenic compounds are of great interest to the pharmaceutical industry. A particularly important area of interest is the discovery and development of novel antimicrobial agents, as the number of effective antimicrobial agents is declining due to increased antimicrobial resistance. A series of novel polyacetylene-substituted 2-hydroxy acids and derivatives have been prepared and characterised. The alkylation of butane-2,3-diacetal protected glycolic acid with iodoalkyl-substituted polyacetylene compounds gave the corresponding diacetal protected polyacetylene substituted 2-hydroxy acids. Diacetal deprotection via acid-mediated hydrolysis, *trans*-esterification or aminolysis resulted in the 2-hydroxy-polyacetylenic acid, ester or amide derivatives. Twenty-one of these novel compounds were tested against 10 microbes of clinical importance and several of them showed good antimicrobial activity, in particular, against *Pseudomonas aeruginosa* [80]. Conducting polymers with graphene oxide nanocomposites and its blend with natural or synthetic rubber are also found as an suitable candidate for supercapacitor and other industrial applications [81, 82].

7.3 Conclusion

CP are known for their diverse applications in many fields, including biomedical applications, such as drug delivery and antimicrobial polymers. Functional CP exhibit potential advantages over smaller analogous molecules and their potential use in

a wide range of applications is related to both their functional groups and polymeric nature, and these characteristic properties depend upon the extraordinarily large size of the molecules. The most commonly known CP are PANI, Ppy, PTh and polyacetylene. PANI is used in biomedical applications because of its insolubility in common laboratory solvents, thereby it is difficult to degrade. f-PANI-based polymers are potentially well suited for use as biocompatible scaffolds for tissue engineering and as antimicrobial wound dressings, with the advantage of being able to kill microbes without the use of antiseptics such as iodine or silver. The antibacterial activity of nanocomposite (PANI ZSP) material is an effective antimicrobial agent against *Escherichia coli* bacteria. PANI-doped fluconazole shows more antifungal activity than PANI. The biological activity of dye-substituted PANI has been observed to be greater compared with the individual activities associated with either PANI or dyes. Similar to PANI in Ppy, one positive charge is formed for each three to five repeat units. Positive charges are counterbalanced by counterions, namely anions present in the polymerisation solution that are embedded in the polymer matrix. The positive charges appear to be responsible for the antibacterial activity of these types of polymers. Polyacetylene-containing three conjugated unsaturated moieties, such as yne–ene–yne, yne–yne–ene and yne–yne–yne, display a diverse range of biological activities, including cytotoxicity, antifungal, antimicrobial, herbicidal and antibacterial; hence, polyacetylenic compounds are of great interest to the pharmaceutical industry. A particularly important area of interest is the discovery and development of novel antimicrobial agents, as the number of effective antimicrobial agents is declining due to increased antimicrobial resistance. It is worth noting that CP are reported to cause cell death by disrupting microbial cell walls via electrostatic contact.

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Narendra Singh Chundawat and Narendra Pal Singh Chauhan

8 Antimicrobial activities of plastics and elastomers

Abstract: An antimicrobial plastic and elastomer additive offers valuable protection for polymers. It is incorporated into a wide variety of plastics and polymeric materials to prevent growth of microorganisms. This prolongs their life, maintains their aesthetic appeal and reduces surface bioburden. An antimicrobial masterbatch can be supplied in carriers suitable for most polymers, including PVC, PE, PMMA, PP, polyamide, polyester, PEG, polycarbonate and polyurethanes. Antimicrobial additives for plastics are also used to treat elastomeric polymers and rubbers such as TPEs and TPVs.

Keywords: PMMA, polycarbonate, PEG, polyacrylonitrile, biocides, polyurethane

8.1 Introduction

The versatile properties and manufacturability of polymers have evoked immense interest in developing a class of biomaterials with the potential to interface with biological systems [1]. However, polymers are prone to pathogenic attack resulting in deterioration of properties, malfunction and so on. Various methods such as the ionic binding technique, incorporation of metal particles/metal oxides/nanoparticles (NP) and physico-chemical modification via, for example, the addition of quaternary ammonium salts and blending with antimicrobial polymers, have been explored for the fabrication of bactericidal materials [2]. However, these methods can result in reduced biocompatibility, cytotoxicity and eco-toxicity.

Polymers are widely used as biomaterials in prostheses, bone replacement implants, drug delivery, catheters and tissue engineering [3]. Among them, polymethyl methacrylate [4], polyethylene glycol (PEG) [5], polytetrafluoroethylene [6] and polyurethane(s) (PU) [7] are commonly used due to their biocompatibility, mechanical properties and ease of moulding into desirable shapes. Polyurethane and polyethylene glycol polymers are widely used in cell culture applications because of its non-toxicity and high transparency, as indicated by the number of brand names available in this area of research. Although the use of polystyrene (PS) as a biomaterial is uncommon in clinical practice, PS is used for medical applications

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such as artificial liver support and controlled release devices [8]. PS is also used in applications such as wound dressings and coatings for implantable medical devices [9], and in implantable medical devices for the controlled delivery of therapeutic agents [10]. When a biomaterial is implanted, it can become a site for bacterial adhesion, colonisation and the formation of a multicellular structure highly resistant to antimicrobials, called a biofilm [11]. According to the National Institutes of Health, 80% of hospital-acquired infections worldwide are attributed to biofilms formed by bacteria [12].

A series of antimicrobial polymethacrylates (PMA) containing quaternary ammonium cations (QUAT) has been synthesised via the *N*-alkylation of thiazole and triazole pendent groups using butyl iodide (BuI), and the chemical composition and distribution of amphiphilic polycations was characterised by nuclear magnetic resonance spectroscopy [13]. The correlation between their structure and antibacterial properties are presented in Figure 8.1, and clearly indicates that polyelectrolytes are responsible for their excellent selective toxicity against bacteria.

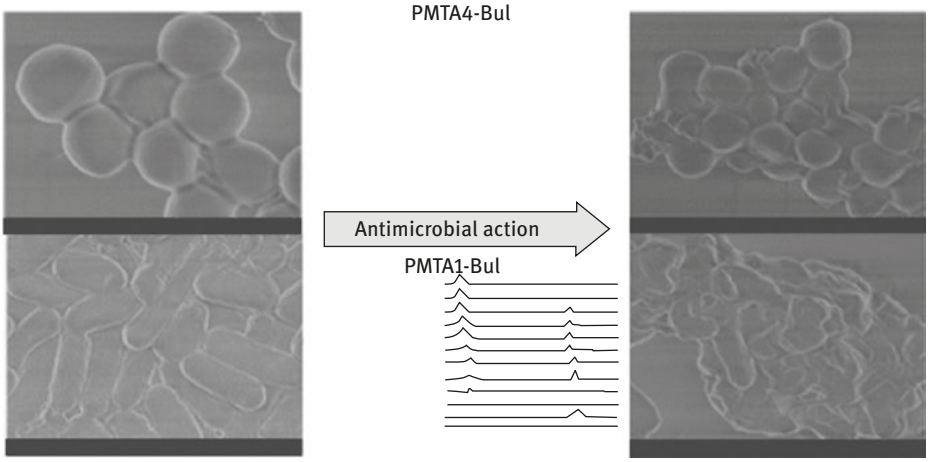

Figure 8.1: Antimicrobial PMA bearing cationic QUAT prepared by the controlled *N*-alkylation of 1,3-thiazole and 1,2,3-triazole pendent groups with BuI. Reproduced with permission from R. Tejero, D. Lopez, F. Lopez-Fabal, J.L. Gomez-Garces and M. Fernandez-Garcia, *Biomacromolecules*, 2015, 16, 6, 1844. ©2015, American Chemical Society [13].

Recently, tobramycin (Tob) was chemically prepared via the site-specific conjugation of PEG to form PEGylated-tobramycin (Tob-PEG); furthermore, confocal laser scanning microscopy and scanning electron microscopy were employed to confirm the data Figure 8.2 [14]. The minimum inhibitory concentration (MIC) of Tob-PEG was found to be very much higher than that of Tob.

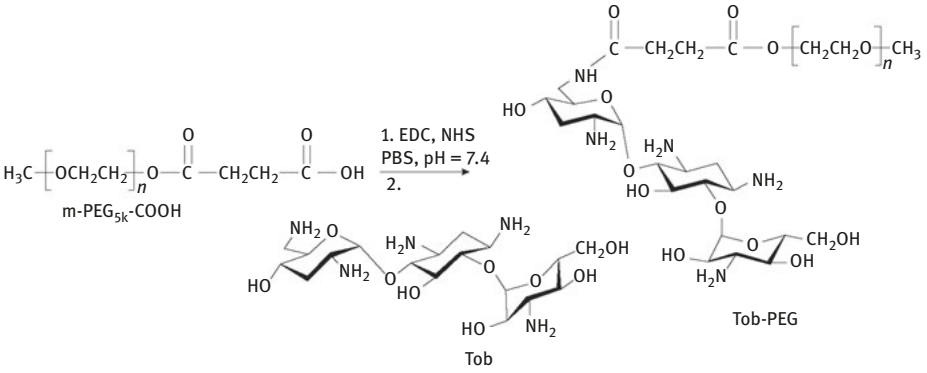

Figure 8.2: Synthetic strategy of chemically producing Tob with PEG via site-specific conjugation to form Tob-PEG. NHS: *N*-hydroxysuccinimide, PBS: phosphate buffered saline and EDC: 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. Reproduced with permission from J. Du, H.M.H.N. Bandara, P. Du, H. Huang, K. Hoang, D. Nguyen, S.V. Mogarala and H.D.C. Smyth, *Molecular Pharmaceutics*, 2015, 12, 5, 1544. ©2015, American Chemical Society [14].

The replacement of synthetic polymers with new biodegradable materials is currently becoming an important challenge. The growing demand for petroleum along with the political uncertainty in many of the most important petroleum-producing countries have resulted in a dramatic increase in the price of petroleum over the last few decades [15]; hence, biopolymers, which are derived from agricultural sources [16], appear to be a promising alternative. Different vegetable (corn, wheat gluten, soy and so on) and animal (milk, albumen, collagen, gelatin and so on) proteins have been used to manufacture bioplastics [17] for applications, including packaging, a matrix for enzyme immobilisation or controlled release and so on [18]. A plasticiser can reduce the bioplastic glass transition temperature, which is advantageous in certain applications [16, 19]. The incorporation of a plasticiser into the protein matrix can be achieved via two methods: (a) physico-chemical or 'casting' method, using a chemical reactant to disrupt disulfide bonds [20] and (b) thermoplastic processing, which consists of mixing proteins and a plasticiser using a combination of heat and shear [21] and, depending on the protein, an additional stage involving further thermomechanical treatments (e.g., compression moulding) may be required to achieve a suitable material [22].

8.2 Polymeric biocides

Biocides are widely used in the coating industry to prevent or inhibit bacterial growth in storage containers and on coated substrate surfaces [23]. The current use of low molar mass biocides is a source of major concern as biocides leach out of the

finished products or substrate films and result in residual toxicity in freshwater systems, such as rivers, dams and groundwater sources [24]. Research has focused on obtaining polymeric biocides that strongly adsorb onto individual particles and upon film formation remain distributed throughout the entire film; examples of these are described in a review by Kenawy and co-workers [25], and work performed by Tiller and Fuchs [26]. It has been found that as a result of large-scale chain entanglement, polymeric biocides mixed in latex eventually form part of the bulk system, allowing antimicrobial activity over a longer period of time due to the lack of leaching of the active ingredients, that is, polymeric biocides and surfactants integrated into the film would be unable to leach from the final substrate film. This material/film could simplify wastewater treatment as macromolecular species could easily be removed from wastewater by means of a suitable flocculent [27]. The copolymer poly(styrene-alt-maleic anhydride) (SMA), contains maleic anhydride (MA) units that are highly reactive towards nucleophilic addition reactions by various amine compounds [28]. SMA is a strongly alternating copolymer and, depending on the excess of styrene relative to MA in the monomer mixture, a block of styrene of varying chain length could be incorporated towards the end of the reaction via controlled living-radical polymerisation [29]. SMA undergoes self-emulsification after the incorporation of a suitable nucleophile via the MA units in the polymer chain, introducing specific functional groups into the polymer chain [30]. Various tertiary amine and quaternary ammonium compounds show a great degree of antimicrobial activity [31]. Amine-functionalised SMA incorporated into synthetic latexes can provide a means of obtaining permanently antimicrobial latexes and coatings. Results showed that incorporating amine-functionalised SMA into synthetic latex results in (1) latexes with inherent antimicrobial and antifungal properties that remain stable over time and (2) the amine-functionalised SMA can replace low molar mass surfactants in ab initio emulsion polymerisation systems. The antibacterial and antifungal properties of synthesised nanosilver-based natural rubber latex foam have been characterised. Roe and co-workers [24] found a method of making antimicrobial plastic catheters using silver NP on standard Pebax® polyamide 20 gauge catheters. Research work on producing silver NP has been extensive [28] and Balan and co-workers [30] have summarised some of the methods of producing silver NP:

- Chemical reduction of silver ions usually in the presence of stabilising agents.
- Thermal decomposition in organic solvents.
- Reversed micelle processes.
- Photoreduction.
- Ultrasonic radiation.
- ^{60}Co-*g*-irradiation.
- Microwave irradiation.

8.2.1 Polymers with silver ion-exchanged zeolites

It has been reported that silver ion-exchanged zeolites exhibit antibacterial activity [32]. The mechanism of antibacterial action of the zeolite is initiated when moisture or liquid film comes into contact with the ion exchange material and silver ions are exchanged with sodium (Na) or other cations from the environment [33]. The released silver ions attach to the bacteria by forming chelate complexes with deoxyribonucleic acid, which blocks the transport processes in the cell [34]. The use of zeolite as a filler in polymeric materials has been reported in the literature and it has been proved that they enhance the antibacterial activity of the polymer [35]. Furthermore, the effect of zeolite content on the physical and thermal properties of the polymer was also examined [35]; increasing the silver/zeolite ratio in the polymer led to an increased antimicrobial activity (due to the higher silver ion concentration), but depending upon the application the zeolite content may influence physical, thermal and/or chemical properties of the polymeric material.

8.2.2 Antimicrobial fibres

Electrospun fibres cannot technically be considered nanoscale due to their comparatively long length; however, the unique properties imparted by the electrospinning process render them a particularly interesting class of materials with a wide variety of potential applications [36]. The high surface area-to-volume ratios make electrospun fibres potentially useful materials in the fields of biomedical [37], energy [38], catalysis [39], sensing [40], filtration [41] and numerous others. The unique morphological properties of electrospun nanofibres have been shown to be particularly promising with respect to medical applications, as fibrous scaffolds have been implicated as potential materials for tissue engineering [37], drug release, wound dressings and enzyme immobilisation. Unfortunately, due to the high affinity for cellular attachment to such scaffolds, many potential applications of nanofibres are limited by the possibility of bacterial adhesion and resulting infections [42]. Biocidal activity is not always directly proportional to fibre size. In particular, for poly(2-hydroxy-5-methylquinone)(polyHMQ)-4 fibres, a3.3 average log reduction of microbes was observed for both fibres electrospun from a chloroform/dimethylformamide mix and nanoribbons electrospun from a chloroform/ethanol mix, despite the diameter of the chloroform/ethanol ribbons being over 3× larger. Both polyHMQ-6 and polyHMQ-8 fibres, which exhibited the most significant activity, also displayed the lowest degrees of apparent crystallinity compared with fibres of the same composition. Additionally, gradual decreases in activity were correlated with concomitant increases in relative crystallinities for specific formulations of polyHMQ-10, polyHMQ-8 and polyHMQ-6. As the molecules within the biocides are relatively large, even a small amount of structural ordering may inhibit their overall

antimicrobial activity and availability to bond with microbes. The short alkyl chains of the biocide were less able to become entangled with other polymer chains leaving them free to act as biocidal moieties, in effect, generating the most biocidal materials investigated.

8.2.3 Polymer–nanosilver for coating applications

Within the human body, bacterial activity is one of the key factors in the initiation of biofilm formation and growth on a polymeric material, resulting in its subsequent degradation. Once the biofilm has formed on the surface of the material it is very hard, or sometimes impossible, to remove it by washing or antibiotic treatment [43]. Several solutions have, however, been presented in the literature, which enhance the antimicrobial properties of polymeric materials. Nanosilver coatings have frequently been used and exhibited enhanced antimicrobial properties. The antimicrobial activity of silver is based on the release of silver ions, which is an ion exchange process [44]; the mechanism behind the silver ion release is, however, at present not well understood, and requires elucidation. Some studies have confirmed that a high concentration of silver released from nanosilver coatings might be toxic to both human and animal cells [45]. Impregnating silver ions into the polymer surface is another way to prevent bacterial adhesion and silver-sulfadiazine is usually used for this purpose [46]. However, clinical studies have proved that the antibacterial activity of silver-sulfadiazine–impregnated catheters decreased rapidly.

Some studies have demonstrated the strong antimicrobial activity of nanosilver [47]; however, the serious threat to aquatic life due to NP seeping, in the form of ionic silver, Ag^{+}, into the environment is an important issue [48]. *Curcuma longa L.* (Zingiberaceae) is an indigenous herb in Southern Asia and is widely recognised for its use in therapeutic remedies against a wide spectrum of diseases such as cancer, dermatitis, acquired immune deficiency syndrome, cholesterol, hepatic disorders, diabetic wounds, rheumatism, sinusitis and so on [49]. The essential oil of *Curcuma longa* contains an active ingredient called curcumin (diferuloylmethane), a natural polyphenolic compound. This pleiotropic molecule interacts with numerous molecular targets demonstrating versatile properties such as antibacterial, anti-inflammatory, antioxidant, antinociceptive, antiprotozoal, nematocidal, antivenom, chemopreventive and so on [50]. Antimicrobial polyacrylonitrile (PAN) films containing curcumin, and its possible interactions with PAN, have been developed and investigated by the Materials Studio developed by Accelrys Ltd. The antimicrobial signature of PAN is similar to natural herbs; hence, potential applications include biomaterials, dialysis membranes, intelligent textiles and so on [51]. Elucidation of the interactions between the additive and polymer was achieved via modelling

studies, whereas the antibacterial efficacy is quantified by measuring the zone of inhibition(s) (ZOI).

Antibacterial potency is dependent on the particle size and dispersion of the active agent. Silver NP/nanosilicates platelet (NSP) nanohybrids have been fabricated with different weight ratios using fully exfoliated clay, which acts as a dispersing agent for NSP and as a carrier for silver NP[52]. Furthermore, the biocompatibility, immunological and antibacterial activities of silver NP/NSP hybrids were evaluated; enhanced antibacterial activity was demonstrated along with an increased biocompatibility upon embedding the silver NP/NSP further into the poly(ether) urethane (Figure 8.3).

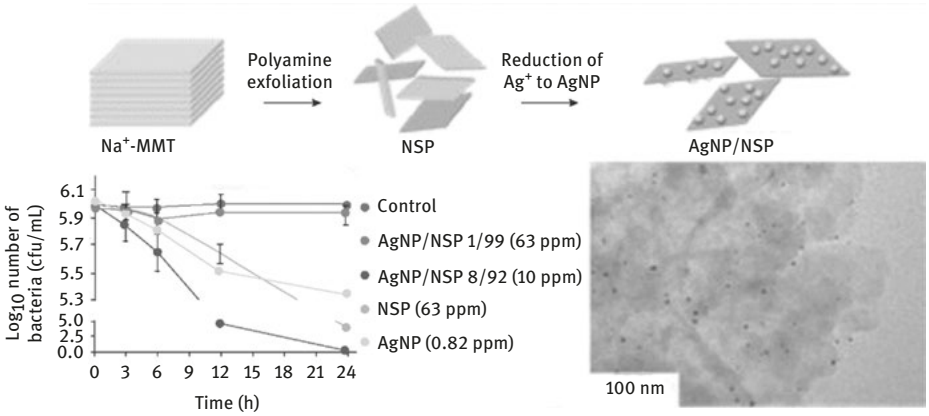

Figure 8.3: NSP as a dispersing agent and carrier for silver NP. MMT: montmorillonite. Reproduced with permission from J.J. Lin, W.C. Lin, S.D. Li, C.Y. Lin and S.H. Hsu, *ACS Applied Materials & Interfaces*, 2013, 5, 2, 433. ©2013, American Chemical Society [52].

8.3 Polyurethane-based materials as antimicrobial agents

PU and silicone rubber are biocompatible materials, which are commonly used in a variety of medical applications [53], for example, as a raw material for central venous catheters and tracheotomy tubes. Although these materials are biocompatible, the side effects which occur during clinical use include inflammation, infection and biofilm formation and growth. This, in turn, initiates the degradation of the material, for example, previous studies have proven that the degradation of PU catheters is caused by either oxidation or hydrolysis of the material [54]. The degradation of silicone rubber is a hydrolysis phenomenon [55], which could be catalysed by an acidic environment.

The antimicrobial activities of metal-containing PU were tested against various microorganisms in dimethylsulfoxide as the solvent. The sample concentration was 50 μg/mL for antibacterial investigations and 100 μg/mL for antifungal studies. The antibacterial activity was screened against *Staphylococcus aureus, Bacillus subtilis* (Gram-positive) and *Escherichia coli* (Gram-negative), while antifungal screening was performed against *Aspergillus niger, Candida albicans* and *Aspergillus flavus*. Bacterial strains were nourished in a nutrient broth (Difco™) and yeasts in malt extract broth (Difco™) and incubated for 24 and 48 h, respectively. The agar diffusion method was followed, that is, bacteria were incubated on Müeller–Hinton agar (MHA) and yeast on Sabouraud dextrose agar.

A range of PU foams and elastomers have been used as cushion insole material in footwear [56]. Surface modification is an effective way to improve the surface property of polymers, whilst retaining the bulk properties [57]. The covalent attachment of polymers or pharmaceutical agents to the polymer surface has been achieved through the use of diisocyanate bridges, such as hexamethylenediisocyanate (HMDI), which can react with urethane, hydroxy or amine groups at the PU surface resulting in the formation of covalent bonds. The unreacted functional isocyanate (–N=C=O) groups can react with hydroxyl or amine groups on other molecules and a large number of molecules have been grafted onto the surface of polymers in this manner. Graft photopolymerisation has been used to graft PEG, polyvinyl alcohol (PVA), poly(2-hydroxyethyl methacrylate) (p-HEMA) and chitosan (CS) onto PU [58]. The radiation-induced grafting of p-HEMA and other methacrylates onto PU elastomers has also been achieved [59]. Wang and co-workers [60] modified the surface of PU by coating it with blends of a stearyl polyethylene oxide coupling polymer in CS as coating materials to optimise the surface biocompatibility of the intravascular catheter. Ananthu and co-workers [61] reported the surface modification of medical-grade PU using a cyanurchloride-activated tetraether lipid. The surface was initially modified with HMDI and subsequently treated with water or hexamethylenediamine to generate free amino groups on the surface. Jiang and co-workers [62] graft polymerised the zwitter ionic monomer of sulfobetaine onto a PU surface, via the vinyl bonds of acrylic acid (AA) or hydroxyethyl methacrylate, in a three-step heterogeneous system, which was then immobilised with HMDI. Yang and co-workers [63] worked on a four-step surface modification method to create a thin lubricious layer of CS/PVA hydrogel on segmented PU urethral catheters. Porous viscoelastic PU sheets were developed and reported to have better physical and mechanical properties than that of conventional PU insoles [64]. A porous viscoelastic PU sheet of 3 mm thickness and 75% porosity was prepared using the reported procedure [65] and used as a base material for surface modification in this study. The surfaces of the PU sheet were modified to incorporate water absorption and antimicrobial properties into the PU insole for application in athletes' trainers to control sweat accumulation and subsequent foot infection. The surfaces of the porous viscoelastic PU sheets were modified with hydrophilic polymers where the

PU acted as the insole material and the hydrophilic polymer on the surface acted as a moisture absorbent and drug carrier. The following hydrophilic polymers were selected for modification of the PU surface: p-HEMA, PEG, PVA and CS; CS was chosen for its antiseptic properties.

Several surface properties, including roughness, wettability, composition, electric charge and surface free-energy seem to contribute to the complex mechanism of bacterial adhesion [66]. In addition, the zeolite concentration influenced the degree of decrease of the contact angle. Water absorption of the zeolite-PU composite is a reasonable explanation for the significant decrease in contact angle; the phenomena that caused the observed plateau are believed to consist of three stages.

8.4 Plastics as antimicrobial agents

It is commonly known that food spoilage is mainly caused by microbial contamination. Most food preservation methods, such as fermentation, drying, thermal processing, freezing, refrigeration and modified atmosphere, are effective but have limitations, especially when applied to fresh meat, as consumers demand minimal changes to the meat texture [67]. As microbial contamination primarily occurs on the food surface, many studies have suggested the addition of antimicrobial agents onto food surfaces to suppress microbial growth to prolong the shelf life of food. However, the direct application of antimicrobial agents onto foods is not an effective method to inhibit microbial growth due to the rapid diffusion of the antimicrobial agent into the food and deactivation of the active substances by food constituents. In order to maintain meat quality, a variety of alternative packaging systems have been developed to preserve foods, as well as ensuring their safety. In fact, the latest development in food packaging involves utilisation of speciality films, which contains bioactive ingredients to suppress the reactivity of degrading agents [68]. In this context, antimicrobial agents, that is, the active agents, are normally added as the active agent into the packaging [69]. Antimicrobial packaging film is defined as packaging containing an antimicrobial agent where the agent is embedded inside the polymer chains and offers a slow and continuous migration to form an antimicrobial layer on the food surface. In other words, the antimicrobial agent can maintain a high concentration over the period required by the application. In general, there are various chemical components of plant origin, which exhibit antimicrobial effects, including saponins and flavonoids, thiosulfinates and glucosinolates. Using the disc diffusion method, spices such as cumin, cinnamon and cloves have shown the greatest antimicrobial effects, of this class of antimicrobial agent, against bacteria such as *Escherichia coli, Enterococcus faecalis, Mycobacterium smegmatis, Staphylococcus aureus, Klebsiellapneumoniae, Pseudomonas aeruginosa, Micrococcus luteus* and *Candida albicans* [70]. In addition, Fisher et al. [71] concluded that *Zingiber officinale, Alpiniagalanga, Curcuma longa* and *Boesenbergia*

pandurata extracts exert antimicrobial effects against Gram-positive and Gram-negative pathogenic bacteria, especially in the range of 0.2–0.4% v/v and 8–10% v/v for finger root spices and other spices, respectively [71]. In general, essential oils are mainly made up of terpenoids and sesquiterpenes along with different groups of aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, which also exhibit antimicrobial effects [72]. For instance, Rahman et al. [73] found that olive leaves (*Oleaeuropaea*) exhibited antimicrobial effects against *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus*, and [74] have shown that an ethanolic leaf extract of *Lonicera japonica* has also shown biocidal properties. *Allium sativum* essence oil (AEO) was found to be an equally effective antimicrobial agent and was added into packaging film. *Allium sativum*, commonly known as garlic, is well known for its antibacterial functionality in widely available supplementary nutrients. They are natural and complex compounds normally formed by aromatic plants as secondary metabolites and are of benefit to humans due to their antibacterial, antifungal and antioxidant properties, which help build up the body's immunity [75]. The antimicrobial properties of garlic were first studied in 1844; it was subsequently found that allyl sulfides are the major constituent of crushed garlic and allicin is responsible for its antibacterial activities [76]. In addition to its antimicrobial benefit, garlic has been reported to reduce blood lipids, modulate cardiovascular and enhance immune functions, and exhibit antioxidant and anticancer properties. To date, there are only a few published papers regarding the use of AEO as an antimicrobial agent in food packaging systems. Most of the studies have been focused on edible film, and as far as we know, research focusing on the production of AEO-incorporated plastic film via the blown film extrusion technique is lacking. The reason may be due to the fact that allicin is very unstable and decomposes easily under high processing temperature and pressure. However, the antimicrobial activity may not be lost as allicin tends to transform into diallyl sulfide, diallyl disulfide, diallyltrisulfide and/or ajoene, which are relatively more stable under high temperature; these compounds also exhibit a comparable antimicrobial effect to allicin [77]. Overall, this study was focused on the effectiveness of AEO-incorporated plastic films to inhibit beef-related pathogenic and spoilage bacteria in vitro. The mechanical and thermal properties of films were assessed to determine the compatibility between AEO and polyethylene (PE).

Studies within the area of active food packaging are currently experiencing considerable attention as a result of consumer demands and market trends. Active food packaging systems are based on materials in which some additives, acting as an antimicrobial and/or antioxidant agent, are combined with a polymer matrix with the aim of extending the shelf life of the foodstuff and improving consumer safety [78]. Antimicrobial packaging has received increased attention from the food and packaging industries due to increasing consumer demand for minimally processed and preservative-free products [79]. Food packaging films allow a controlled release of the additives into the food over prolonged periods (including storage and distribution operations), hence limiting the possible undesirable flavours caused by the direct

addition of these additives to food [80]. Over recent years, the demand for the use of natural additives has led to a clear increase in the number of studies based on natural extracts such as essential oils, which are categorised and generally recognised as safe by the US Food and Drug Administration [81], and could be considered as potential alternatives to synthetic additives such as butylatedhydroxytoluene [82]. The essential oils extracted from plants or spices are rich sources of biologically active compounds, such as terpenoids and phenolic acids, and it is well known that some of them exhibit antimicrobial properties. In particular, carvacrol and thymol are major compounds in thyme and oregano essential oils [83]; these two compounds are phenolic monoterpenes and isomers that exhibit significant in vitro antimicrobial activity [84]. Carvacrol shows antifungal, insecticidal, antioxygenic and antiparasitic activities [85]; on the other hand, thymol has received considerable attention as an excellent food antioxidant, in addition to being an antimicrobial agent, which exhibits very high antifungal activity [86]. The antimicrobial activity of both compounds against several bacterial strains has been investigated and reported [87]. Active compounds are usually added to packaging materials by incorporating their precursor essential oils [88]. Hence, there is increasing interest in the evaluation and possible application of these compounds for minimising the surface contamination of foods, such as meats, fruits and vegetables, and decreasing the microbial growth rate of microorganisms responsible for food degradation [89]. In particular, the use of carvacrol and thymol might be advantageous due to a possible synergistic effect produced by the addition of both additives into the polymer matrix; this effect has been confirmed against several microorganisms for food-packaging applications [90]. The use of high initial concentrations of these volatile additives has been previously reported, as some loss during processing is expected [91].

Antimicrobial packaging reduces, inhibits or retards the growth of pathogenic microorganisms in packed foods [92] through (1) the incorporation of volatile and non-volatile antimicrobial agents into polymers and (2) coating or adsorbing antimicrobials onto polymer surfaces [93]. A variety of compounds, including organic acids, enzymes, such as lysozyme, and natural antimicrobials, such as spices, have been proposed to be components of active food packaging [94]. However, essential oils, which contain high concentrations of phenolic compounds, such as carvacrol, eugenol and thymol, show the strongest antimicrobial activity against foodborne pathogens [95], and their antimicrobial properties have been demonstrated in numerous studies [96]. In addition, various organic acids and their salts (formic acid, sorbates, benzoates and propionates) are used to inhibit microbial growth [97] and increase the shelf life of fresh dough in combination with cooling, as these products are packaged without thermal treatment. The compounding of bioplastics has been performed using a Polylab torque-rheometer equipped with a Rheomix 3000p kneading tool (Thermo-Haake GmbH, Germany), which allowed the evolution of mixing temperature and torque to be monitored. Neither heating nor cooling was supplied to the kneading chamber (filled to 85% of its full capacity) during

compounding. Dynamic mechanical thermal analysis, tests were conducted on three compounded bioplastic samples using a DMS 6100 (Seiko Instruments Inc., Japan) in double cantilever (bending) mode. To assess antimicrobial activity, the Kirby–Bauer test was firstly carried out on pure or 10 vol% aqueous solution samples. As previously mentioned, if bacteria are susceptible to a particular antimicrobial agent, an area of clearing is evident surrounding the paper disc soaked in the biocide, which is called the ZOI (where bacteria are not capable of growing). In an analogous manner to biocides, the antimicrobial effectiveness of the active wheat gluten-based bioplastics containing formic acid was ascertained by measuring the ZOI diameter using the Kirby–Bauer test.

Various biodegradable polycarbonate(s) (PC) polymers have been fabricated via the organocatalytic ring-opening polymerisation of functional cyclic carbonate monomers, which were quarternised to create cationic polymers with various pendent structures such as alkyl, aromatic and imidazolinium (Figure 8.4). These polymers have shown excellent antimicrobial properties and haemolytic characteristics when assayed using rat red blood cells [98].

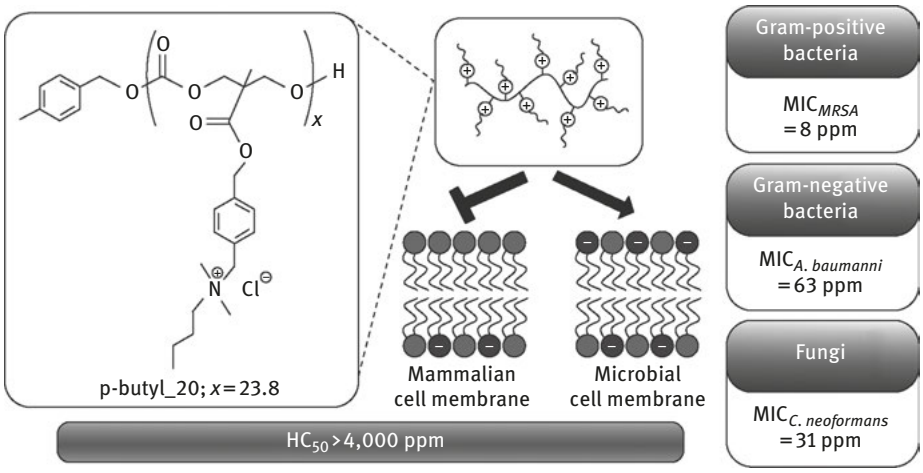

Figure 8.4: Biodegradable PC polymers and their MIC data. MRSA: methicillin-resistant *Staphylococcus aureus*. Reproduced with permission from W. Chin, C. Yang, V.W.L. Ng, Y. Huang, J. Cheng, Y.W. Tong, D.J. Coady, W. Fan, J.L. Hedrick and Y.Y. Yang, *Macromolecules*, 2013, 46, 22, 8797. ©2013, American Chemical Society [98].

The polymer network is stimuli responsive, that is, changes in mesh size can contribute to the regulation of loading and release processes of the active substance [99]. Methacrylic acid-grafted silk sutures have been shown useful for the loading of 8-hydroxy quinoline hydrochloride [100], while 1-vinylimidazole-, acrylonitrile- or

AA-grafted polypropylene (PP) monofilaments could absorb ciprofloxacin and tetracycline hydrochloride [101]. Anchoring vancomycin has been previously coupled to titanium implants and bone allografts to successfully prevent colonisation by Gram-positive bacteria [102]. PP-*g*-glycidyl methacrylate (GMA) and vancomycin-immobilised PP-*g*-GMA sutures were found to exhibit effective antibacterial activity against a *Staphylococcus aureus* strain.

Wood–plastic composites (WPC) are increasingly employed as interior and exterior building materials, for example, deckings. Their composition generally varies between 40% and 70% wood particles, 30% and 50% thermoplastic polyolefin (PO) polymer and 0.5% and 15% additives. Soft and hard wood, such as spruce, pine, maple, beech and oak, are commonly used as wood flour or shavings, which are embedded in a polymeric matrix such as PP, PE and polyvinyl chloride. WPC materials were developed to combine the advantages of wood and PO polymers, in particular, for their long-term performance, cost-effectiveness, shape flexibility and 'carbon footprint' [103]. Embedding wood particles in a polymeric matrix restricts moisture uptake, which is beneficial as moisture levels below 20% are recommended to prevent fungal decay. Most fungi optimally decay wood at moisture levels above fibre saturation (usually around 25–30%) but well below fully saturated conditions [104]. The fungal colonisation and decay of WPC was occasionally observed after outdoor exposure, especially under favourable conditions of warm temperatures and high moisture conditions [105]. Fungi can degrade a diverse range of wood compounds and use them as a source of nutrition. White rot fungi primarily degrade lignin and leave the cellulose fibres [106], whereas brown rot fungi mainly metabolise cellulose fibres and leave the brownish lignin.

8.4.1 Plastics as antifouling agents

The fouling phenomenon can be defined as the accumulation of micro- and macroorganisms on surfaces after prolonged immersion in an aquatic environment. More specifically, materials immersed in seawater are rapidly, that is, within 24 h, coated by a macromolecular film, which then favours colonisation by prokaryotes (mostly bacteria), and unicellular (microalgae) and multicellular eukaryotes, such as macroalgae, barnacles and mussels [107]. Biofouling represents a major problem for the maritime industry, especially for shipping, as the presence of biofouling on ship hulls increases the weight of the boat leading to elevated fuel consumption and maintenance costs [108]. Biofouling is also of great concern for offshore structures and aquaculture equipment [109]. This phenomenon is estimated to cause losses, directly and/or indirectly, to a country with a marine industry, as high as 7% of the gross national product [110]. To combat biofouling, various copper and tributyltin (TBT)-based coatings have been widely used. TBT-self-polishing copolymer paints

have been the most successful solution to the problem of biofouling on ships [111]; unfortunately, these formulations have detrimentally affected marine eco-systems [112]. As a consequence, TBT use on small boats (less than 25 m in length) has been prohibited in many countries since the mid-1980s. An International Convention held on 5th October, 2001 banned the application of TBT-based antifouling paints from 1st January, 2003 and imposed a complete prohibition of such paints on ship hulls from 1st January, 2008. The paint industry has been urged to develop TBT-free products and some less toxic materials have already been used, such as low molecular weight molecules (organic biocides, e.g., Irgarol® 1051, zinc pyrithione, diuron, Sea-Nine™ 211TM, zineb) incorporated in a matrix [113]. A promising alternative is to covalently bind biocidal functions onto a polymer chain to avoid the release of biocide products and to maintain permanent antifouling activity. A number of biocidal agents, such as quaternary ammonium salts, phosphonium salts, sulfonium salts, chlorophenyl derivatives and *N*-halamine have been introduced into ordinary polymers. In antifouling paints, quaternary ammonium salts are usually mixed with polymers such as PU [114], polysiloxane [115] and PMA. It has been shown that ionic PU containing oligoisoprene-bearing ammonium groups exhibited antibacterial activities and such an alternative has been further investigated by fixing quaternary ammonium salt functions to a polymer made from renewable resources (natural rubber). Selective degradation of *cis*-1,4-polyisoprene using well-controlled oxidative chain cleavage leads to new carbonyltelechelic polyisoprenes. Chemical modifications of carbonyl end-groups and carbon–carbon double bonds led to new photosensitive oligomers (with or without quaternary ammonium salt groups). These oligomers have been crosslinked using a radical method (via acrylate groups) and/or a cationic method (via epoxy groups) [116]. The ionic and non-ionic polyisoprene-based coatings were screened for their antibacterial activity. Five marine bacterial strains, *Pseudoalteromonaselyakovii* (ATCC® 700519™), *Vibrio aestuarianus* (ATCC® 35048™), *Polaribacterirgensii* (ATCC®700398™), *Cobetia marina* (ATCC® 25374™) and *Shewanellaputrefaciens* (ATCC® 8071™), were used as target species for the antibacterial assays as these microorganisms are representative of fouling species in both estuarine and marine environments [117]. Bacteria were cultivated in marine broth (5% tryptone in filtered natural seawater) and incubated for 5 days at 25 °C to allow their development [118]. The bacterial concentrations used for inoculation (2×10^8 colony forming units (CFU)/mL) were estimated by measuring the optical density (OD) at a wavelength of 630 nm according to the methodology described by Amsterdam and Loman [119]. Antibacterial testing of the coatings was performed using the disc diffusion technique in Petri dishes containing agar. Film samples (6 mm diameter) of the materials (sterilised for 2 h using ultraviolet [UV] radiation in a sterilising tube: 40 W, emission at 254 nm) were placed onto the surface of an agar plate (13% agar and 5% tryptone in filtered natural seawater) and the plate was inoculated with bacterial culture (2 mL, 2×10^8 CFU/mL). After

incubation at 25 °C for 48 h, the antibacterial activity was estimated by the ability of the coating to inhibit bacterial growth; PS surfaces were used as the control. All coatings were tested using six different discs and the results were assessed based on visual observations and were ranked following the procedure described by Hellio and co-workers [120].

Five coatings were also tested for their inhibitory activity against five strains of marine fungi obtained from the collection of the University of Portsmouth, UK (School of Biological Sciences): *Halosphaeriopsismediosetigera*, *Asteromyces-cruciatus*, *Lulworthiauniseptata*, *Zalerion* sp. and *Monodictyspelagica*. The fungal strains were maintained on maize meal agar (Sigma) slopes. For the antifungal assay, the protocol described by Hellio and co-workers [120] was used and the polymer material was incorporated into 200 mL of maize agar 12%, pH 6 (Sigma, USA). The Petri dish was then inoculated aseptically at the centre with a 2 mm diameter agar plug of mycelium. All assays were performed in triplicate and incubated at 25 °C for 4 weeks. The growth zones were then recorded and compared with the controls.

New triblock PC polymers consisting of antifouling PEG, antimicrobial cationic PC and an anchoring maleimide functional block have been synthesised and their antimicrobial and antifouling properties were investigated (Figure 8.5). As antibacterial activity was demonstrated against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli* bacteria, the antifouling polymer coating is a potential candidate for the prevention of catheter-associated bloodstream infections [121].

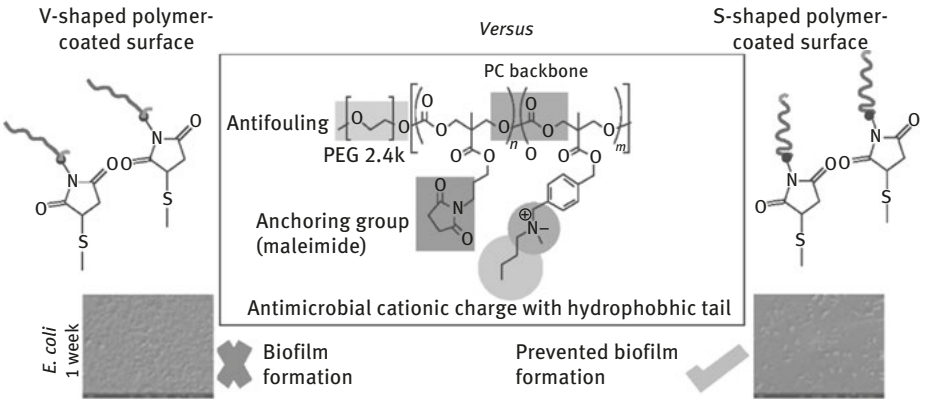

Figure 8.5: Synthetic production of triblock PC polymers consisting of three critical components, including antifouling PEG, antimicrobial cationic PC and a tethering or functional block. Reproduced with permission from Z.X. Voo, M. Khan, K. Narayanan, D. Seah, J.L. Hedrick and Y.Y. Yang, *Macromolecules*, 2015, 48, 4, 1055. ©2015, American Chemical Society [121].

8.5 Antimicrobial testing methods for plastics and elastomers

The performance of an antimicrobial coating has been tested in vivo without optimising its efficacy in vitro [122]. The most common method to test the efficacy of a device coating in vitro involves the use of stagnant broth solutions or hard agar surfaces. Depending on the application, if stagnant broth solutions or hard agar surfaces are used, an antimicrobial that elutes out of a device coating will likely reach concentrations that are not physiologically relevant. In a physiological environment, it has been shown that fluid flow is more likely to occur than static conditions, thus antimicrobial coatings may benefit from being tested under fluid flow conditions in vitro prior to testing them in vivo [123]. Investigators have at times performed in vivo testing with a reliance on MIC data; however, MIC values are wholly based on data from planktonic bacteria [124] and are not likely to translate to levels that are required to eradicate biofilm bacteria [125]. Furthermore, Williams and Costerton have recently hypothesised that wound and surgical sites are at risk of being contaminated with bacteria that reside in the biofilm phenotype, which may further reduce the efficacy of prophylactic antibiotics that are administered based on MIC values [126]. These three limitations were addressed in this study by first optimising an active release coating against biofilms of MRSA using an in vitro flow cell system. The developed coating comprised a silicone polydimethylsiloxane polymer and an active release antimicrobial agent – cationic steroid antimicrobial-13 (CSA-13), which is a synthetic analogue of naturally occurring antimicrobial peptides (AMP). Unlike AMP, CSA-13 is not a peptide, thus it is not a target for proteases, it can be produced in commercial quantities at relatively low cost, and it has a very rapid, broad spectrum and non-specific method of attacking bacterial cell membranes, which reduces the risk of initiating bacterial resistance [127]. CSA-13 has been shown to have superior activity to antibiotics and AMP in head-to-head comparisons [128], it has a shelf life of several years, and can be sterilised by gamma radiation, autoclaving or ethylene oxide treatment. Based on these characteristics, CSA-13 may be a promising clinical alternative to traditional antibiotics, AMP and other antimicrobial compounds. The in vitro data that were generated and the CSA-13 coating itself were translated to a sheep model of a simulated Type IIIB open fracture [129]. In this study, the method of infecting the sheep model was to first grow well-established biofilms of MRSA in a membrane biofilm reactor [130]. Using biofilms as the initial inocula more accurately modelled the phenotype of bacteria from natural eco-systems that may contaminate open fracture sites. The ability of the CSA-13 coating to prevent biofilm implant-related infection was tested. Specifically, it was hypothesised that when CSA-13 was used as the active release agent of an active release coating on metal plates, it would prevent biofilm implant-related osteomyelitis from developing in an animal model of a simulated Type IIIB open fracture.

8.5.1 Shake-flask method

A 100 mg sample of each coating was cut into small pieces, sterilised by UV light, and then dispersed in 9 mL of sterile saline water (0.85 wt%). 1 mL of bacterial (*Escherichia coli* or *Staphylococcus aureus*) culture (10^6 CFU/mL) was subsequently added to this solution and finally the concentration of polymer in the suspension was diluted to reach 10 mg/mL. The flasks were shaken at 90 rpm for 24 h and the temperature was maintained at 37 °C; blanks without the coating were also run. The surviving bacteria before and after shaking were counted using the plate count method.

8.5.2 Agar diffusion method

Modified natural foam rubber exhibiting antimicrobial activities and unmodified foam materials were placed in separate positions on MHA plates under aseptic conditions. Another thin layer of MHA was poured onto the foam samples, so that foam materials were sandwiched between the MHA layers. For the lower layer, 15 mL of MHA was poured into sterilised Petri dishes under aseptic conditions. For the upper thin layer, 10 mL of MHA was poured onto the two pieces of rubber and material. The bacterial concentration was determined by measuring the OD at 600 nm. An OD value of 0.3 corresponded to a bacterial concentration of 10^8 CFU/mL of the bacterial solutions of *Escherichia coli* and MRSA were inoculated onto MHA plates and evenly spread. The inoculated agar plates were incubated at 37 °C for 24 h [131].

8.5.3 Parallel streak method

Antifungal activity was tested using the parallel streak method and was performed according to the research work reported by Lee [132]. Modified and unmodified foam rubber samples were placed on Suborn dextrose agar (SDA) culture medium and a thin SDA gel layer (10 mL) was again poured on top of the foam rubber samples. Parallel streaks were drawn on the top gel layer using a loop containing fungal colonies of *Aspergillus niger*. The samples were incubated at 37 °C for 72 h and the fungal growth on the streaks was visualised on textile samples.

8.5.4 Zone of inhibition method

Using the ZOI method, antibacterial activity was investigated against *Staphylococcus aureus* (MTCC-96) and *Bacillus subtilis* (MTCC-441) as standard Gram-positive bacteria, and *Escherichia coli* (MTCC-1650) and *Klebsiellapneumoniae*

(NCIM-5432) as standard Gram-negative bacteria strains. The culture media for the microorganisms was prepared using Müeller–Hinton broth [133]. A single colony of each test strain was grown in the medium at 37 °C for 24 h in a rotary shaker (200 rpm). The cultured strains were adjusted to 0.5 OD at 600 nm [134]. The PAN/*Curcuma longa* samples were placed in the Petri dish and incubated for 24 h at 37 °C; pristine PAN film was used as a control. The ZOI were determined by measuring the areas around the disc where no microbial growth was observed. All experiments were carried out in triplicate.

8.5.5 Antifungal activity using the powder test

For the powder test, fungal spore suspensions were prepared by pouring 5 mL of sterile deionised water on each fungal plate and gently scraping the surface to obtain sporulating fungi. This step was repeated 3× for each fungal species. Spore suspensions were then filtered through a glass wool filter. The number of spores was adjusted to 10^6 mL spores/mL for each fungal spore suspension. Equal volumes of each fungal spore suspension were blended and denoted 'powder test fungal spore suspension'. Fungal blends from Sweden, Sri Lanka and Tanzania were prepared and each was denoted by the name of the country. Each fungal spore suspension was prepared using the same procedure as for the powder test. However, for the biodegradation test, the fungal plates were wetted with a mineral–salt solution containing a wetting agent and prepared according to the International Organization for Standardization (ISO), ISO 846:1997(E). After filtering the spore suspension of each fungus through glass wool, the fungal spore suspensions were centrifuged and the supernatant liquid was discarded. ????The residue was resuspended in the mineral–salt solution and centrifuged. Finally, the washed residue was suspended in the mineral–salt solution. The number of spores was adjusted to 10^6 spores/ml for each fungal spore suspension. Equal volumes of each fungal spore suspension were blended and used to inoculate the samples. This suspension was denoted 'standard fungal spore suspension'. The powder test was conducted to study the antimicrobial efficiency and efficacy of the antimicrobial agents against fungi. Malt extract agar plates were prepared and sodium benzoate, 4,5-dichloro-2-octyl-4-isothiazolin-3-one and *para*-aminobenzoic acid were placed on the top of the agar plates at concentrations of 1, 3 and 5% (w/w). Agar plates containing antimicrobial agents were inoculated by pipetting 100 ml of the powder test fungal spore suspension and standard fungal spore suspension onto agar plates, which were then incubated for 28 days at 29 °C and 90% humidity. Control growth agar plates were also prepared and incubated under the same conditions.

8.5.6 Biodegradation test

Prepared control and antimicrobial samples were analysed for their susceptibility to fungal attack, i.e., growth, according to the standard test, ISO 846: Plastic–Evaluation of the action of Microorganisms. Samples were cleaned prior to the test *via* dipping in 70% ethanol for 1 min. The samples were then dried for 72 h under constant air flow. Sample inoculations were carried out according to three methods: A, B and B$^{\#}$. In method A, agar without nutrients was used, while for B and B$^{\#}$, agar containing nutrients (glucose) was used. For methods A and B, the samples were placed on the agar surface after which 100 ml of standard fungal spore suspension was pipetted onto the sample. For method B$^{\#}$, fungal inoculation was performed prior to placing the samples on the agar plate, i.e., after observing visible growth (3–4 days of sample incubation). Viability growth agar plates were also prepared to assess the growth of all studied fungi. Samples in methods A, B and B$^{\#}$ were incubated for 28 days at 29 °C and 90% humidity. Control growth plates were incubated under the same conditions.

8.5.7 Broth microdilution method

In order to assess the bacterial susceptibility profile, the MIC for different drugs was established using the broth microdilution method [135]. The surface hydrophobicity of the bacterial strains was determined using the microbial adhesion to hydrocarbon test [136]. The bacterial suspension was cultured in tryptone soya broth (Oxoid Ltd., UK) for 24 h at 37 °C. After plasma treatment, PS microtitre plates were used as substrates for the bacterial adhesion assay following a modified protocol [137]. To assess if the plasma treatment affected bacterial growth, two assays were performed: OD at 600 nm and cell viability (resazurin indicator). The difference between the final and initial absorbance was taken as a measure of bacterial growth. To evaluate cell viability, the contents of the wells were transferred after incubation for the bacterial adhesion assay into another standard sterile 96-well PS plate, to which 25 µl of a resazurin solution (0.1 mg/ml) was added.

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9 Functionalised antimicrobial polymers

Abstract: Antimicrobial polymers, also known as polymeric biocides, have been recently developed to overcome problems associated with the growth of microorganisms such as bacteria, fungi or protozoa. The research on this class of materials is growing fast due to their advantages over conventional antibacterial agents. Among different strategies, functionalised antimicrobial polymers attracted a lot of attention as evidenced in many researches. This chapter aims to introduce and describe the developments in the field of functionalised antimicrobial polymers for different applications.

Keywords: Polymers, antibacterial, functionalization

9.1 Introduction

Antimicrobial polymers are also known as polymeric biocides and are a class of polymer, which can restrict or inhibit the growth of microorganisms such as bacteria, fungi or protozoa. Over the last two decades great advances have been made in the field of macromolecules, which exhibit antimicrobial properties, including the synthesis of novel constructions and modifications of known polymers, as well as biological, physico-chemical and biochemical research, and engineering design. These polymers have been designed to mimic antimicrobial peptides (AMP), which are utilised by the immune systems of living things to destroy bacteria. Functionalised polymers have gained considerable interest over the last few decades due to their functional groups and polymeric nature, which give them unique characteristics and advantages over the use of similar smaller molecules. Antimicrobial polymers can be generated by attaching or inserting an active antimicrobial group onto a polymer backbone via an alkyl or acetyl linker. Antimicrobial polymers can improve the output and selectivity of currently used antimicrobial agents, while reducing their associated environmental hazards as antimicrobial polymers are mostly non-volatile and chemically stable. This property makes antimicrobial polymers an ideal candidate for use in the field of medicine to fight infection, in the

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food industry to inhibit bacterial contamination and in water hygiene processes to inhibit the growth of microorganisms in drinking water [1]. Tashiro studied the synthesis flow of antimicrobial polymers such as polyionenes (polymers with positively charged nitrogen atoms localised in the backbone of a macro chain) and cationic macromolecules comprising pendent positively charged active groups, including biguanide, quaternary ammonium salts, and quaternary pyridinium or phosphonium salts; their antibacterial activity was also investigated [2]. In another study, Kenawy and co-workers focused on the chemical composition of antimicrobial macromolecular systems and various approaches to the preparation of antimicrobial polymers or copolymers, as well as the antimicrobial activities of the polymers and the main uses of antimicrobial macromolecular systems, were reported [3]. In 2005, Tew and co-workers studied the biological activities of antimicrobial polymers and found that they were influenced by the amphiphilicity of the polymer or oligomer as an entity rather than the activity of one antimicrobial portion, either embedded or covalently attached, and mostly contained antimicrobial macromolecules that can mimic the biological action of antimicrobial natural host-defence peptides (e.g., synthetic polyphenyleneethynylenes, polynorbornenes and polymethacrylates) [4].

9.1.1 Basic requirements for antimicrobial polymers

The essential characteristics that an ideal antimicrobial polymer should include: (1) simply and economically synthesised, (2) stable in long-term applications and storage at the temperature of its anticipated application, (3) not soluble in water for water disinfectant applications, (4) does not decompose to release toxic products, (5) should not be toxic or harmful to people who handle it, (6) can be regenerated upon loss of activity and (7) biocidal against a broad spectrum of pathogenic microorganisms after brief contact periods.

9.1.2 Mechanism of action and factors of activity

The principal strategy for modelling synthetic antimicrobial polymers depends upon the general structural properties of the outer envelope of different bacterial cells. The main characteristic of the outer envelope of the cells is a net negative charge (often stabilised by the presence of divalent cations such as Mg^{2+} and Ca^{2+}) due to the presence of teichoic (or lipoteichoic) acid molecules in the Gram-positive bacteria cell wall, the lipopolysaccharides and phospholipids in the Gram-negative bacteria outer membrane, and the cytoplasmic membrane itself, which is a combination of a phospholipid bilayer with embedded necessary functional proteins, such as enzymes. The cytoplasmic membrane has selective permeability properties (i.e., it is semipermeable) and regulates the transfer of solutes and metabolites in

and out of the cell cytoplasm [5]. As mentioned above, due to the features of the cell wall/outer membrane and cytoplasmic membrane of a cell, the main portion of antimicrobial polymers are designed to be cationic hydrophilic–hydrophobic macromolecular systems, that is, to target the cytoplasmic membrane (so-called membrane-active agents). Some polymers have links with a hydrophilic polar functional block, which tolerates cationic charge, and a hydrocarbon non-polar hydrophobic block (a hydrophobic structure, which acts as a whole link), or random copolymers formed by a hydrophobic monomer and a hydrophilic comonomer, which contained functional groups. Surface–activity properties and adsorption/absorption ability (important properties for surfactants), and the high binding affinity for bacterial cells exhibited by these polymer/copolymer structures is improved in the presence of high lipophilicity, which results in serious damage to the structural organisation and integrity of cell membranes, leading to cytoplasmic membrane disruption (in most cases), leakage of cytoplasmic contents and cell lysis.

9.1.3 Factors affecting antimicrobial activity

9.1.3.1 Effect of molecular weight

Molecular weight (MW) plays an important role in determining antimicrobial properties of a polymer. Ikeda and co-workers studied the antimicrobial activity of polyacrylates and polymethyl acrylates, containing side-chain biguanide groups, and their copolymers with acrylamide groups [6]. They found that the biocidal action of the polymethyl acrylate containing pendent biguanide groups against *Staphylococcus aureus* was obviously dependent on the MW. The optimal MW region required for action was $5 \times 10^4 - 1.2 \times 10^5$ Da. When the MW was $\leq 5 \times 10^4$ Da, the antibacterial property increased with MW up to maximum activity; conversely, the antibacterial activity decreased sharply when the MW of the polymer was over 1.2×10^5 Da [7, 8].

9.1.3.2 Effect of counterions

Kanazawa and co-workers investigated the dependency upon counteranions of poly [tributyl(4-vinylbenzyl)phosphonium] salts against *Staphylococcus aureus*; it was shown that the antibacterial activity was dependent upon the counteranion structure [9]. The counteranion activity was low when a tight ion-pair with a phosphonium ion was formed, whereas the activity was high for those counteranions liberating free ions. The antimicrobial characteristics, which were governed by the solubility products of the polymers, were in the following order: chloride >tetraflouride> perchlorate >hexafluorophosphate.

9.1.3.3 Effect of spacer length and alkyl chain

Antimicrobial activity is reliant upon spacer length as this changes the composition and charge density of the polymer, which subsequently affects the mode of interaction with the cytoplasmic membrane [10]. Ikeda and co-workers investigated poly (trialkylvinylbenzylammonium chloride) and observed that the antimicrobial activity was highest in the presence of the longest chain (C12) [11]. The explanation for the parabolic relationship between antibacterial features and alkyl chain length is related to (1) dual binding sites on the surface as the binding positions at each site differ for long and short alkyl chain replacements or (2) different aggregation behaviour for long and short hydrophobes [12].

9.1.3.4 Preparation of antimicrobial polymer

- Synthesis from antimicrobial monomers
  This synthetic method involves covalently linking antimicrobial agents that contain functional groups with high antimicrobial activity, such as hydroxyl, carboxyl or amino groups, to a variety of polymerisable derivatives or monomers prior to polymerisation. Depending on the mechanism of action of the active agent, such as depleting the bacterial food supply or bacterial membrane disruption, polymerisation may enhance or reduce the antimicrobial activity of the active agents.
- Synthesis by adding antimicrobial agents to preformed polymers
  This synthetic method has two steps: the first step involves synthesising the polymer and the second includes modification with active groups. Some monomers generally used to form the backbone of homopolymers or copolymers include vinyl benzyl chloride, methyl methacrylate, 2-chloroethyl vinyl ether, vinyl alcohol and maleic anhydride. The polymers are then activated by anchoring antimicrobial groups, such as phosphonium salts, ammonium salts or phenol groups, via quaternisation, chloride substitution or anhydride hydrolysis.
- Synthesis by adding antimicrobial agents to naturally occurring polymers
  Chitin is an abundant natural biopolymer and exhibits excellent antimicrobial properties. To obtain enhanced antimicrobial properties, deacetylated products of chitin–chitosan (CS) have to be synthesised, which includes the introduction of alkyl groups to the amine groups to produce quaternised *N*-alkyl CS derivatives, the introduction of extra quaternary ammonium grafts to CS and modification with phenolic hydroxyl moieties.
- Synthesis by insertion of antimicrobial agents into the polymer backbone
  This method involves chemical reactions to incorporate antimicrobial agents into polymeric backbones; polymers such as polyamides, polyesters and polyurethanes, which have biologically active groups, are used due to their ability to hydrolyse active drugs and small harmless molecules.

9.2 Quaternary pyridinium-functionalised polynorbornenes

Polymers exhibiting quaternary nitrogen functionalities have commonly been used as biocidal agents [13–21]. A number of polymeric disinfectants containing quaternary pyridinium groups, either in the backbone or as pendent groups, have been prepared and exhibit good antibacterial activity.

Gao and co-workers investigated the random copolymers of acrylamide and vinyl pyridine, of varying MW and pyridine content, which were subsequently quaternised with dimethyl sulfate [22]. Investigations proved that polymers with higher cationic functionality showed stronger antibacterial activity. In addition, depending on their alkyl chain length, methacrylate-based polymers with pendent pyridinium moieties were found to exhibit antibacterial activity [23].

Quaternary pyridinium polymers can show biological activity when bound to surfaces, for example, poly(4-vinyl pyridine)-modified glass surfaces, which were modified using different alkyl bromide derivatives. Suitable polymers for antibacterial applications not only exhibit antibacterial activity, but also non-toxicity to human cells (i.e., selectivity).

The structure–activity importance of synthetic pyridinium-based polymers synthesised via the ring-opening metathesis polymerisation (ROMP) of norbornene has been reported; these polymers were expected to directly affect the bacterial membrane [24–26]. The interaction of these polymers with a phospholipid membrane model has been investigated using the fluorescent dye, calcein, embedded in unilamellar vesicles.

By controlling the hydrophobic/hydrophilic equilibrium of the polymers, their selectivity for bacterial cells over red blood cells (RBC) can be improved. This property is related to the effect that the ionic nature and hydrophobic character of these polymers has on cell membrane activity. The length of the alkyl substituents in the polymer repeat units affects antibacterial effectiveness; in more hydrophobic polymers (hexyl and higher alkyl chain lengths in the repeat unit), disruption of the membrane integrity occurs more effectively. ROMP is an attractive method of synthesis, which is widely used to prepare well-defined polymers with controlled MW and low polydispersity index values (PDI) [27–30].

Work has been performed in which the quaternary pyridinium functionality was attached to 7-oxanorbornene-5,6-exo-dicarboximide-functionalised monomers, which were subsequently polymerised using a Grubbs' catalyst (Grubbs' catalysts are a series of transition metal carbene complexes used as catalysts for olefin metathesis). The polymer series, a–f, and quaternised polymer, a', (Figure 9.1 and Table 9.1) was designed to study the effect of the hydrophobic alkyl substituent on the antibacterial and haemolytic activities of polymers using dimethylformamide (DMF) as the solvent. Six different alkyl substituents from 2 to 10 carbons, which included ethyl, butyl, hexyl, octyl and decyl, as well as the aromatic phenylethyl,

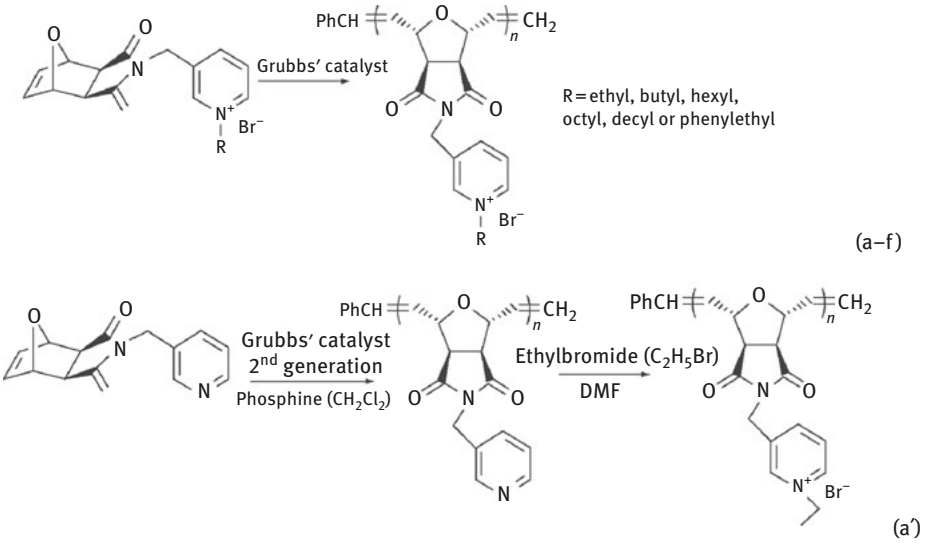

Figure 9.1: Monomers and polymers based on oxanorbornene derivatives [33].

Table 9.1: Antibacterial and haemolytic activities of polymers. Mn values are measured based on full conversion.

Mn[a)] (kDa)	Polymer	R	MIC (mg/mL)		HC_{50} (mg/mL)	Selectivity (HC_{50}/MIC)	
			Escherichia coli	Bacillus subtilis		Escherichia coli	Bacillus subtilis
3	a	Ethyl	200	200	4,030	≈20	≈20
10	a	Ethyl	200	200	>2,000	>10	>10
3[a)]	a’	Ethyl	200	200	2,000	10	10
3	b	Butyl	200	200	2,000	10	10
10	b	Butyl	200	200	1,653	8	8
3	c	Hexyl	12.5	4	<50	<4	<12.5
10	c	Hexyl	12.5	4	202	16	50
3	d	Octyl	4	4	8	1.7	1.7
10	d	Octyl	6	4	<50	<8.3	<12.5
3	e	Decyl	12.5	6	7	0.6	1.3
10	e	Decyl	12.5	6	<50	<4	<8.3
3	f	Phenylethyl	12.5	12.5	240	19	19

Table 9.1 (continued)

Mn[a] (kDa)	Polymer	R	MIC (mg/mL)		HC_{50} (mg/mL)	Selectivity (HC_{50}/MIC)	
			Escherichia coli	Bacillus subtilis		Escherichia coli	Bacillus subtilis
10	f	Phenylethyl	12.5	12.5	108	8	8
2.4	MSI-78		12.5	–	120	10	–

Mn[a] value was observed as 3 kDa by matrix-assisted laser desorption ionisation–time-of-flight mass spectrometer and synthetic pathways for (a–f) and (a') are shown in Figure 9.1.
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were polymerised using a Grubbs' third-generation catalyst in trichloroethylene or methanol at room temperature.

The activity of all monomers and polymers against *Escherichia coli* (Gram-negative bacteria) and *Bacillus subtilis* (Gram-positive bacteria), as representative bacteria, was investigated. Increasing the number of carbons in the alkyl substituent led to the generation of more potent polymers.

Both the antibacterial and haemolytic activities increased when the alkyl chain length was ≥C6. The activity of each polymer at two different MW (*Mn, th* = 3 and 10 kDa) was determined against *Escherichia coli*, *Bacillus subtilis* and human RBC (Table 9.1). Haemolytic activity was assessed as HC_{50} (the concentration of peptide required for 50% haemolysis), while the reported minimum inhibitory concentration (MIC) caused greater than 90% inhibition and typically resulted in >99% inhibition. Increasing the alkyl substituent chain length caused an increase in activity, which indicates that hydrophobicity plays a major role in the antibacterial activity of the polymers.

Increasing the hydrophobicity improves the ability of the polymers to bind into the lipid membrane; hence, increasing the concentration of bound polymer, which leads to enhanced membrane perturbation and finally cell death [31]. For similar reasons, in each polymer series, increasing the alkyl chain length led to decreased HC_{50} values (i.e., increased hydrophobicity) [32].

9.3 Functionalised antimicrobial polyethylene surfaces

Low MW biocides such as chlorinated phenols, derivatives of isothiazolone, chlorine-releasing *p*-halamines, as well as salts and complexes of metals (typically zinc

and silver), have been used over the past few decades. Materials with the ability to kill harmful microorganisms are currently receiving more attention. Although these agents work by affecting cell metabolism, their disadvantages include causing environmental toxicity and bacterial resistance. In most cases, it is possible to substitute low MW substances for macromolecular antimicrobial agents, such as polycationic substances, which have a very clear mechanism of action involving inducing a phase separation of charged and uncharged lipids inside the bacterial cytoplasmic membrane [34]. Polymers functionalised with pendent amino groups display high antimicrobial activity [35] and the polymer, poly(2-*tert*-butylaminoethyl) methacrylate (TBAM) acts as a very effective contact biocide.

9.3.1 Preparation of antimicrobial linear low-density polyethylene compounds

Linear low-density polyethylene (LLDPE) was compounded with TBAM and the surfaces of the resulting polymers were then subjected to microbiological investigation (colony forming units [CFU], biofilm formation). LLDPE and TBAM were compounded to prepare LLDPE samples containing 0, 1.5, 3 and 5 wt% of TBAM. Transmission electron microscopy micrographs of TBAM particles, dispersed in a polyethylene (PE) matrix showed that the diameter of these particles range from 0.05 to 0.5 μm. For all compounds containing less than 5 wt% of TBAM, similar results were observed.

9.3.2 Antimicrobial properties

Staphylococcus aureus and *Escherichia coli*, a Gram-positive bacteria and Gram-negative bacteria, respectively, were used to determine the antimicrobial properties of LLDPE/TBAM compounds. Pristine LLDPE was used as a control sample in all experiments. For all LLDPE compounds containing TBAM, the CFU/mL of *Staphylococcus aureus* was reduced to zero after a contact time of 24 h. For the LLDPE control surface, only a slight reduction of bacteria to 6.6×10^4 CFU/mL, from their starting concentration of 1×10^6 CFU/mL, was observed. The concentration of *Escherichia coli* was also considerably reduced during contact with the antimicrobial LLDPE samples. The compound containing 5 wt% TBAM reduced the bacterial count to 0 CFU/mL, while LLDPE compounds containing 3 and 1.5 wt% TBAM reduced the concentration to 1.4×10^3 and 1.2×10^5 CFU/mL, respectively. In contrast, *Escherichia coli* bacteria in contact with pristine LLDPE surfaces led to an increase from the initial concentration (1×10^6 CFU/mL) to 1.4×10^8 CFU/mL after 24 h.

9.3.3 Biofilm formation

Maintaining hygienic surfaces (or contact surfaces) is dependent upon the antimicrobial activity of the active agents. Deposition of microbial biomass at these surfaces allows the formation of biofilms that mainly consist of water, polysaccharides, cell fragments, dead microorganisms and other components [36]. The deposition of biofilms depends upon the ability of the surface antimicrobial agent to kill microorganisms, as well as surface roughness, and the polarity and hydrophilicity of the substrate. This means that the formation of biofilms on these surfaces reduces the antimicrobial activity of the polymer. Mucous biofilms are suitable colonisation environments for bacteria; however, the formation and adherence of biofilms to plastic substrates are undesired in many applications (e. g., water tubing). Biofilm formation on modified LLDPE surfaces was investigated. The formation of biomass (adhering biofilm) on a test substrate was determined by measuring adenosine triphosphate (ATP) levels after 8, 12 and 16 weeks of incubation in water; glass was used as a negative control (low biofilm formation), while plasticised polyvinyl chloride (PVC) plates were employed as a positive control (strong biofilm formation). After 8 weeks of testing, no adhering biofilms were detected on any of the LLDPE surfaces under investigation. After 12 and 16 weeks of incubation, biofilm formation was confirmed via ATP measurements. The results obtained after 16 weeks of incubation showed that biofilm formation on pristine LLDPE was lower (145 picogram [pg] ATP cm^{-2}) than on soft PVC (21,000 pg ATP cm^{-2}). The addition of the antimicrobial polymer TBAM to LLDPE led to a further reduction of biofilm formation to approximately 70 pg ATP cm^{-2}; however, the obtained values were higher than those recorded for glass plates (negative control; 32 pg ATP cm^{-2}). In conclusion, LLDPE compounds containing TBAM not only lowered the bacterial concentration but also led to a significant reduction of biofilm formation [37].

9.4 Functionalised antimicrobial polymers based on poly(hydroxystyrene-*co*-methyl methacrylate) derivatives

Poly(hydroxystyrene-*co*-methyl methacrylate) [poly(HS-*co*-MMA)] can be modified to add amino groups onto the side chain of the polymer. The amine-modified polymer can react with two classes of active compounds; the first class is aldehydes such as vanillin, *p*-hydroxybenzaldehyde, *p*-chlorobenzaldehyde and anisaldehyde, and the second class is phenolic esters such as *p*-hydroxymethylbenzoate, 2,4-dihydroxymethyl benzoate and methyl salicylate.

9.4.1 Synthesis of modified poly(hydroxystyrene-co-methyl methacrylate) polymers

This modification of poly(HS-*co*-MMA) aimed to add an active amino group to the copolymer by reacting it with ethylenediamine (EDA). The aminatedpoly(HS-*co*-MMA) was expected to show high reactivity to aldehyde and esters.

9.4.2 Modification of poly(hydroxystyrene-co-methyl methacrylate) with ethylene diamine

Modification of the copolymer poly(HS-*co*-MMA) with EDA resulted in aminated modified poly(HS-*co*-MMA) (I) (Figure 9.2); the amination reaction occurred in methanol (a solvent). Results obtained by elemental analysis were in good agreement with the calculated values. The infrared (IR) shows peaks at 3,365 cm^{-1} for (NH_2), a strong absorption band at 1,718 cm^{-1} for (C=O), 2,940 cm^{-1} for (CH aliphatic), a band at 1,661 cm^{-1} for (C–N) and peaks at 1,551–1,554 cm^{-1} for the –NH secondary amine. The ^{1}H-nuclear magnetic resonance (NMR) spectrum of (I) in (d_6-dimethylsulfoxide [DMSO]) was characterised by the appearance of singlet signal at 0.9 ppm due to the CH_3 proton, whilst NH_2 protons resonated at 3.1 ppm, 1.4 ppm (CH_2doublet), 2.2 ppm (CH_2 triplet), 3.3 ppm (CH_2 triplet), 2.5 ppm (CH_2 duplet), 2.4 ppm (CH triplet), 6.4–7 ppm (m,H, ArH), 4 ppm (signal for H in OH) and 8 ppm (NH signal).

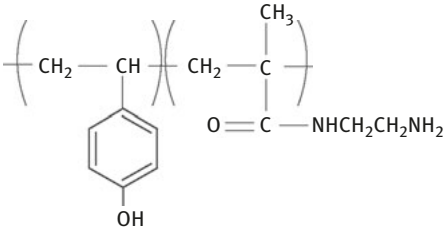


Figure 9.2: The aminated modified poly(HS-*co*-MMA) polymer (I) [38].

9.4.3 Modification of the modified poly(hydroxystyrene-co-methyl methacrylate) with aromatic aldehyde derivatives

The Schiff base reaction between modified poly(HS-*co*-MMA) and different aldehydes has been carried out using absolute ethanol. A series of derivatives of aminated poly(HS-*co*-MMA) (Figure 9.3) were prepared by condensation with vanillin (II), or *p*-hydroxybenzaldehyde (III), or a combination of *p*-chlorobenzaldehyde (IV) and *p*-methoxybenzaldehyde (V), as shown in Table 9.2, in the presence of a catalyst, glacial acetic acid, in an oil bath at 80–90 °C with stirring. Results obtained by

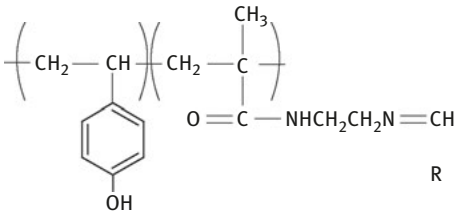
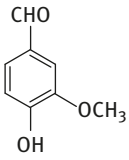
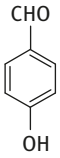
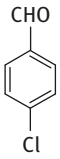
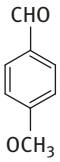


Figure 9.3: Aminated poly(HS-*co*-MMA) [38].

Table 9.2: Modified poly(HS-co-MMA) with an aromatic aldehyde [41].

Polymer	Aldehyde	R
II	Vanillin	
III	*p*-Hydroxybenzaldehyde	
IV	*p*-Chlorobenzaldehyde	
V	Anisaldehyde (*p*-methoxybenzaldehyde)	

elemental analysis were in good agreement with the calculated values. The IR spectra of the polymer (II–V) showed a strong band at 3,263–3,383 cm^{-1} due to the OH of the secondary amine, a band at 1,713–1,718 cm^{-1} due to (C=O), at 2,841–2,942 cm^{-1} due to (CH aliphatic) and the appearance of a band at 1,649 cm^{-1} due to

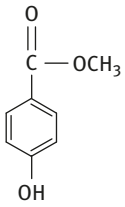
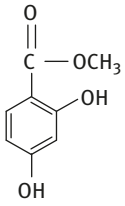
(C=N). The IR spectra of polymer (II) and (V) showed absorption bands at 2,618 and 2,608 cm^{-1}, respectively, due to the methoxy group (OCH_3), and polymer (IV) showed a strong band at 770 cm^{-1} due to the C–Cl group, peaks at 1,552–1,554 cm^{-1} were for (–NH–) and 3,365 cm^{-1} for (NH_2) in polymer (I).

9.4.4 Modification of the amine modified poly(hydroxystyrene-co-methyl methacrylate) (I) with various hydroxy aromatic esters

The reaction of absolute methyl alcohol with the corresponding acid in the presence of sulfuric acid results in the production of various phenolic esters and the esterification process was accompanied by a high conversion yield (90–95%). The ^{1}H-NMR spectrum of *p*-hydroxymethylbenzoate (VII) in DMSO indicates peaks at 10.3 ppm (1H, singlet, OH in position 4), 7.8 ppm (2H, doublet, CH in positions 3 and 5), 6.8 ppm (2H, doublet, CH at positions 2 and 6) and 3.8 ppm (3H, singlet, CH_3).

The ^{1}H-NMR spectrum of 2,4-dihydroxymethylbenzoate (VIII) (Table 9.3) in DMSO indicates peaks at 10.7 ppm (1H, singlet, OH in position 2), 10.42 ppm (1H, singlet, OH in position 4), 7.62 ppm (1H, singlet, CH in position 3), 6.25 ppm (1H, doublet, CH in position 5), 6.35 ppm (1H, doublet, CH in position 6) and 3.82 ppm (3H, singlet, OCH_3). The antimicrobial activities of the polymers can be enhanced by introducing phenol onto the polymer side chains; hence, the reaction of amine-

Table 9.3: Modified poly(HS-co-MMA) (I) with different hydroxy aromatic esters [41].

Polymer	Aldehyde	R
VII	*p*-Hydroxymethylbenzoate	
VIII	2,4-Dihydroxymethylbenzoate	

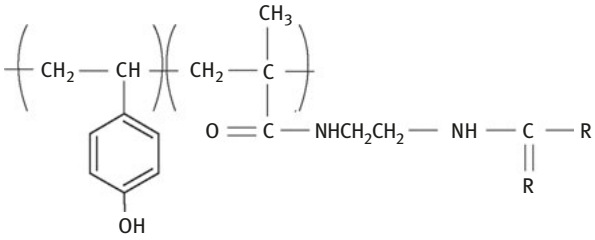


Figure 9.4: Amine modified poly(HS-*co*-MMA) [38].

containing copolymer I (Figure 9.4) with esters was conducted to introduce phenol groups to the side chains of the polymer.

Any unreacted ester was removed by washing with diethyl ether. The modified polymers were then collected, dried and characterised. In general, the reactions occurred easily and without any problems, and the predicted higher yields were achieved. The products were characterised by elemental analysis. Absorption bands at 3,395–3,490 cm^{-1} were due to the stretching vibrations of the OH group and a strong band at 1,714–1,716 cm^{-1} was due to the C=O group. The absorption band at 2,940–2,942 cm^{-1} was due to the CH aliphatic group and peaks at 1,551–1,554 cm^{-1} indicated (NH).

9.4.5 Antimicrobial activity of poly(hydroxystyrene-co-methyl methacrylate) and its derivatives

The antimicrobial activity of the synthesised derivatives of poly(hydroxystyrene-*co*-methyl methacrylate) were mainly anticandidal (for the treatment of candidal infection) and antifungal rather than antibacterial. Polymers I and II exhibited the highest antimicrobial activity against all the tested microorganisms. Polymers III and IV showed higher activity against bacterial species than fungi. Polymer I showed the highest activity against *Trichophytonrubrum*, while polymer II showed the highest activity against *Fusarium oxysporum*. At high concentrations these synthesised derivatives showed toxicity to the larvae of Brine shrimp [38].

9.5 *p*-chloroacetophenone oxime-based polymers exhibit biological activity

Polymer additives can improve manufacturing processes and product quality, as they aid the formation of a continuous coating phase without any

detrimental effect to the polymer. Biomedical applications of acrylic terpolymer with arginylglycylaspartic acid, that is, a tripeptide composed of L-arginine, glycine and L-aspartic acid peptides, have been studied by Fussell and Cooper [39]. Chauhan and co-workers have investigated the biological activities of synthesised terpolymers based on *p*-hydroxybenzaldehyde oxime [40]; the synthesis and characterisation of a monomer, (*p*-chloroacetophenone oxime), and its copolymer with formaldehyde (CAO-F), and terpolymer with formaldehyde and benzoic acid (CAO-F-BA) were discussed. The molecular structures of CAO, CAO-F and CAO-F-BA were assessed using Fourier-Transform infrared (FTIR) and ^{1}H-NMR data. The ^{1}H-NMR spectra of the monomer, copolymer and terpolymer are shown in Figure 9.5.

9.5.1 Antimicrobial activity

The antibacterial activity of the synthesised resins was tested against *Bacillus subtilis, Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*; antifungal activity was tested against *Alternaria solani* and *Fusarium oxysporum*. The inhibition zone and activity index were measured (see Figure 9.6 for antibacterial activity and Figure 9.7 for antifungal activity) compared with a standard antibacterial drug (ciprofloxacin) and a standard antifungal drug (amphotericin-B).

Both CAO-F and CAO-F-BA demonstrated good activity against all bacteria and fungi used; CAO-F-BA showed excellent antifungal and antibacterial activities in comparison to CAO-F.

9.6 Hydroxyquinoline-based polymers

Newly discovered functional terpolymers are utilised in a wide range of applications, such as electronic devices and as an additive to biological materials [41, 42]. In the biomaterial field, terpolymers are assessed according to their drug-carrying potential, treatment applications and ability to be desirable crosslinking materials and molecular switches [43–45]. 8-Hydroxyquinoline (HQ)-based polymers exhibit various properties, which include being an excellent cation exchanger for selective metal ions [46], and good luminescence [47], photovoltaic [48] and antimicrobial activities [49]. Functional terpolymers obtained from HQ and 4-chloroacetophenone (CA) are applicable for antimicrobial coating applications when formaldehyde (F) is used as the reagent.

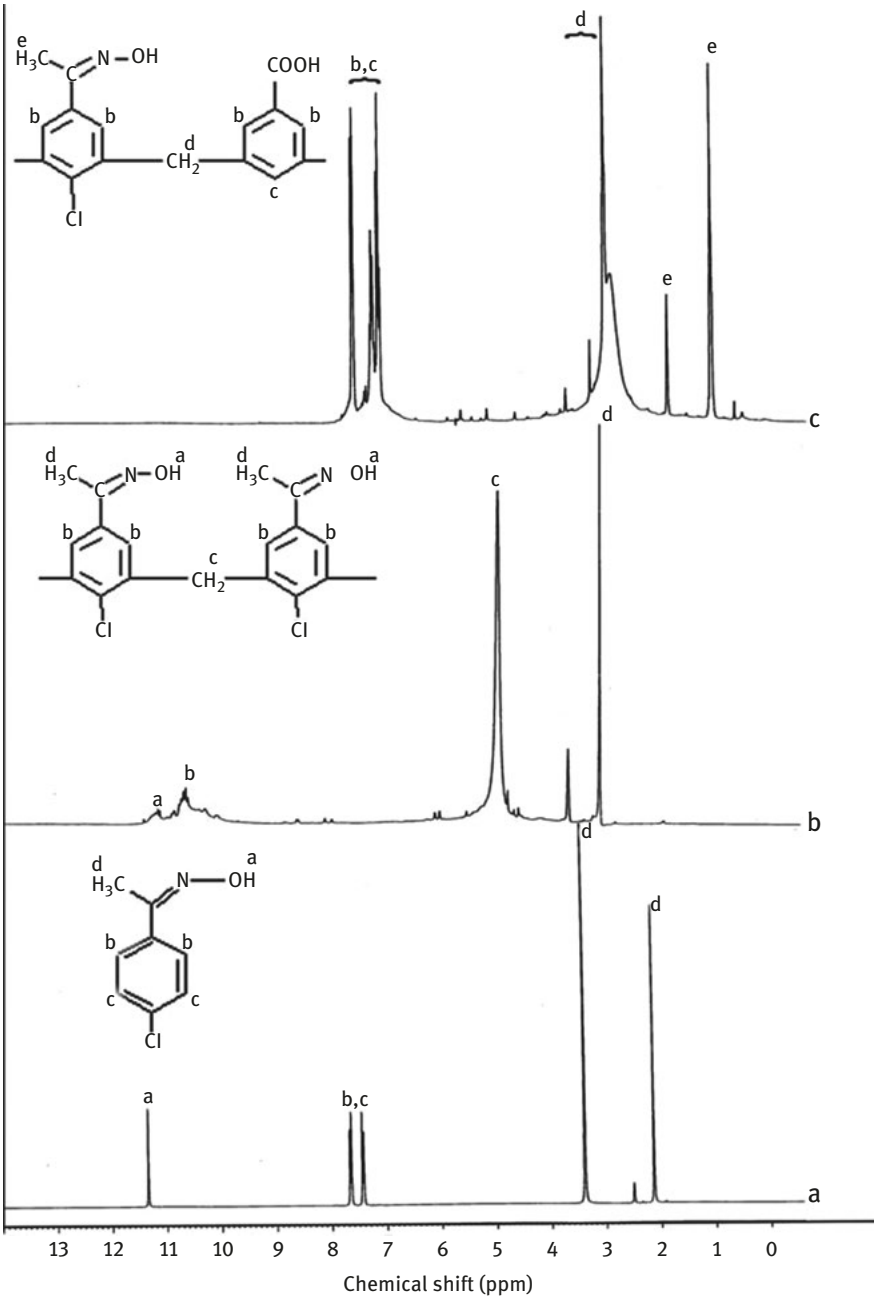


Figure 9.5: [1]H-NMR spectra of (a) the monomer, CAO, (b) the copolymer, CAO-F and (c) the terpolymer, CAO-F-BA. Reproduced with permission from N.P.S. Chauhan, R. Ameta and S.C. Ameta, *Journal of Applied Polymer Science*, 2011, 122, 573. ©2011, John Wiley & Sons [40].

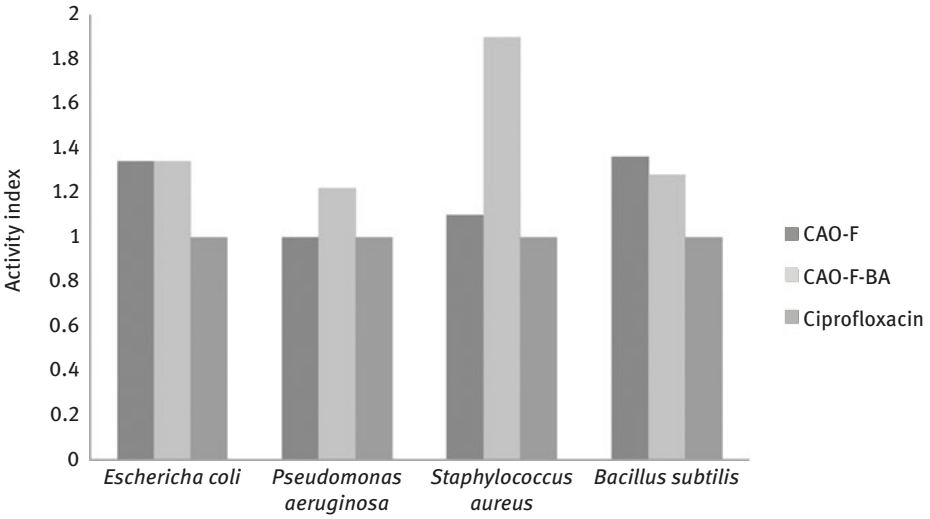


Figure 9.6: Copolymer CAO-F, terpolymer CAO-F-BA and comparison with standard drug antibacterial activity. Reproduced with permission from N.P.S. Chauhan, R. Ameta and S.C. Ameta, *Journal of Applied Polymer Science*, 2011, 122, 573. ©2011, John Wiley & Sons [40].

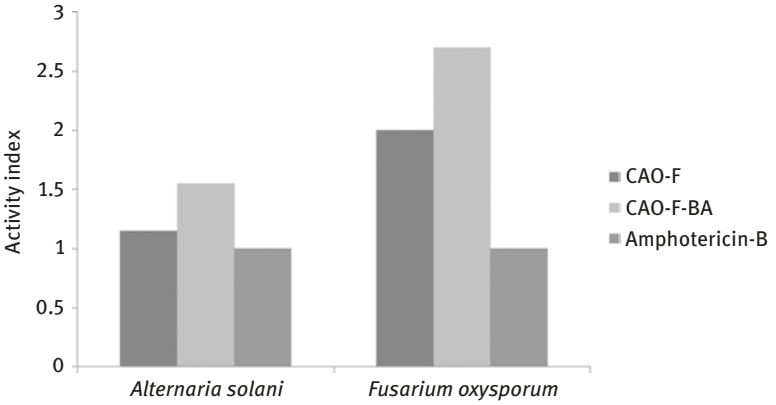
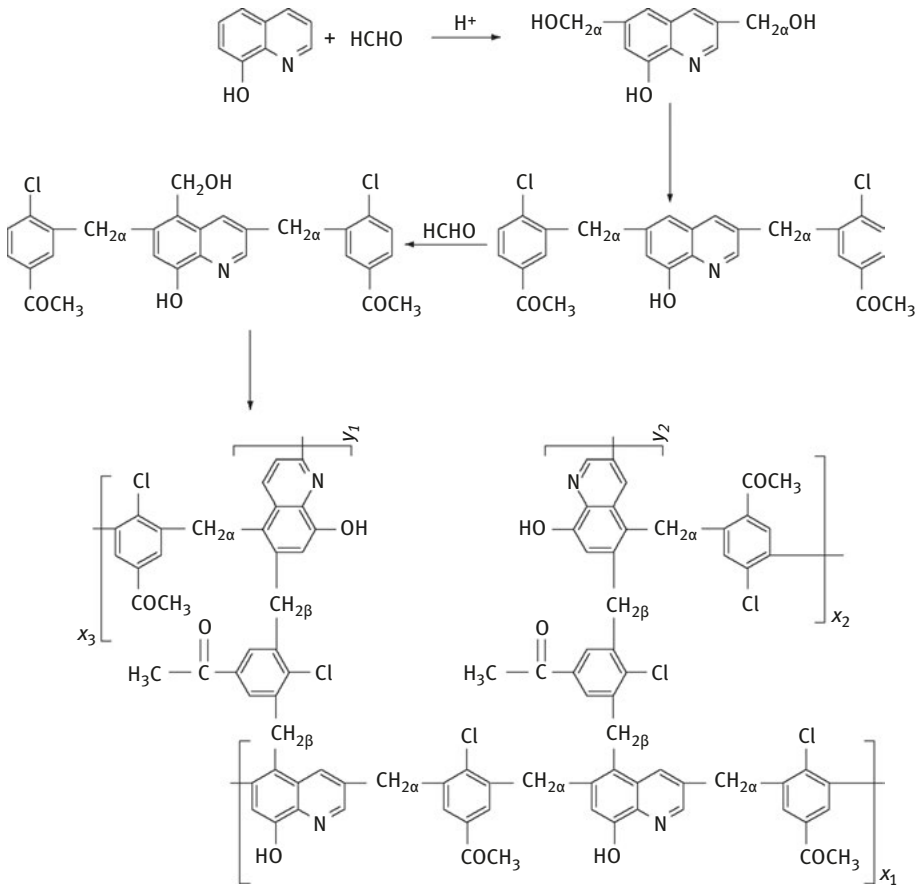


Figure 9.7: Copolymer CAO-F, terpolymer CAO-F-BA and comparison with standard drug antifungal activity. Reproduced with permission from N.P.S. Chauhan, R. Ameta and S.C. Ameta, *Journal of Applied Polymer Science*, 2011, 122, 573. ©2011, John Wiley & Sons [40].

9.6.1 Antimicrobial activities

In general, the antibacterial mechanism of a terpolymer disinfectant involves six steps [50]: (1) adsorption onto the bacterial cell surface, (2) diffusion through the cell wall, (3) connection to the cytoplasmic membrane, (4) rupture of the



Scheme 9.1: Overall strategy of synthesising the terpolymer HQ-F-CA. Reproduced with permission from N.P.S Chauhan, Structural and thermal characterization of macro-branched functional terpolymer containing 8-hydroxyquinoline moieties with enhancing biocidal properties *Journal of Industrial and Engineering Chemistry*, 2013, 19, 1014–1023. ©2013, Elsevier [51].

cytoplasmic membrane, (5) further action of the quaternary iminium groups permeate the intracellular contents, such as K^+ ions, deoxyribonucleic acid and ribonucleic acid, and (6) bacterial cell death (Figure 9.8). The results for the antibacterial and antifungal activity are shown in Table 9.4, ciprofloxacin and amphotericin-B were used as the controls. Antimicrobial screening data confirms that HQ-F-CA exhibits better activity compared with other resins, such as the self-crosslinkable terpolymer 4-acetylpyridineoxime-formaldehyde-acetophenone (4-APO-F-A), which could be due to the presence of hydroxyl, acetyl and chloro groups [51].

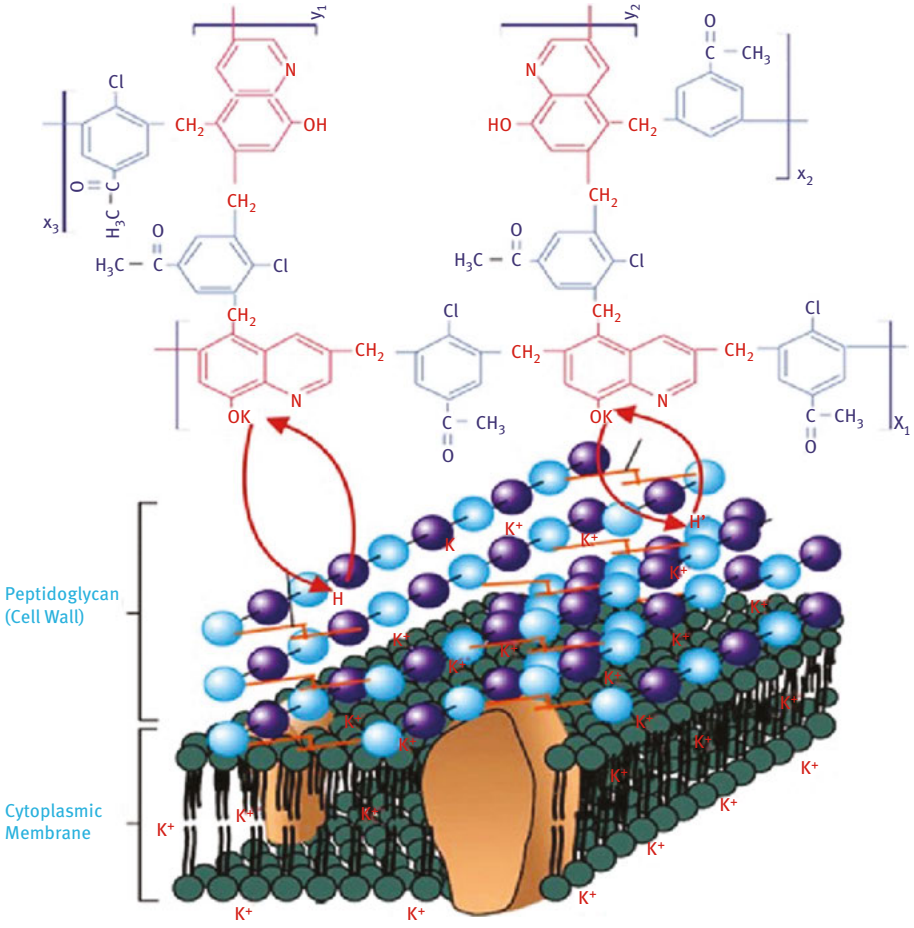


Figure 9.8: Biocidal ion exchange mechanism of the terpolymer. Reproduced with permission from N.P.S Chauhan, *Journal of Industrial and Engineering Chemistry*, 2013, 19, 1014. ©2013, Elsevier [51].

9.7 Antifouling copolymer brushes based on 2-(2-methoxyethoxy) ethyl methacrylate and hydroxyl-terminated oligoethylene glycol methacrylate

Bacterial adhesion onto a material surface is a significant stage in various applications, such as ship hull fouling, and contamination of medical devices, industrial cooling water systems and food processing equipment [52]. When bacteria adhere onto a

Table 9.4: Antimicrobial data for 4-APO-F-A and HQ-F-CA with respect to standard drugs (in brackets).

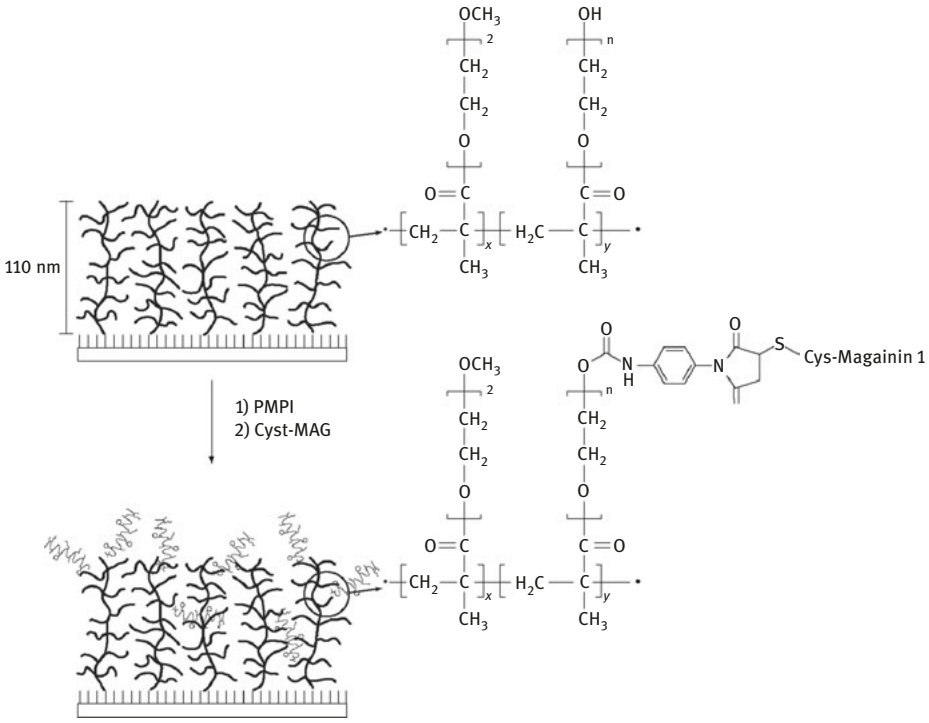
Terpolymer	Inhibition zone in mm (activity index)					
	Gram-negative bacteria		Gram-positive bacteria		Fungi	
	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Bacillus subtilis	Alternaria solani	Fusarium oxysporum
HQ-F-CA	29 (2.07)	31 (2.58)	27 (2.70)	35 (3.18)	25 (2.08)	23 (3.67)
4-APO-F-A	25 (1.78)	25 (2.08)	20 (2.00)	27 (2.48)	18 (1.50)	19 (3.17)

Reproduced with permission from N.P.S Chauhan, *Journal of Industrial and Engineering Chemistry*, 2013, 19, 1014. ©2013, Elsevier [54].

solid surface, they form colonies and then biofilms, which enables the development of pathogenic infections [53]. Many investigations have focused on producing thin coatings that reduce bacterial adhesion onto solid surfaces. The applied methods were mostly based on two different strategies: (i) the immobilisation of biocidal substances and (ii) the deposition of an antifouling coating, which prevents proteins and subsequently cell adhesion onto the surface. The most common techniques to immobilise antibacterial substances onto solid surfaces include chemical grafting, surface impregnation or physical entrapment [54]. Similar to non-adhesive coatings, they mainly comprise self-assembled monolayers (SAM) or polymer brushes based on polyethylene glycol or its derivatives [55–57]. Polymer brushes are of interest due to their role in the engineering of the surface properties of a material [58]. They present higher mechanical and chemical robustness in comparison to other surface modification methods (e. g., SAM), which leads to greater long-term stability. Among the techniques used to prepare polymer brushes, the 'grafting from' approach, based on surface-initiated atom transfer radical polymerisation (ATRP), offers an experimentally straightforward route to dense brushes with a narrow PDI, a controlled architecture and a well-defined thickness and composition [59]. The oriented end-grafting of an AMP onto poly[2-(2-methoxyethoxy)] ethyl methacrylate-*co*-hydroxyl-terminated oligoethylene glycol methacrylate [poly(MEO_2MA-*co*-HOEGMA)] brushes has been described. In addition to their non-adhesive behaviour, these copolymer brushes contain reactive hydroxyl groups, which allow the controlled loading of the brushes with AMP resulting in the activity of the obtained coatings.

Magainin I, a 23 residue defence peptide was first isolated from the skin of the African clawed frog *Xenopuslaevis* [60]; it was selected for its broad biocidal activity against Gram-positive and Gram-negative bacteria and its non-haemolytic properties at its effective antimicrobial concentration. Magainin-functionalised copolymer brushes of various compositions were tested against two different Gram-positive bacteria. To prepare antibacterial coatings, which combined both biocidal and non-

adhesive properties, the tethering of a natural defence peptide, magainin I, on poly (MEO_2MA-*co*-HOEGMA) brush platforms was explored. Magainin I exerts its toxic effect against bacteria by permeabilising the cell membranes via the formation of pores or structural defects in the membranes [61], it is therefore important to maintain its mobility and accessibility after attachment onto the copolymer brushes. To maintain the properties that magainin I displays in solution when attached to the brushes, the peptide derivatives were tethered on the hydroxyl extremities of the relatively long water-soluble and flexible oligoethylene glycol side chains of the poly(MOE_2MA-*co*-HOEGMA) brushes (Scheme 9.2). Furthermore, to position the peptide on the surface and allow it to easily access bacterial membranes, a cysteine residue was attached to the C-terminal side of magainin I; as magainin I does not contain a cysteine residue, the magainin I derivative MAG-Cys could be exclusively attached to the brushes via its C-terminal portion, by use of an *N*-(*p*-maleimidophenyl)isocyanate (PMPI) heterolinker (Scheme 9.2). The isocyanate moiety of the linker reacts with the hydroxyl group of the copolymer brushes and the maleimide



Scheme 9.2: Oriented grafting of a MAG-Cys derivative onto poly(MOE_2MA-*co*-HOEGMA) brushes via a PMPI heterolinker. Reproduced with permission from K. Glinel, A.M. Jonas, T. Jouenne, J. Leprince, L.G, Wilhelm and T.S. Huck, *Bioconjugate Chemistry*, 2009, 20, 71. ©2009, American Chemical Society [69].

reacts with the thiol function of the MAG-Cys or biotinyl-MAG-Cys derivative [62]. An interesting feature of the poly(MOE_2MA-*co*-HOEGMA) brushes is the possibility of changing their functionality via the amount of immobilised magainin I present on the hydroxyl-reactive groups, which can be achieved simply by changing the composition of the monomer mixture used. Various copolymer brushes with an increasing content of the reactive HOEGMA were prepared this way and subsequently functionalised by magainin I derivatives.

To qualitatively investigate the effect of altering the amount of magainin I grafted onto the copolymer brush surface, the brushes were functionalised with a biotin-tagged derivative (biotinyl-MAG-Cys) and then incubated with streptavidin-fluorescein isothiocyanate (streptavidin-FITC); the surface-immobilised streptavidin-FITC was detected using fluorescence microscopy. For all tested biotinyl-MAG-Cys-functionalised brushes, the surfaces were uniformly fluorescent at the micrometre scale, which indicates the uniform immobilisation of the biotin-tagged magainin I molecules on the surface. In contrast, the absence of fluorescence caused by brushes not functionalised by magainin I was evidence of their inactivity toward protein adsorption. It was therefore concluded that the fluorescence detected on functional brushes arises from the streptavidin binding to the biotin-tagged brushes, not from unspecific adsorption. The concentration of grafted peptide was indirectly estimated as a function of the composition of the copolymer brush (e.g., HOEGMA content) by extracting the average fluorescence intensity via image analysis. To achieve this, a deliberate scratch was made on the sample surface to determine the background fluorescence signal. The antibacterial properties of the MAG-Cys-modified brushes were tested against two strains of Gram-positive bacteria: *Listeria ivanovii* and *Bacillus cereus*; the first causes serious food poisoning [63], the other could cause foodborne diseases and exhibits a strong ability to adhere to and form biofilms on stainless steel [64] and glass [65].

In the experiment, the functionalised silicon substrates were incubated in bacterial suspensions for 3 h and immediately observed using optical microscopy. A control adhesion test using unmodified copolymer brushes was also performed for comparison. The inspection of recorded micrographs (Figure 9.9) revealed the absence of bacteria on the unmodified poly(MOE_2MA-*co*-HOEGMA) brush for both tested strains, which is evidence of the inertness of this brush platform. A similar result was obtained for unmodified copolymer brushes of varying composition. In contrast, some bacteria were observed on magainin-functionalised brushes, but the cell coverage did not exceed 1% of the surface, independent of the copolymer brush composition. This result indicates that the bacterial attachment onto the brushes is exclusively due to the presence of the magainin I peptide, which interacts with cell membranes. To check the viability of the bacteria observed on the magainin-functionalised brushes, the adhering cells were stained with the fluorescent LIVE/DEAD® viability kit before observation via confocal laser scanning microscopy (CLSM) [66]. Inspection of the images obtained using the green and

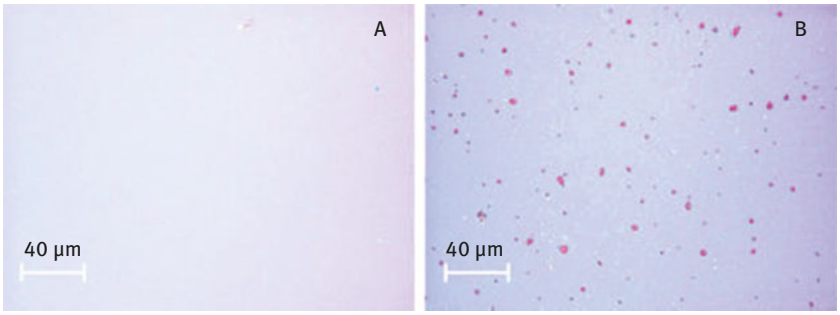


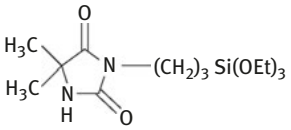
Figure 9.9: Optical micrographs of the brush surface after 3 h incubation in a suspension of *Listeria ivanovii*: (a) non-functionalised and (b) MAG-Cys-functionalisedpoly(MEO_2MA-*co*-HOEGMA) brushes. Reproduced with permission from K. Glinel, A.M. Jonas, T. Jouenne, J. Leprince, L.G, Wilhelm and T.S. Huck, *Bioconjugate Chemistry*, 2009, 20, 71. ©2009, American Chemical Society [69].

red channels of the CLSM of magainin-functionalised brushes exposed to *Listeria ivanovii* revealed only the presence of red stained cells corresponding to dead bacteria; this observation was also made for the sample of lower grafting ratio. Similar assays were performed on *Bacillus cereus*. CLSM observations showed that all *Bacillus cereus* cells adhering to the surface of the functionalised brushes were partially red stained, which is evidence of damage to the membrane. The fact that *Bacillus cereus* cells were not seen to be completely red stained is most probably due to the staining process, which depends on the experimental conditions and the bacteria used, as previously noted [67, 68]. Bacterial cell counts performed with *Listeria ivanovii* after 3 h of contact, confirmed the antimicrobial properties of the magainin-grafted brushes; the assays performed using both bacterial species demonstrated the high efficiency of the magainin-functionalised copolymer brushes to kill all bacterial cells adhering to the surface. Biocidal activity was detected even for the lower grafting ratio of the tested magainin I peptide. Magainin-functionalised brushes provide efficient protection against bacterial growth and progression by killing cells, which are in contact with them. The presence of the AMP combined with the intrinsic antifouling properties of the brushes should result in a long delay in biofilm formation, which is of interest for many applications in the medicine and food industries.

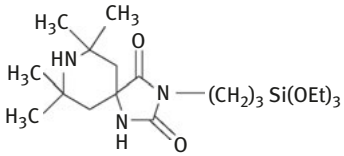
9.8 *N*-halamine acrylamide monomer and its copolymers for antimicrobial coatings

N-halamines are excellent antimicrobial materials with regards to biocidal efficacies, stability in aqueous solution and dry storage, lack of surface corrosion,

low toxicity and relatively low production cost [70]. *N*-halamine chemistry has been applied to numerous materials to impart antimicrobial properties [71]. Antimicrobial *N*-halamine moieties have been attached to surfaces such as cellulose fibres by several grafting, tethering [72] and polymerisation methods [73]. One of the more successful methods is to bond the *N*-halamine precursor to a silane (Figure 9.10), which can then tether to a surface via covalent ether linkages. Among the various *N*-halamine compounds, heterocyclic structures such as hydantoins, oxazolidinones, imidazolidinones and triazines have been widely studied to obtain higher stable aliphatic structures [74]. In addition, acyclic halamine structures have also been added to materials to produce more stable cyclic *N*-halamines [75].



5,5-Dimethyl-3-(3′-triethoxysilyl propyl)-hydantion



3-(3′-Triethoxysilyl propyl)-7,7,9,9-tetramethyl-1,3,8-triazaspiro[4.5]-decane-2′4-dione

Figure 9.10: Structure of previously synthesised *N*-halamine compounds [79].

A new *N*-halamine monomeric compound, hydantoin acrylamide (HA), was synthesised by forming a hydantoin ring from the ketone moiety of a secondary amide monomer, *N*-(1,1-dimethyl-3-oxobutyl)acrylamide (DA), which has been used for coatings and textile applications [76]. Amide and imide moieties of the hydantoin ring halogenate more easily than sterically hindered secondary amides; this method can be used to impart antimicrobial properties to films, coatings or paint. HA can be copolymerised with many commercial monomers.

HA was copolymerised with a siloxane monomer, poly(hydantoin acrylamide siloxane), in various ratios to impart tethering functionality. Testing of the obtained cellulose-coated copolymers included hydrolysis during washing tests, stability to ultraviolet (UV)-A irradiation in a weathering chamber and biocidal efficacy against Gram-positive and Gram-negative bacteria.

9.8.1 Synthesis and characterisation of hydantoin acrylamide

HA was synthesised via the Bucherer–Berg reaction. *N*-halamine coatings and polymers have been synthesised using a similar approach, which led to the formation of a hydantoin ring from a ketone moiety [77]. The initial material, DA, only has one amide group and, during the reaction, a hydantoin ring was formed on the moiety, which increased the number of N–H groups from one to three, and thus increased the theoretical chlorine loading of the compound from 17 to 31 wt%. DA has a melting point of 57 °C, but HA has a melting point of 178 °C due to the increased hydrogen bonding and formation of hydantoin rings; NMR and FTIR analysis were used to investigate the reactions. There are several additional signals following hydantoin ring formation. First, a signal for a methyl group (a) at 2.02 ppm and methylene group (b) at 2.97 ppm, adjacent to the carbonyl moiety, were shifted to 1.23 and 2.15 ppm, respectively, due to the disappearance of the neighbouring electron-withdrawing carbonyl moiety. Two additional signals at 7.58 and 10.58 ppm can be assigned to the amide (c) and imide (d) proton moieties, respectively, of the hydantoin ring. ^{13}C-NMR spectra of HA also clarified the formation of the hydantoin ring from the ketone moiety of DA by the disappearance of resonance at 206.92 ppm and the appearance of two signals at 155.99 and 178.65 ppm. The FTIR spectrum of HA has an additional N–H stretching band at 3,203 cm^{-1} and two carbonyl stretching bands of the hydantoin ring at 1,759 and 1,704 cm^{-1}.

9.8.2 Synthesis and characterisation of copolymers

Different compositions of HA were copolymerised with siloxane monomer (SL) and the feed ratio of copolymers (M_1/M_2) varied from 0.2 to 5. The elemental viscosities of copolymers were 0.55, 0.63 and 0.34 dl/g, respectively (in 2-methoxyethanol at 25 °C), indicating that polymerisation had occurred. The amount of HA in the copolymer contributed to the halogen-loading capability (antimicrobial property), whereas the tethering SL contributed to the adhesion property of the copolymers. The average composition of monomers in the copolymers was determined from the corresponding ^{1}H-NMR spectra. The mole fraction of HA in the copolymer was calculated by comparing the signal area of total methyl group protons attached to the silicon atom [-Si-$(OCH_3)_3$] to the imide proton signal area of the HA moiety.

9.8.3 Stability of poly(hydantoin acrylamide siloxane) after washing and ultraviolet light irradiation

Three types of washing experiments were performed on coated fabrics – prechlorinated coatings (C) at concentration levels indicated at 0 machine washes,

prechlorinated and rechlorinated after a given number of machine washes (R) and unchlorinated after a given number of machine washes (U). Several observations can be made; first, the initial chlorine loading of the coated fabrics (0 machine washes) was increased by increasing the HA concentration in the copolymer, due to increased N–H sites in the copolymers. The prechlorinated coatings (C) lost most of their initial chlorine loadings within 10–25 washes; however, this rate refers to the N–Cl bond dissociation and is not a result of the dissociation of tethering groups (siloxane) from cotton as rechlorination of the copolymers resulted in chlorine loadings (R) at about their initial values. All of the unchlorinated copolymers (U) were also very resistant to decomposition during washing cycles. The stability of the copolymers on cotton was increased by increasing the amount of the siloxane groups (SL) in the copolymer composition.

9.8.4 Antimicrobial efficacies

The treated cotton swatches were challenged with *Escherichia coli* O157:H7 and *Staphylococcus aureus* at concentrations of about 10^8 CFU. The unchlorinated control samples resulted in only about 0.10 log reduction, due to the adhesion of bacteria to the cotton swatches within a 30 min contact time interval. All of the chlorinated coated samples with chlorine loadings of 0.23–0.24% exhibited excellent antimicrobial activity. Chlorine inactivated all *Staphylococcus aureus* growth with a log reduction of approximately 8.2 in a contact time of 10 min and 5 min, in Experiment 1 and Experiment 2, respectively. The two repeated experiments indicated a negligible difference in results between the experiments. When the experiment was run a third time, a 7.98 log reduction was obtained at the 5 min contact interval, which could be due to performing repeated bacterial testing on textile surfaces, which are inherently variable [81]. On the other hand, all *Escherichia coli* O157:H7 growth was inactivated with a log reduction of around 8.2 at a contact time of 5 min in repeated experiments. It is notable that a sample of Cl containing a chlorine loading of only 0.10 wt% produced log reductions of 7.98 and 8.01 for *Staphylococcus aureus* and *Escherichia coli* O157:H7, respectively, at contact times of 5 and 10 min [79].

9.9 Conclusion

The interest in antimicrobial polymers as alternatives to existing biocides, and in some cases, to antibiotics has increased. Investigations focusing on the initial synthesis of pyridine-functionalised oxanorbornene-based monomers, their ROMP, biological activity (MIC, HC_{50}) and model membrane behaviours, prove that alkyl

substituents exert a unique impact on activity. To study antimicrobial PE surfaces, LLDPE was compounded with the polymeric biocide TBAM (bulk modification with 1.5–5.0 wt% of TBAM); the prepared surfaces were then subjected to microbial assays. It was determined that the terpolymer CAO-F-BA requires a higher activation energy compared with the copolymer CAO-F; hence, this product is applicable in the field of advanced materials. CAO-F-BA shows long-lasting antibacterial and antifungal properties. 8-hydroxyquinoline, formaldehyde and 4-CA can be used in antimicrobial coating applications and potentially other applications in the bioengineering field. Surface-initiated ATRP is applicable to the synthesis of non-adhesive poly(MEO_2MA-*co*-HOEGMA) brushes, which are used as a basis to covalently immobilise a natural antibacterial peptide. The peptide can be tethered in various concentrations by changing the composition of the copolymer brushes, which allows variation of the biocidal activity of the coatings; even for low levels of grafted peptide, a high biocidal activity of the peptide-functionalised brushes can be obtained and this property is very useful in the medical and food industries. A new *N*-halamine monomer, HA, has been successfully synthesised from a commercial acrylamide monomer without damaging the vinyl bonds. On a cotton fabric, chlorinated copolymers caused an approximately 8-log reduction of growth of both Gram-negative and Gram-positive bacteria within 5–10 min; *N*-halamine compounds seem to be efficient antimicrobial agents for controlling infection.

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10 Antimicrobial activities of *N*-halamine-based polymers

Abstract: *N*-helamine–based polymers have various properties such as long-term stabilities, regenerability and high stability, relatively lower cost and long-lasting biocidal properties. The bactericidal activities of these polymers are due to oxidative halogen moieties.

Keywords: N-helamine, biofilm, coating, antibacterial

10.1 Introduction

Bacterial contamination is of great concern in numerous fields, including medical devices, healthcare products, water purification systems, food storage and packaging, hospitals, dental office equipment and household sanitation. As a response to widespread microbial threat, antibacterial materials of various kinds have attracted significant research interest (Desnose et al 2019, Chen et al 2011) [1–5]. Among the many antibacterial materials currently available, *N*-halamine is an important compound and is the most promising due to several advantages such as powerful antibacterial activity, long-term stability, high durability, low cost and regenerability [6]. *N*-halamine is an effective oxidising agent and its bactericidal action is considered to be a result of a chemical reaction involving the transfer of positive halogen from *N*-halamine to an appropriate bacterial receptor [7]. This reaction effectively inhibits or, in some cases, destroys cellular enzymatic or metabolic processes, resulting in microbial death [8]. Consequently, considerable research effort has been devoted to fabricating various *N*-halamine-based antibacterial materials [9]. In this chapter, recent developments are described and emphasis is placed on the structure of monomeric *N*-halamines and *N*-halamine-based polymers, which exhibit biocidal properties.

10.2 Structure of *N*-halamine

The initial concept of *N*-halamine compounds was proposed by Kovacic and co-workers in 1969 [10, 11]. An *N*-halamine compound can be defined as a compound

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containing one or more nitrogen–halogen (N–X) covalent bonds, which are usually formed via the halogenation of imide, amide or amine groups [12–14]. *N*-halamine exhibits biocidal properties due to the +1 oxidation state of the halide atoms in the chloramine (>N–Cl) or bromamine (>N–Br) groups. Various structures of inorganic *N*-halamine monomers are presented in Figures 10.1 and 10.2. *N*-halamine can contain inorganic groups such as phosphate, sulfate and so on, and organic groups such as alkyl and carbonyl, which are referred to as inorganic and organic *N*-halamines, respectively.

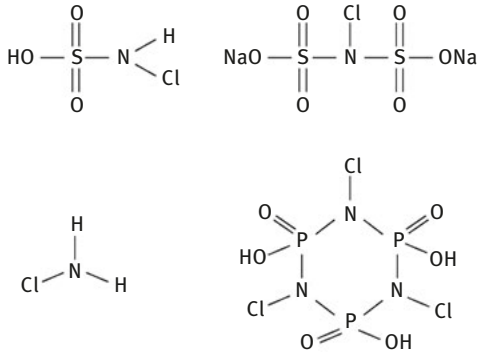

Figure 10.1: Structure of some inorganic *N*-halamines.

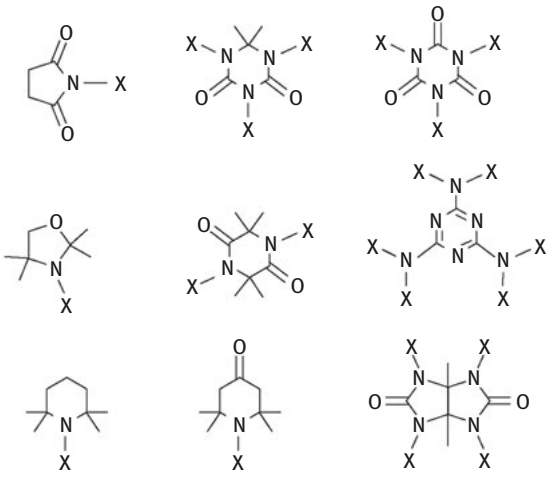

Figure 10.2: Structures of some cyclic organic *N*-halamines; X = Cl, Br or H.

Polymers can be emulsified in water to produce coatings which, once chlorinated, act as contact disinfectants. These coated surfaces efficiently inactivate microbes

within relatively brief contact times, that is, several minutes. Antimicrobial latexes can be formed via the copolymerisation of an *N*-halamine precursor monomer with other monomers in water, in the presence of a surfactant, or by chemically grafting the *N*-halamine precursor monomer onto an emulsified polymer backbone, followed by chlorination.

N-halamine polymers are the derivatives of polymeric materials, which contain haloamine functional groups. Because of their diverse applications, they have received considerable attention over the past few decades. In general, biocidal *N*-halamine polymers can be prepared via four methods. The first approach is the polymerisation of *N*-halamine monomers, or monomers bearing the N–H bond, by themselves or with other monomers to generate biocidal homopolymers or heteropolymers, respectively. The second method, which is less common, involves the electrochemical generation of biocidal coatings, where the monomer is a protein. The third method is to graft or coat *N*-halamine precursor monomers onto polymer backbones or to modify the polymer units to form *N*-halamine derivatives, for example, biocidal moieties can be grafted onto a commercial polymeric resin by modifying its surface. The fourth method involves adding *N*-halamine monomers or polymers to the host polymer just before polymer processing or fibre extrusion. It has been reported that hydantoin (imidazolidine-2,4-dione) and dimethyl hydantoin are the basic moieties of many *N*-halamine polymers used for coating-based applications.

Many biocidal *N*-halamine polymers have been prepared by homo- or heteropolymerisation. *N*-halamine polyurethane was synthesised by copolymerising a heterocyclic ring-based monomer with toluene-2,6-diisocyanate or toluene-2,4-diisocyanate. In addition, an *N*-halamine acrylamide monomer, a hydantoin acrylamide (HA), was forming using a hydantoin ring on the ketone moiety of a secondary amide monomer, *N*-(1,1-dimethyl-3-oxobutyl) acrylamide. HA has been copolymerised with a siloxane monomer (SL) using different feed ratios [15] and the resulting copolymers were covalently coated onto cotton fabric via the siloxane groups. Because of the advantage of the polymeric coatings tethering onto surfaces via multiple bonds, their stability against washing was exceptional. However, surfaces can also be coated using the layer-by-layer (LbL) technique, which was initially used for *N*-halamine biocidal coatings by Cerkez and co-workers [16]. Because of the fact that bleached cotton is inherently negatively charged, as indicated by a negative zeta potential, positively charged *N*-halamine copolymers were able to be deposited without surface modification. In fact, since the charged copolymers are very soluble in water, coating via the LbL technique may have potential use in the industrial production of antimicrobial functional or multifunctional textile coatings.

A series of water-dispersible biocidal polymers has been prepared via the copolymerisation of HA with the sodium salt of 2-acrylamido-2-methylpropane sulfonic acid [17, 18]. The addition of the synthesised copolymers, at a concentration of 1.5 wt%, to a commercial water-based latex paint resulted in antimicrobial copolymers upon chlorination with dilute household bleach.

Another innovative way to prepare organic coatings, which exhibit antibacterial properties, due to the presence of *N*-halamine groups, is to modify proteins using electrochemically generated hypochlorite or hypobromite ions, notably at a tin oxide electrode, and is particularly effective if the protein contains numerous amino acid residues bearing amine groups such as histidine or lysine, as is the case with bovine serum albumin (BSA). In fact, in the presence of hypochlorous or hypobromous acid, electrochemically generated by the anodic oxidation of chloride or bromide ions, respectively, and the polymerisation of protein leads to poly(BSA) and the formation of nanoclusters of modified proteins, that is, proteins containing chlorine or bromine depending on the nature of the electrolyte used. When an antimony-doped tin (II) oxide electrode is polarised in an aqueous solution containing chloride/bromine, the chloride and/or bromide ions (X^-) are oxidised at the electrode surface, and this leads to hypochlorous and hypobromous acid.

$$2X^- \rightarrow X_2 + 2e^- \tag{10.1}$$

In water, chlorine (Cl_2) and bromine (Br_2) dissociate into HOCl (at pH >2.5) and HOBr (at pH >5.7):

$$X_2 + H_2O \rightarrow HOX + H^+ + X^- \tag{10.2}$$

Therefore, the chemical modification of the tin dioxide surface during halide ion oxidation (Eqs. 10.1 and 10.2) is due to the reaction of hypohalogenous acid with the protein side-chains. Actually, during the process, two types of reaction occur: (i) the oxidation of sulfur atoms and (ii) the substitution of the hydrogen (H) of some amine/imine/amide groups, that is, oxidation and halogenation of the protein side-chains occurs.

A great number of biocidal polymers have been prepared using a grafting or coating procedure. *N*-halamine precursors can be bonded to substrates via different groups, such as epoxide, hydroxyl groups (diol) and alkoxy silane (siloxane). Sun and co-workers synthesised 2-amino-4-chloro-6-hydroxy-s-triazine (ACHT) via the hydrolysis of 2-amino-4,6-dichloro-s-triazine. ACHT was then immobilised onto cellulosic fibrous materials using a simple pad-dry-cure approach. After treatment with diluted chlorine bleach, the resulting fibrous materials effectively prevented biofilm formation. Biocidal polypropylene was prepared by incorporating *N*-halamine onto its backbone, in the form of 2,4-diamino-6-diallylamino-1,3,5-triazine, as well as several acyclic *N*-halamine precursors, such as acrylamide, methacrylamide, *N-tert*-butylacrylamide and *N-tert*-butylmethacrylamide [19, 20].

10.3 *N*-halamine as an antimicrobial and biofilm-controlling additive for polymers

A biofilm can be defined as a microbial community enclosed in a self-produced polymeric matrix and bathed in fluid [21, 22]. Microorganisms readily colonise

conventional polymeric materials and form biofilms on a wide range of industrial, environmental, institutional and medical/hygienic applications, which can cause serious problems, including transferring infectious agents, reducing heat transfer in industrial cooling towers, corroding pipes and blocking filters. A number of polymers exhibiting antibiofilm properties have been reported and some of these studies have achieved encouraging results [17, 20, 23–33]. Upon contact, *N*-halamine can transfer positive halogens to appropriate receptors in microbial cells (either directly or indirectly), resulting in microbial death.

The increasing occurrence of microbial and nosocomial infection has stimulated research activities into antimicrobial polymers and textiles [19, 25, 34]. Most medical textiles and polymeric materials used in hospitals are conductive to cross-transmission of diseases, as most microorganisms can survive on these materials for hours to several months [17, 26]. Thus, it would be advantageous for polymeric surfaces and textile materials to exhibit antibacterial properties so as to reduce and prevent disease transmission and cross-contamination within and from hospitals. *N*-halamines exhibit a similar antimicrobial potency to chlorine bleach, one of the most widely used disinfectants, but they are much more stable, less corrosive and have a considerably reduced tendency to generate halogenated hydrocarbons, making them attractive candidates for the production of antimicrobial polymeric materials. *N*-halamine compounds are currently used as antimicrobial additives to produce polymers with antimicrobial and biofilm-limiting activities.

10.4 *N*-halamine-based antibacterial coatings

The applications of natural and synthetic antibacterial polymers to surfaces can be restricted by a lack of suitable conditions to sustain an antimicrobial environment; in order to solve the problem, there is a need to develop durable antibacterial polymers [35–37]. Antimicrobial coatings can be generated by attaching antimicrobial agents to polymer surfaces via physical adsorption, ironic interaction or hydrogen bonds; however, these fabrication methods are usually limited due to the fast leaching of the antibacterial agents into the product environment and their short-term effectiveness due to the exhaustion of these agents [38, 39]. Therefore, the fabrication of durable antibacterial surfaces is of great importance to minimise the spread of infection [40–42]. Long-lasting biocidal surfaces are usually fabricated by bonding surface molecules with specific antibacterial moieties. Although it is relatively easy to chemically bind antimicrobial agents to polymers, which contain reactive sites, such as hydroxyl groups in cellulose and amino groups in nylon, most polymers are inert and do not have the necessary functionality. The current methods to generate reactive groups on polymers are mainly based on oxidation reactions, such as chemical treatment and high-energy irradiation; however, these methods

are complex and do not provide specific functionality, that is, the type of the functional groups produced and the depth of the functionalisation. The surface functionality obtained by these methods is thermodynamically unstable, and hence can be lost after a short period of time. It is highly desirable to develop a simple yet effective technique to functionalise inert polymers using arbitrary chemistry to obtain the required surface properties.

Block copolymers have been employed as an effective delivery vehicle to transfer functional groups onto polymer substrates [43–47]. The first block copolymers are usually identical to the substrate and anchor the block copolymer onto it; the second block is a lower surface tension polymer containing functional groups and forms a surface layer at the air–polymer interface via preferential surface segregation. The functional groups of the lower surface tension block can be used to bond antibacterial agents to generate biocidal coatings on the polymer substrate. In this chapter the specific example used to illustrate the design of an antibacterial surface involves polystyrene (PS)–poly(*tert*-butyl acrylate) (PtBA) delivering its *tert*-butyl groups onto the surface of PS when spin-coated using toluene solutions. The *tert*-butyl groups are then hydrolysed onto reactive carboxyl groups by exposure to trifluoroacetic acid (TFA); *tert*-butylamine molecules are conjugated with surface-bound carboxyl groups via amide bonds, using 2-chloro-4,6-dimethoxy-1,3,5-triazine as the linker [48]. Antibacterial *N*-halamine is then generated by the chlorination of amide groups using a sodium hypochlorite solution. *N*-halamine was chosen for two reasons: it is a very promising antibacterial candidate, as it quickly kills a wide range of microorganisms whilst causing minimal environmental concern; in addition, it is also difficult for microorganisms to develop resistance [49–52]. *Tert*-butylamine was used to ensure that there was no α-hydrogen atom on the vicinal carbons of the formed *N*-halamines, so that elimination of chlorine by the α-hydrogen was impossible. This method successfully fabricated an antibacterial surface. Spin-coated films of functional block copolymers are an effective means to quantitatively control the thickness and surface density of functional groups on polymer substrates. Polymers functionalised with reactive groups can be used as templates for conjugation with antibacterial agents to obtain self-cleaning surfaces. In the example described, during spin-coating, a PS–PtBA block copolymer self-assembled at the surface of a PS substrate and formed a surface layer of PtBA. The thickness of the PtBA layer was linearly dependent on the concentration of the solution used for spin-coating until a saturated monolayer of PtBA was formed at 0.35% (w/w). The *tert*-butyl groups on PtBA blocks were converted to reactive carboxylic acid groups by exposure to TFA to conjugate with *tert*-butylamine molecules via amide bonds. The NH groups in amide bonds were transformed into *N*-halamine after treatment with sodium hypochlorite, which resulted in powerful antibacterial efficacy. In addition, over 50% of the chlorine lost after irradiation with ultraviolet-A rays for 7 days could be regained upon rechlorination. This antibacterial design concept is applicable to polymers using arbitrary chemistry to achieve the required biocidal activities.

Moreover, a water-based polymer synthesised from *N*-chloro-2,2,6,6-tetramethyl -4-pipridinyl methacrylate (Cl-TMPM) was prepared via emulsion polymerisation (Figure 10.3). Furthermore, the addition of this antimicrobial material to commercial water-based latex paints led to antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *enterococcus, Escherichia coli, Candida tropicalis*, bacteriophage MS2 virus and *Staphylococcus chartarum* [53].

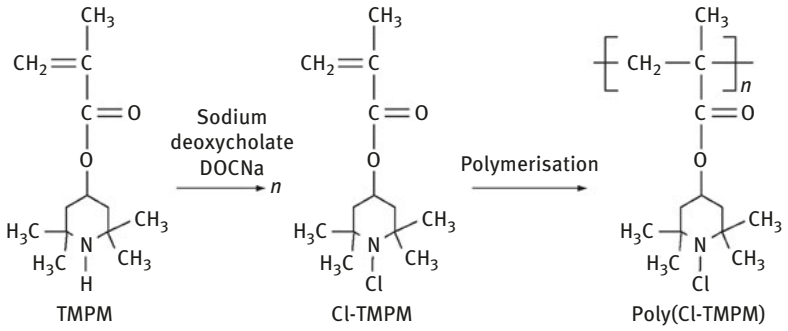

Figure 10.3: Synthesis of Cl-TMPM via emulsion polymerisation. Reproduced with permission from *Z. Cao and Y. Sun, ACS Applied Materials & Interfaces*, 2009, 1, 494. ©2009 American Chemical Society [53].

It has been proposed that the mode of action of *N*-halamine biocidal polymers depends on contact between the polymer and bacteria, which leads to halogen exchange and bacterial death. *N*-halamine biocidal polymers are very stable, do not decompose in water to form toxic products or release halogen until contact with bacteria. The strong bacterial toxicity of *N*-halamines is due to the oxidative halogen moieties [54]. *N*-halamines can be composed of one or more imide/amide/amine *N*-halamine bonds and their stability is amine > amide > imide (Figure 10.4). As detailed in this chapter, an amine *N*-halamine polymeric NP based on 4-

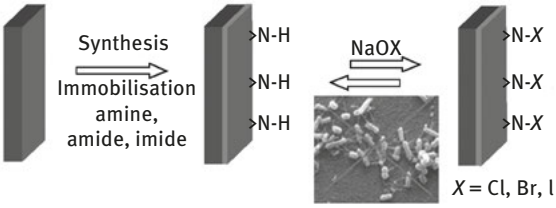

Figure 10.4: Biocidal mechanism of *N*-halamine-based polymers. Reproduced with permission from F. Hui and C. Debiemme-Chouvy, *Biomacromolecules*, 2013, 14, 585. ©2013, American Chemical Society [54].

(allyloxy)-2,2,6,6-tetramethylpiperidine was successfully developed as an antimicrobial agent against pathogenic bacteria [55]. Polymer blends have been synthesised by N-halamine incorporated into TPU structure via a solvent casting method having rechargeable antimicrobial functions (Figure 10.5) [56].

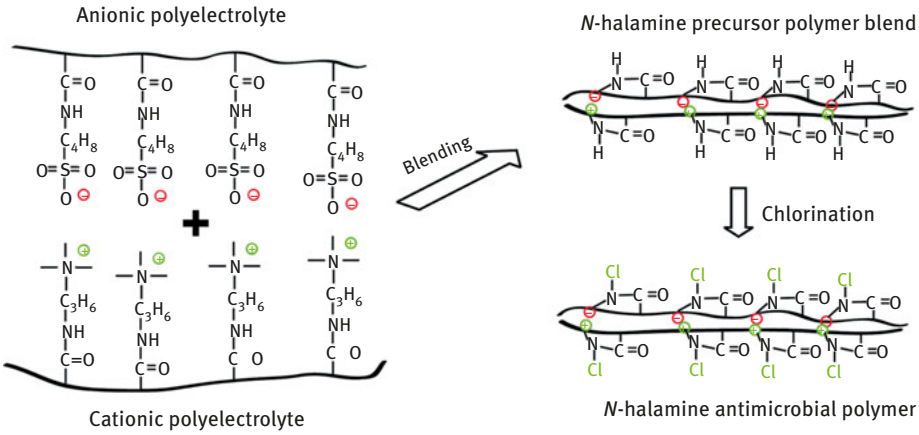

Figure 10.5: Preparation of N-helamine antimicrobial polymers [56].

10.5 Conclusion

The antimicrobial *N* -halamine biocidal polymers described in the literature are water-insoluble and therefore can be safely used to sterilise drinking water and many other water-based applications, such as disinfecting water supplies, swimming pools, hot tubs, industrial water systems and other applications where a sanitised water supply is required. Due to their antimicrobial characteristics, hydantoin-based *N* -halamines and silicon dioxide-based *N*-halamine NP, have been developed and have exhibited contact/release-killing mechanisms leading to microbial death. The reasonably good antibacterial activities against several pathogenic bacteria are possibly due to the oxidative chlorine content; *N*-halamine polymers exhibit similar antimicrobial potency to chlorine bleach, a widely used disinfectant.

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11 Antimicrobial testing methods

Abstract: As of late, there has been a developing enthusiasm for looking into and growing new antimicrobial operators from different sources to battle the rise of the microbial obstruction. The objective of in vitro antimicrobial susceptibility testing (AST) is to give a dependable indicator of how a life form is probably going to react to antimicrobial treatment in the tainted host. In this manner, a more prominent consideration was made on antimicrobial movement screening and assessing strategies. A few bioassays are notable and ordinarily utilized, for example, disk-diffusion, well diffusion, broth or agar dilution, poison food technique and so on. A few techniques give quantitative outcomes (e.g., minimum inhibitory concentration), and all give subjective appraisals utilizing the classes susceptible, intermediate or resistant. This kind of data helps the clinician in choosing the proper antimicrobial operator, helps in creating antimicrobial use arrangements and gives information to epidemiological observation. The culture medium is a fluid answer for which all the fundamental supplements have been included, which assumes a vital job in antimicrobial defenselessness testing. The choice of an anti-toxin board for vulnerability testing depends on usually watched powerlessness designs and is intermittently reexamined. In any case, fresher or developing instruments of obstruction require steady watchfulness with respect to the capacity of each testing technique to precisely identify opposition. In this part, a comprehensive rundown of in vitro AST techniques and definite data on their focal points and constraints are referenced.

Keywords: Antimicrobial susceptibility test, minimum inhibitory concentration, antimicrobial polymers, broth dilution, diffusion, poison food technique, culture media

11.1 Introduction

Treatment failure due to resistance to antimicrobial agents has resulted in elevated rates of morbidity and mortality, in addition to increased health care costs [1]. Although defining the precise public health risk and estimating the increase in cost is a complex task, there is little doubt that emergent antibiotic resistance is a serious global issue [2].

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Appropriate antimicrobial therapy has unquestionable benefit; however, physicians and the public frequently use these agents inappropriately [3]. This misuse is a result of physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for the diagnosis of infections that potentially have bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents and not following established recommendations for using chemoprophylaxis. The availability of over-the-counter antibiotics, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs on a global scale. The widespread availability of antimicrobial drugs leads to their incorporation into herbal or 'folk' remedies, which also increases the inappropriate use of these agents [4].

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections and resistant community acquired infections. As resistance to 'first-line' antibiotics develops, treatment using new, broader spectrum, more expensive antibiotics increases, but is followed by the development of resistance to the new class of drugs [4].

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens are mobile on a local and global scale, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally; hence, surveillance data needs to be collected from selected sentinel sources. These patterns can change rapidly and need to be monitored closely due to their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area [5].

Upon introduction of a variety of antimicrobials, it became necessary to routinely perform antimicrobial susceptibility testing (AST); this involves the antimicrobial, contained in a reservoir, being allowed to diffuse into a medium, on a Petri dish, and interact with freshly seeded test organisms. Even now, a variety of antimicrobial reservoirs are used but the most common method involves an antimicrobial-impregnated absorbent paper disc. The AST disc diffusion method is the most practical method and is still the method of choice for the majority of laboratories worldwide. Automation may force the method out of the diagnostic laboratory but in India, as well as in smaller laboratories of even advanced countries, it will certainly be the most commonly performed microbiological test for many years to come. It is, therefore, imperative that microbiologists fully understand the principles of the test and keep updating information regarding antimicrobial resistance as and when necessary. All techniques involve either diffusion of the antimicrobial agent in agar or dilution of the antibiotic in agar or broth; even automated techniques are variations of these techniques [6].

The goal of in vitro AST is to provide a reliable predictor of how an organism is likely to respond to antimicrobial therapy in the infected host. This type of information aids the clinician in selecting the appropriate antimicrobial agent, aids in developing antimicrobial use policies and provides data for epidemiological surveillance; data from the latter provides a foundation to choose appropriate empirical treatment (first-line therapy). The results of in vitro AST guide clinicians in the appropriate selection and use of initial regimens and drugs for individual patients in specific situations [7]. The selection of an antibiotic panel for susceptibility testing is based on commonly observed susceptibility patterns and is periodically revised [8].

11.2 Culture media

The culture medium is an aqueous solution to which all the necessary nutrients have been added. A nutrient medium, designed by Koch, was based on the fact the microbes grow within host animals, including humans, hence meat infusions and extracts were chosen as basic nutrients; the basic medium contained 0.5% peptone (an enzyme digest of meat), 0.3% meat extract (concentrated water-soluble contents of meat) and 0.8% sodium chloride (the same salt concentration as found in meat). Depending on the type and combination of nutrients, different categories of media can be made. The mixture of necessary nutrients can be used as a liquid medium or a solidifying agent can be added. 'Agar' is a natural polysaccharide produced by marine algae and is the most commonly used solidifying agent added to media (usually at a final concentration of 1.5% w/v). If the hydrolysis of agar is suspected, a silica gel can be used as a replacement solidifying agent. Solid media are useful for identifying bacteria via colony characteristics. Isolation of pure culture can be achieved using solid media, whereas liquid media yield a mixture of all types of bacteria present in the sample [9].

11.2.1 Components of media

The components of media fall into four categories:

1. Nutrients: carbohydrates, serum, whole blood and ascitic fluid, yeast extract, peptone and beef extract.
2. Solidifying agent: agar.
3. Water: tap water with low mineral content, gas distilled water or demineralised water.
4. Additives or supplements: dyes, vitamins, amino acids, growth factors and antibiotics.

11.2.2 Role of substances added to the media

The roles of substances added to the media are described as follows:

1. Carbohydrates: provide energy, act as a carbon source and also indicate fermentation reactions.
2. Serum, whole blood and ascitic fluid: promote the growth of less vigorous organisms.
3. Dyes: act as selective growth inhibitors for certain bacteria and indicate pH change due to acid formation, for example, phenol red is red in alkaline media and yellow in acidic media. Gentian violet inhibits the growth of Gram-positive bacteria.
4. Agar: solidifies media – it is made from red algae Gelidium (Rhodophyta). Chemically it mostly comprises galactose and very few microbes can degrade it. It remains liquid at 100 °C (easy to pour) and solidifies at 40 °C (incubation temperatures).
5. Yeast extract: an aqueous extract of yeast cells, available as a powder. It is a rich source of B vitamins and organic nitrogen, as well as carbon compounds.
6. Peptone: this is a product, which results from the digestion of protein-rich materials such as meat, casein, gelatin and so on. It is a source of nitrogen as well as vitamins and growth factors.
7. Beef or meat extracts: an aqueous extract of lean beef tissue concentrated into a paste. It contains water-soluble substances of animal tissue, which include carbohydrates, organic nitrogenous compounds, water-soluble vitamins and salts.
8. Casein hydrolysate: contains amino acids obtained by the hydrolysis of the milk protein, casein and can be used instead of peptone. Its constitution is more clearly defined than other peptones.
9. Malt extract: consists of soluble substances extracted from barley sprouts at 55 °C. The obtained liquid is concentrated by heating at 55 °C to form a thick brown viscous material, which contains maltose, starch, dextrin, glucose, 5% proteins and protein breakdown products, minerals salts, growth factors and inositol.
10. Media supplements: substances such as vitamins, amino acids, growth factors and antibiotics are added to media for a definite purpose, for example, actidione (cyclohexamide) – an antibiotic added after filter sterilisation, L-(+)-arabinose, arginine – absorbs carbon dioxide, asparagines, biotin, cysteine, dextrin, Ehrlich's reagents, fuchsin, galactose, glucose, glycerol, glycogen, lactose, maltose, mannitol, ornithine, phenylalanine, resazurine (indicator), ribose, citric acid, sorbitol, starch, sucrose and ethylenediaminetetraacetic acid – a disodium salt used to complex iron in media [9, 10].

11.2.3 Types of media

Media are classified in various ways; they may be classified as aerobic and anaerobic culture media, on the basis of molecular oxygen and reducing substances in the media, or solid or liquid, based on the physical state.

Various types of media are as follows:

1. Broths: liquid media.
2. Semisolid: containing <1%.
3. Solid: containing >1% agar. Solid media include the following types:
   - Slants: test tubes filled with liquid agar and allowed to solidify at an angle. Slants are used to maintain pure cultures.
   - Deeps: test tubes filled with liquid agar and allowed to solidify while upright. Depps are used to identify bacterial gas production.
   - Plates: Petri dishes filled with agar. Provides a large surface area for microbial growth and are used to isolate pure cultures. Plates should always be incubated in an inverted position to prevent condensation dripping onto the media surface (water droplets on the surface can spread bacteria and ruin the streak).
4. Complex: support the growth of most heterotrophic organisms.
5. Defined: support the growth of specific heterotrophs and are often mandatory for chemoautotrophs, photoautotrophs and for microbiological assays.
6. Reducing: support the growth of obligate anaerobes.
7. Simple or basal media: nutrient broth is an example of basal media, which contains peptone – 1%, meat extracts – 1%, sodium chloride – 0.5% and distilled water. It has a pH of 7.4–7.5. When 2–3% agar is added to nutrient broth it is called nutrient agar.
8. Natural or empirical media: the concentration of various nutrients is not exactly known, for example, milk, urine, diluted blood, vegetable and fruit juices, meat extracts and infusions and so on.
9. Semisynthetic media: contain both natural and chemical nutrients, for example, potato dextrose agar (PDA).
10. Synthetic media: made up of entirely synthetic substances, for example, Sabourad agar.
11. Dehydrated media: these are commercially available, powdered, ready to use media. It only requires the addition of a specified amount of distilled water to a specified amount of powder.
12. Living culture media: obligate parasites are cultured in living cells, callus, organs of animals or plants, for example, a chick embryo can be used to culture viruses.
13. Minimal media: a defined medium, which has just enough ingredients to support growth. Types of ingredients vary and depend upon the microbial species.
14. Special media: when certain ingredients are added to a basal medium to study special characteristics or to provide special nutrients required for the growth of

the organism, it is called a complex medium. Virtually all special media are complex media, these are further divided into:

- Enrichment media: similar to selective media but are designed to increase the number of desired microorganisms to a detectable level without stimulating the rest of the bacterial population. When some special nutrients such as blood or serum, egg or meat pieces are added to basal media, the latter are termed enriched media. It favours the multiplication of a particular species of bacteria by incorporating special substances, which selectively favour its growth or inhibit the growth of competitors, for example, selenite broth, alkaline peptone water, Blood agar, Loeffler's serum media.
- Selective media: suppresses the growth of unwanted microbes or encourages the growth of desired microbes. In addition to basal media, they contain certain substances such as bile salt or deoxycolate citrate, which inhibits all bacteria except those of a particular type or group. Isolation of a particular species of bacteria from a mixed inoculum is possible using selective medium, for example, MacConkey agar, deoxycolate citrate agar.
- Indicator media: when a certain indicator (neutral red or bromothymol blue) or reducing agent (potassium tellurite) is incorporate into the culture medium it is called an indicator medium. The colour of the medium changes with alterations of pH due to bacterial growth.
- Differential media: these distinguish colonies of specific microbes from others. When a culture medium containing certain substances helps to distinguish the differing properties of different bacteria, it is called differential media, for example, MacConkey agar. It is also an indicator medium. It contains peptone, agar, lactose, sodium taurocholate and neutral red. The lactose fermenters form pink colonies while non-lactose fermenters produce colourless or pale colonies. Blood agar: it serves both as an enriched, as well as an indicator medium and shows different types of haemolysis.
- Transport media: when specimens, for example, faeces, throat swabs and so on are sent to a laboratory from distant places, the bacteria may not survive the time taken for transit or may be overgrown by non-pathogenic bacteria. For transporting specimens, special media have been devised, which are called transport media and this type of media preserves the viability of the organism. The medium is non-nutrient and therefore other commensal bacteria are not able to grow in it, for example, Stuart's transport medium for urethral discharge (gonococci), deep semisolid thioglycollate medium for anaerobes and so on.
- Sugar media: a standard media used for biochemical tests and contains 1% sugar in peptone water along with an indicator (Andrade's indicator). A small tube (Durham's fermentation tube) is kept inverted within the large tube containing the sugar media. Upon production of acid by bacteria the colourless medium turns pink and gas production is indicated by the accumulation of

gas bubbles at the top of the inverted tube. Glucose, sucrose, lactose and mannitol are routinely employed for sugar fermentation tests.
- Assay media: prescribed composition used for assaying vitamins, amino acids and antibiotics.
- Maintenance or storage media: maintains the viability and physiological characteristics over a period of time without accelerating growth; it contains only the basic nutrients. Substances which enhance growth or substances which when degraded result in the production of harmful substances, such as acids and so on, are omitted.
- Selective-differential media: some media have both properties, for example, MacConkey agar. It is selective as it contains crystal violet and bile salts, which inhibit the growth of bacteria other than coliforms. It is differential as it contains neutral red and lactose, which is degraded by coliforms to acid and is detected due to a change in pH. At acidic pH, colourless neutral red becomes red and the colonies develop a red colour. *Shigella* and *Salmonella* colonies remain colourless and therefore can be easily distinguished [11].

11.3 Methods of antimicrobial susceptibility testing

AST methods are divided into types, based on the principle applied in each system [12], and are detailed in Sections 11.3.1–11.3.4.

11.3.1 Diffusion

11.3.1.1 Disc diffusion test

The disc diffusion susceptibility method is simple and practical and has been well standardized [13]. The test is performed by applying a bacterial inoculum of approximately 1–2 × 10^8colony forming units (CFU)/mL to the surface of a large (150 mm diameter) Müeller–Hinton agar plate. Up to 12 commercially prepared, fixed concentration, paper antibiotic discs are placed on the inoculated agar surface. Plates are incubated for 16–24 h at 35 °C prior to determination of results. The zones of growth inhibition around each of the antibiotic discs are measured to the nearest millimeter [14]. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI) (formerly the National Committee for Clinical Laboratory Standards) or those included in the US Food

and Drug Administration approved product leaflets for the discs [15]. The results of the disc diffusion test are 'qualitative', in that a category of susceptibility (i.e., susceptible, intermediate or resistant) is derived from the test rather than the minimum inhibitory concentration (MIC) [16]. However, some commercially available zone reader systems claim to calculate an approximate MIC of some organisms and antibiotics by comparing zone sizes with standard curves of that species and drugs stored in an algorithm [17]. The advantages of the disc method are test simplicity as it does not require any special equipment, the provision of categorical results, which can be easily interpreted by clinicians and flexibility in the selection of discs for testing [18, 19].

- Media for the disc diffusion test: Müeller–Hinton agar medium

Müeller-Hinton agar is considered to be the best for the routine susceptibility testing of non-fastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing.
- It is low in sulfonamide, trimethoprim and tetracycline inhibitors.
- It allows the satisfactory growth of most non-fastidious pathogens.

11.3.1.2 Agar well diffusion method

In this method, 25 mL of a molten sterile medium is poured into presterilised Petri dishes and allowed to solidify at room temperature (RT) [20]. The agar plates are seeded with 0.1 mL of (1×10^8 spores/mL) of a fungal/bacterial culture using the spread plate method. Subsequently, 10 mm wide wells are bored into these agar plates using a sterile cork borer. 250 mL of a stock solution of an antimicrobial compound is filled into the wells and the plates are incubated at 25 ± 2 °C. The antimicrobial activity is interpreted from the size of the zone of inhibition(s) (ZOI) measured to the nearest millimetre, that is, the clear zones surrounding the wells, as shown in Figure 11.1 [21].

- Media for the agar well diffusion method

General purpose media are used for the agar well diffusion method, such as a nutrient agar medium for bacteria and a PDA medium for fungi [22].

11.3.2 Dilution methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial required to inhibit or kill the microorganism. This can be achieved by dilution of an antimicrobial in either agar or broth media [20].

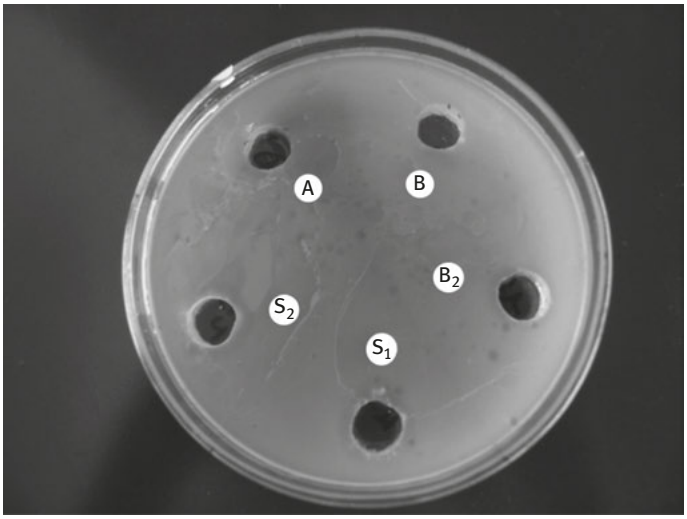

Figure 11.1: Agar well diffusion method.

11.3.2.1 Minimum inhibitory concentration

The MIC is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation [23].

Diffusion tests are used to determine the susceptibility of microorganisms but have their limitations; when equivocal results are obtained or in the case of prolonged serious infection, for example, bacterial endocarditis, the elucidation of antibiotic action in relation to the pathogen needs to be more precise. Also the terms 'susceptible' and 'resistant' are open to interpretation. Thus, when in doubt, a precise assessment is needed and involves determining the MIC of the antimicrobial compounds to the organisms using the twofold serial dilution method, which is shown in Figure 11.2 [24].

There are two methods of testing for MIC:

a) Broth dilution method.
b) Agar dilution method.

– Broth dilution method

The broth dilution method is a simple procedure for testing a small number of isolates, even a single isolate. It has the added advantage that the same tubes can also be used for minimum bactericidal concentration(s) (MBC) tests.

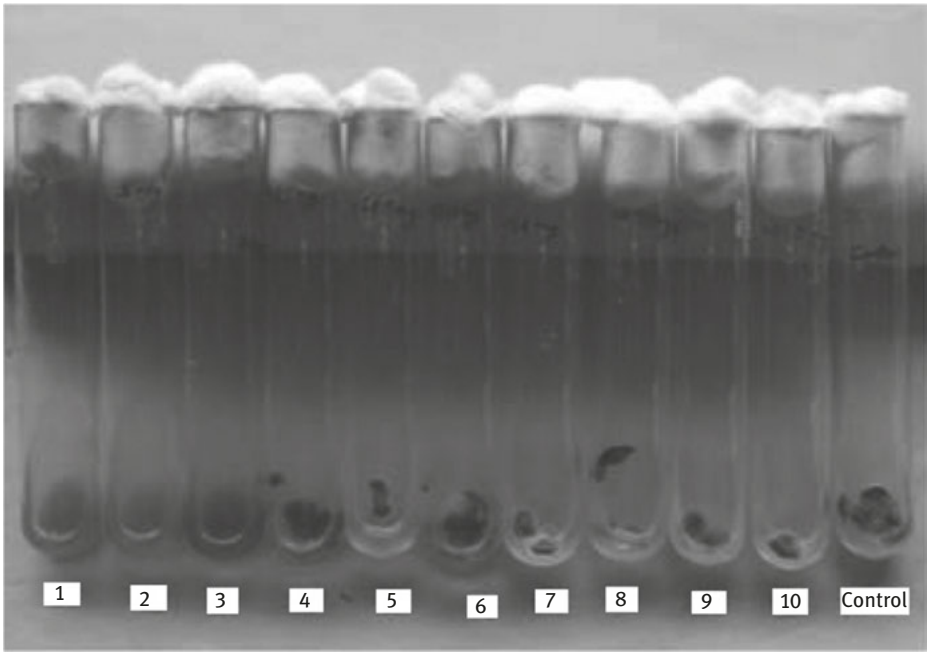

Figure 11.2: Minimum inhibitory concentration.

– Materials

Sterile graduated pipettes of 10, 5, 2 and 1 mL, sterile capped 7.5 × 1.3 cm tubes/small screw-capped bottles, Pasteur pipettes, an overnight broth culture of the test and control organisms (same as for disc diffusion tests), the required antibiotic in powder form, the required solvent for the antibiotic, sterile distilled water – 500 mL and suitable nutrient broth medium. A suitable rack to hold 22 tubes in two rows, that is, 11 tubes in each row.

– Method

Prepare stock dilutions of antibiotic concentrations of 1,000 and 100 µg/L, as required, from the original stock solution (10,000 mg/L). Arrange two rows of 11 sterile 7.5 × 1.3 cm screw-capped tubes in the rack. In a sterile 30 mL (universal) screw-capped bottle, prepare 8 mL of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made [25]. Mix the contents of the universal bottle using a pipette and transfer 2 mL to the first tube in each row. Using a fresh pipette, add 4 mL of broth to the remaining 4 mL in the universal bottle, mix and transfer 2 mL to the second tube in each row. Continue preparing the dilutions in this way but if 10 or more are required, the series

should be started again half way down the concentration dilution series. Place 2 mL of antibiotic-free broth in the last tube in each row. Inoculate one row with a specific amount of an overnight broth culture of the test organism diluted approximately to 1 in 1,000 in a suitable broth and the second row with the similarly diluted control organism of known sensitivity. The result of the test is significantly affected by the size of the inoculum. The test mixture should contain 10^6 organisms/mL. If the broth culture used has grown poorly, it may be necessary to use this undiluted. Incubate tubes for 18 h at 37 °C. Inoculate a tube containing 2 mL of broth with the organism and keep at +4 °C in a refrigerator overnight, this is used as a standard for the determination of complete inhibition [13, 26].

– Reading of the results

The MIC is expressed as the lowest dilution, which inhibited microbial growth and is judged by a lack of turbidity in the tube. Because very faint turbidity may result from the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard to determine complete inhibition. A standard strain of known MIC value, run with the test, is used as the control to check the reagents and conditions allowed microbial growth.

11.3.2.2 Minimum bactericidal concentrations

The main advantage of the 'broth dilution' method for MIC determination lies in the fact that it can easily be converted to determine the MBC as well.

– Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (before incubation) by evenly spreading a loopful over a quarter of the plate on a medium suitable for the growth of the test organism and is then incubated at 37 °C overnight. The MIC of the control organism is determined to check that the drug concentrations are effective. Note the lowest concentration, which has inhibited the growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate overnight at 37 °C. Compare the amount of growth from the control tube before incubation, which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity. These tubes are not subcultured; the purpose of the control is to confirm via its MIC that the drug level is correct, whether or not this organism is killed is immaterial [27, 28].

– Reading of the results

These subcultures may show:

– Similar number of colonies indicating bacteriostasis only.
– A reduced number of colonies indicating partial or slow bactericidal activity.
– No growth if the whole inoculum has been killed.
– The highest dilution showing at least 99% inhibition is taken as the MBC.

11.3.2.3 Minimum fungicidal concentrations

To estimate the minimum fungicidal concentration (MFC), a loopful of biomass is taken from respective tubes containing the MIC and higher concentrations of antimicrobial compound and inoculated into tubes containing sterile (autoclaved) broth and incubated at 25 ± 2 °C for 72 h (for fungi). Subsequently a loopful from these tubes is then streaked on to the surface of sterile agar plates and incubated at 25 ± 2 °C for 72 h [29, 30].

– Reading of the results

The presence or absence of growth is observed after the respective fungal incubation time. Appearance of growth indicates that the antimicrobial compound concentration is just fungistatic and absence of growth indicates that the antimicrobial compound concentration is fungicidal. Cidal action is indicated by a reduction in the number of colonies by at least 99%, as shown in Figure 11.3 [20].

11.3.2.4 Microbroth dilution test

This test uses double-strength Müeller–Hinton broth, 4× strength of antibiotic solutions, prepared as serial twofold dilutions, and the test organism at a concentration of 2 × 106/mL. In a 96-well plate, 100 mL of double-strength Müeller–Hinton broth, 50 mL each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35 °C for 18–24 h. The lowest concentration showing inhibition of growth is considered to be the MIC of the organism [13, 31].

– Reading of the results

MIC is expressed as the highest dilution, which inhibited growth judged by a lack of turbidity in the tube. Because very faint turbidity may result from the inoculum, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. A standard strain of known MIC, run with the test, is used as the control to check the reagents and conditions allowed microbial growth.

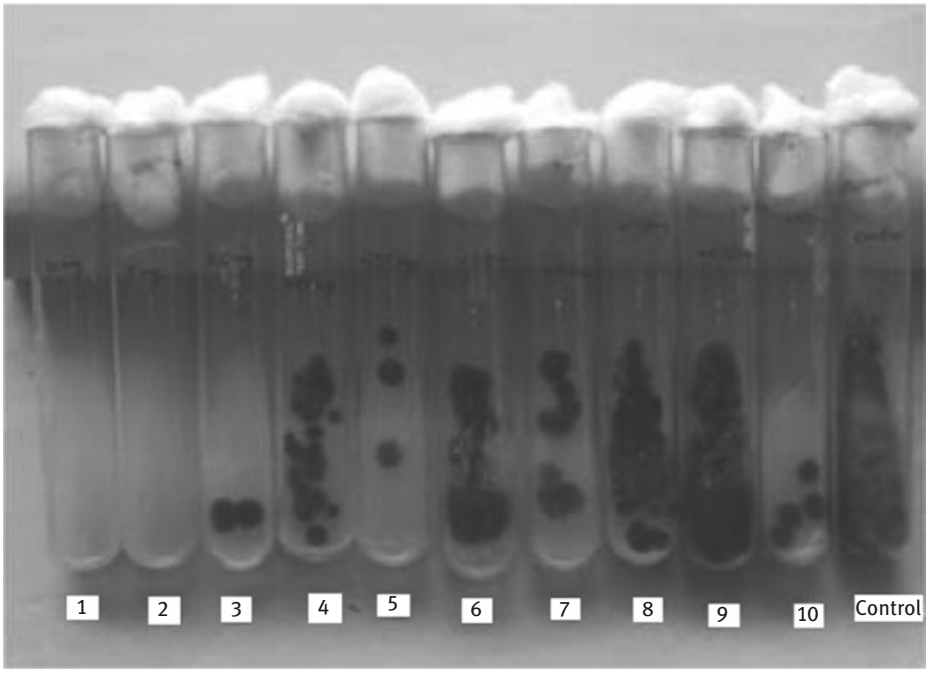

Figure 11.3: Minimum fungicidal concentrations.

11.3.2.5 Agar dilution method

Agar dilutions are most often prepared in Petri dishes and have the advantage that it is possible to test several organisms on each plate. If only one organism is to be tested, for example, *Mycobacterium tuberculosis*, the dilutions can be prepared on agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism. The dilutions are made in a small volume of water and added to agar, which has been melted and cooled to not more than 60 °C. Blood may be added and if 'chocolate agar' is required, the medium must be heated before the antibiotic is added. It would be convenient to use 90 mm diameter Petri dishes and add 1 mL of the desired drug dilutions to 19 mL of broth. Label a sterile Petri dish on the base for each concentration required. Prepare the dilutions in water placing 1 mL of each in the appropriate Petri dish. 1 mL water is also added to a control plate. Pipette 19 mL of the melted agar, cooled to 55 °C to each plate and mix thoroughly. Adequate mixing is essential and if sufficient technical expertise is not available for this skilled manipulation, it is strongly recommended that the agar is first measured into stopper tubes or universal containers and the drug dilution added to these and mixed by inversion before pouring into Petri dishes. After the plates have set, they should be dried at 37 °C, with their lids tipped, for 20 to 30 min in an incubator. The plates are then inoculated

either with multiple inoculators as spots or with a wire or platinum loop calibrated to deliver 0.001 mL, which is spread over a small area. In either case, the culture should be diluted to contain 10^5 to 10^6organisms per mL. With common fast-growing organisms, this can be obtained by adding approximately 5 µL of an overnight broth culture to 5 mL broth or peptone water [20].

– Reading of the results

The antibiotic concentration of the first plate showing 99% inhibition is taken as the MIC for the organism.

11.3.3 Diffusion and dilution

11.3.3.1 E-test method

An E-test is also known as the epsilometer test and is an 'exponential gradient' testing methodology where 'E' in E-test refers to the Greek symbol epsilon (e). The E-test (AB Biodisk) is a quantitative AST method and allows both the dilution and diffusion of the antibiotic into the medium. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E-test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation, a symmetrical inhibition ellipse is produced. The intersection of the ZOI edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy [32].

E-tests can be used to determine MIC for fastidious organisms such as *Streptococcus pneumoniae*, β-haemolytic streptococci, *Neisseria gonorrhoeae, Haemophilus* sp. and anaerobes. It can also be used for non-fermenting Gram-negative bacilli, for example, *Pseudomonas* sp. and *Burkholderia pseudomallei*.

Antimicrobial resistance of major consequence may be detected, for example, the test is very useful in detecting glycopeptide-resistant enterococci and glycopeptide-intermediate *Staphylococcus aureus* and slow-growing pathogens such as *Mycobacterium tuberculosis*. Furthermore, it can be used for detecting extended spectrum β-lactamases. In conclusion, the E-test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents [33, 34].

11.3.4 Poison food technique

11.3.4.1 Inoculum disc

A seven-day-old culture of the test fungus is used for the preparation of a 6 mm diameter inoculum disc.

11.3.4.2 Method

100 mg of antimicrobial compound is dissolved in 10 mL solvent to prepare a 10 mg/mL stock solution. 1 mL of stock solution is mixed with 9 mL of the molten sterile culture medium and this mixture is poured into presterilised Petri plates (9 cm diameter) and allowed to solidify at RT. Prepared Petri plates are inoculated aseptically with a 5 mm disc of test cultures. The plates are then incubated at 28 ± 2 °C for 7 days. A culture control is also maintained along with the test samples. Antifungal activity is measured as a function of the increase in growth of the 5 mm disc of inoculum, as shown in Figure 11.4 [35–38].

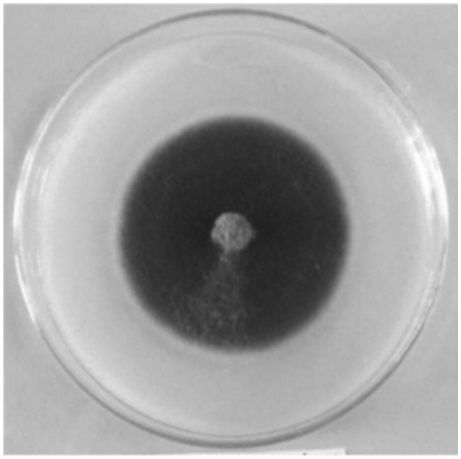

Figure 11.4: Poison food technique.

11.4 Antimicrobial properties of polymers

The development of effective antimicrobial polymers requires understanding the different growth behaviour and treatment susceptibility of microbes [39]. There is an ongoing battle against several microbial strains of infection and the risks of acquiring drug resistance has not ceased despite a considerable number of products developed for new cellular targets. Polymers are widely used as biomaterials in prostheses, bone replacement implants, drug delivery, catheters and tissue engineering [40]. The antimicrobial activity of polymers has been studied by several research groups [41–48].

11.4.1 Antibacterial activity of polyaniline/polyvinyl alcohol/silver

In this study [49], polyvinyl alcohol (PVA)/polyaniline (PANI)/silver nanocomposites were tested for antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The nanocomposite samples resulted in ZOI with diameters of the 7, 8, 12, 9 and 8 mm and 10, 12, 15, 9 and 8 mm against *Escherichia coli* and *Staphylococcus aureus*, respectively. It was observed that the PVA/PANI composite, which was used as a control matrix, exhibited no antibacterial activity when compared with PVA/PANI/silver nanocomposites.

11.4.2 Antifungal activity of polyaniline and polyaniline-doped with fluconazole

All the fungi which were tested, that is, *Candida albicans* (ATCC® 140503™), *Candida tropicalis* (ATCC® 13803™) and *Candida krusei* (ATCC® 34135™) successfully showed consistent ZOI in the presence of PANI- and PANI-doped with fluconazole. As the concentration of PANI- and PANI-doped with fluconazole increased, the susceptibility also increased for all fungi. The results elucidated the inhibitory concentration of PANI and PANI-doped with fluconazole on *Candida tropicalis* (ATCC® 13803™). There were no ZOI of PANI in dimethylsulfoxide, which acts as a control. The ZOI were 7 mm for a concentration of 1.25 mg/mL, 8 mm for a concentration of 2.5 mg/mL, 9 mm for a concentration of 5.0 mg/mL and 11 mm for a concentration of 10 mg/mL. From this we can assume that the MIC of PANI for *Candida tropicalis* (ATCC® 13803™) is 1.25 mg/mL. The ZOI were 9 mm for a concentration of 1.25 mg/mL, 10 mm for a concentration of 2.5 mg/mL, 11 mm for a concentration of 5.0 mg/mL and 13 mm for concentration of 10 mg/mL. From this we can assume that the MIC of PANI-doped fluconazole for *Candida tropicalis* (ATCC® 13803™) is also 1.25 mg/mL [50].

11.4.3 Antibacterial activity of wood flour/polyvinyl chloride composites

The antibacterial activity of composites was assessed against *Escherichia coli* ATCC 8739 and *Staphylococcus aureus* ATCC® 6538P according to the International Organization for Standardization (ISO), ISO 22196: 2007E. First, a test inoculum was prepared by transferring one loop of the preincubated bacteria into a small amount of 1/500 diluted nutrient broth. The test specimens, which included three specimens made from each composite, including

wood flour/polyvinyl chloride (PVC), and three untreated made from pure PVC with dimensions 50 × 50 × 1 mm, were placed in Petri dishes and inoculated with 0.4 mL of the test inoculum. The surface of the inoculated specimens was covered with a thin piece of polypropylene film (40 × 40 mm) and pressed down gently so that the test inoculum is spread to the edges. After incubation for 24 at 35 °C under humid conditions (95%), the test inoculum on the film and test specimens were completely recovered by 10 mL of soybean casein lecithin polysorbate (SCDLP) broth (prepared by adding 1 g of lecithin and 7 g of polysorbate per litre of tryptic soy broth [TSB]). Recovered SCDLP broth was tenfold serial diluted in phosphate buffered physiological saline and 1 mL of each dilution was placed together with 1 mL of undiluted recovered SCDLP into separate Petri dishes. 15 mL of plate count agar was poured into each Petri dish, and was swung gently to disperse the bacteria and then incubated for 48 h at 35 °C under humid conditions (95%). After incubation, the number of colonies on the Petri dishes was counted, which contained 30–300 colonies [51].

11.4.4 Antimicrobial activity of hydrogels

Escherichia coli and *Staphylococcus aureus* were reconstituted from the lyophilised form according to the manufacturer's protocol, and cultured in TSB at 37 °C under constant shaking at 300 rpm, while *Candida albicans* was cultured in yeast nitrogen base at RT under constant shaking at 50 rpm. Prior to treatment, the microbes were first inoculated overnight to enter into the log growth phase. Cationic polycarbonate (PC) ([vitamin E [VE]/BnCl [1:30] or VE/PrBr [1:30])-containing hydrogels were prepared using 4 wt% of the triblock copolymer (5-methyl-5-(α-tocopheryl)carboxyl-1,3-dioxan-2-one) (MTC)-VE)1.25 polyethylene glycol (20k)-(MTC-VE)1.25 and varying amounts of VE/BnCl (1:30), VE/PrBr (1:30) and/or fluconazole. Hydrogels (50 mL) were placed into each well of a 96-well microplate containing an equal volume of microbial suspension (3×10^5 CFU/mL). Prior to this, the concentration of the microbe solution was adjusted to give an initial optical density reading of approximately 0.07 at 600 nm wavelength on a microplate reader (TECAN, Switzerland), which corresponded to the concentration of McFarland 1 solution (3×10^8 CFU/mL). The culture plate was kept either at 37 °C for bacterial samples or RT for *Candida albicans* under constant shaking at 300 or 50 rpm, respectively, for 24 h. After treatment, samples were taken to make a series of tenfold dilutions and then plated onto agar plates. The plates were incubated for 24 h at 37 °C and the number of CFU were counted. Microbes treated with hydrogel without cationic PC were used as the negative control, and each test was carried out in triplicate. MBC is defined as the concentration of the cationic PC that eliminates >99.9% of the microbes [52].

11.5 Standards selected for the antimicrobial testing of materials

Following is a list of standards selected for the antimicrobial testing of materials taken from ISO and the American Society for Testing and Materials (ASTM):

- ISO 22196:2011P: Measurement of the Antibacterial Activity of Plastic and other Non-porous Surfaces.
- ASTM E2180-07: Standard Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) in Polymeric or Hydrophobic Materials.
- ASTM E2149-10: Standard Test Method for Determining the Antimicrobial Activity of Immobilized Antimicrobial Agents under Dynamic Contact Conditions.
- ASTM G21-09: Standard Practice for Determining the Resistance of Synthetic Polymeric Materials to Fungi.
- ISO/CD 16256: Clinical Laboratory Testing and in vitro Diagnostic Systems – Reference Method for Testing the in vitro Activity of Antimicrobial Agents against Yeast Fungi Involved in Infectious Diseases.
- ISO 20645:2004: Textile Fabrics – Determination of Antibacterial Activity – Agar Diffusion Plate Test [53, 54].

11.6 Conclusion

The AST methods described in this chapter provide reliable results when used according to the procedures defined by the CLSI or by the manufacturers of the commercial products. However, there is considerable opportunity for improvement in the area of rapid and accurate determination of pathogenic resistance to antibiotics. There is a need for the development of new automated instruments that can provide faster results and also lower the cost of testing by virtue of lower reagent prices and reduced labour requirement. To accomplish this, it will be necessary to explore different methodological approaches for the detection of pathogenic growth. The direct detection of resistance genes via polymerase chain reaction or similar techniques has limited applicability, as only a few resistance genes are firmly associated with phenotypic resistance, for example, mec A (a gene found in bacterial cells; the mec A gene allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics), van A (resistance to vancomycin and teicoplanin, expressed by *Enterococcus faecalis, Enterococcus faecium* and *Staphylococcus aureus*), van B (resistance to vancomycin only, expressed by *Enterococcus faecalis* and *Enterococcus faecium*) [55]. There are hundreds of β-lactamases and numerous mutations, acquisitions and expression mechanisms that

result in fluoroquinolone, aminoglycoside and macrolide resistance; too many to be easily detected by current molecular techniques [56]. Thus, it seems likely that phenotypic measures of the level of susceptibility of pathogens to antimicrobial agents will continue to be clinically relevant for years to come.

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12 Antimicrobial peptides

Abstract: In the past few decades, several antimicrobial peptides were portrayed successfully. They belong to first-line of obstruction contrasted with forceful dynamic bacterial contamination that remain unaccompanied or synergistic through further unmistakable and insusceptible opposing atoms to challenge virus and to switch for occupant bacterial inhabitants. Blocked off the type of a broad scope of both humble and complex life forms, they are normal, short (less than 50 amino gathering leftovers), amphipathic and totally charged. Expending as a preeminent objective, the bacteriological films showing intracellular signs of receptive antimicrobial peptides were extensively named Antimicrobial Peptides, peptides are extremely short and dangerous than standard mammalian cells. Nowadays, due to intensified bacterial fight against anti-toxins, wide-extending utilization of anti-infectious agents in the virus practices, formed by the expanded measure of immuno-bargained patients are at peril of forceful disease;. This may be an answer for the need of protected and genuine antimicrobial agents. In this section, advancement, portrayal, readiness and different utilization of hostile microbial peptides have been much discussed. Additionally, we assign here the system of abuse of these interesting particles as fine as their perspectives as novel dynamic antimicrobial operators.

Keywords: Antimicrobial Peptides

12.1 Introduction

Antimicrobial peptides (AMPs) are intriguing, the assortment and a decent variety of these peptides normally as particles are found in all objectives and purposes of all types of wide range of life forms beginning from microorganisms to humans. The capability of AMPs are in part credited to the contrast in both prokaryotic and eukaryotic cells, that may typify focuses for AMPs. Overall, these peptides illustrate wide range movements of anti-infectious agents, which can be helpful as novel operators. By and large, AMPs are low, charged and are 6–100 amino acids long. Most AMPs can be able to destroy gram-positive and gram-negative microbes, yeasts, infections, growths and considerably malignant growth cells, while others act legitimately by regulating the host safeguard frameworks [1]. Currently, excess of 3,054 AMPs have been kept in the

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Antimicrobial Peptide Database (http://aps.unmc.edu/AP/main.php) found and detached from humans, plants, creatures of land and water, creepy-crawlies and even microscopic organisms [2, 3]. Generally, AMPs have additionally been mentioned to be as host protection peptides [4], cationic amphipathic peptides [5], anionic antimicrobial peptides/proteins [6], cationic AMPs [7], α-helical antimicrobial peptides [8] and cationic host safeguard peptides [9]. The antimicrobial peptides assume an imperative key job in the control of microbial diseases rely on their capacity to associate with the layers. The AMPs have been extracted as an antimicrobial specialist from soil Bacillus strain that goes back to 1939 found by Rene Dubos [10, 11]. This extraction is likewise viable of ensuring mice against contamination with pneumococci disease. Both Hotchkiss and Dubos fractionated, recognized and separated an AMP in year 1940, named as gramicidin. Alhough there were some harmful causes related with intraperitoneal application [12], gramicidin was proved to be a powerful topical treatment for wounds and ulcers [13]. In the year 1941, another AMP found in tyrocidine showed bactericidal effect against both Gram-positive and Gram-negative microbes [14]. Nonetheless, tyrocidine likewise has been found to be dangerous to human platelets [15]. Later, another AMP was separated from wheat *Triticum aestivum* [16], which was named as purothionin and found to be successful against some pathogenic microscopic organisms and parasites [17].

In 1956, the first predetermined AMP was defensin, which was distinguished and disconnected from rabbit leukocytes [18] and later bombinin from epithelia [19]. In the meantime, lactoferrin was isolated from milk of dairy animals [20]. In the same year, it was additionally discovered that human leukocytes were related to AMPs in their lysosomes [21].

The age of certain AMPs is inducible, yet the greater part of the AMPs are framed by specific inevitable cells. The utlilization of silk moth, for instance, a model framework, Hultmark and partners [22] showed that P9A and P9B can be induced in hemolymph by inoculation with *Enterobacter cloacae*. In another examination [23], epithelial cells from different kinds of tissues in mice depicted arbitrarily expanded rate of mRNA translation for defensin generation after infection with *Pseudomonas aeruginosa* PAO1.

Figure 12.1 shows the prospects of biological function in AMPs; hence, AMPs attach to bacterial films through electrostatic associations, either to break the layer or to enter into the bacterium to inhibit intracellular capacity. A few AMPs have show immunity through selecting/enacting immunocytes or by impacting toll-like receptor (TLR), credit to microbial items and nucleic acids discharged when tissues are harmed. DC, dendritic cell; LPS, lipopolysaccharide; LTA, lipoteichoic corrosive; MAVS, mitochondrial antiviral flagging protein. A few different ways by which eukaryotic cells are generally engaged in AMP generation are similarl to epithelial cells present in lymphs in gastrointestinal and genitourinary frameworks [24, 25], phagocytes [26] and lymphocytes [27]. AMPs likewise have been found to impact

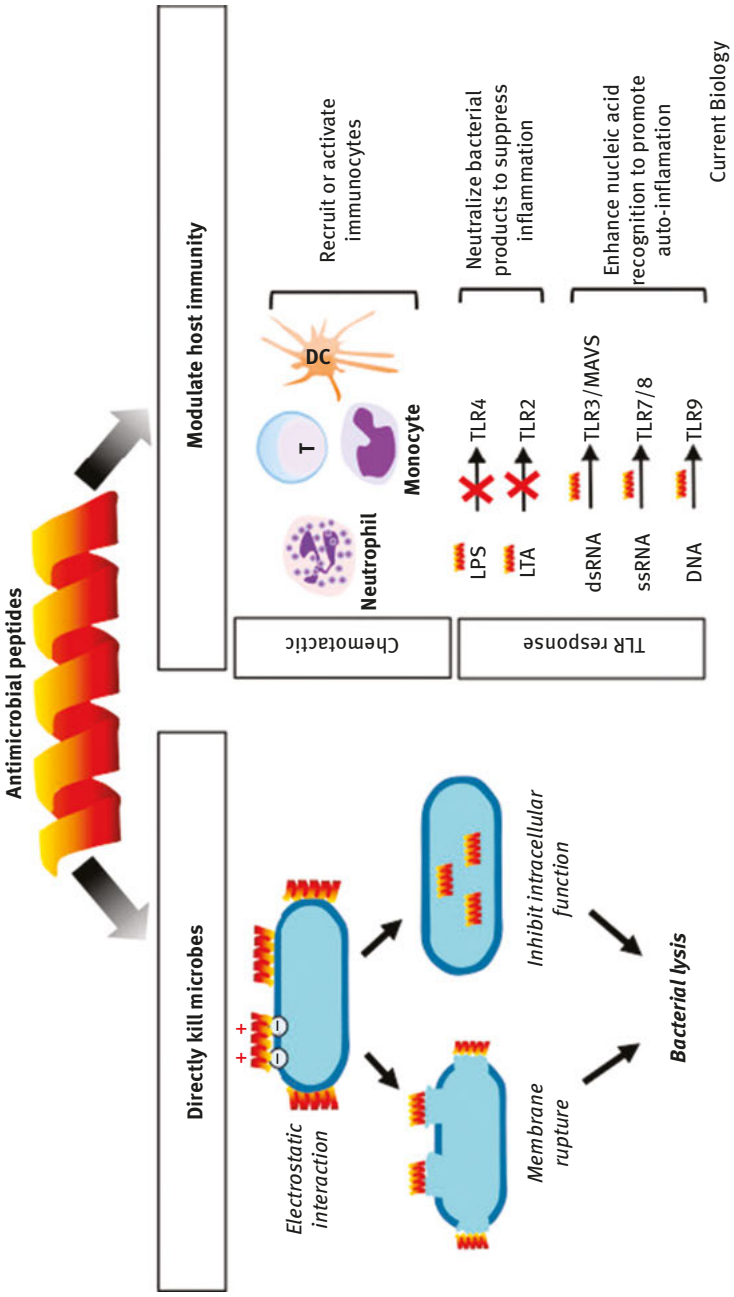

Figure 12.1: The biological function of antimicrobial peptides. (Adapted from [24]).

the host's challenging reactions during the time of contamination [28–30]. Lipopolysaccharide (LPS) is the real particle discharged from the bacterial cell as a result of a solid reaction of anti-infectious treatment, as well as host insusceptibility to clear diseases, which can stimulate AMP created by warm-blooded creatures [26]. For a reasonable precedent, HEK293 cells generally produce defensin in light of LPS stimulation [31]. CAP18 [32], CAP35 [33] and a lactoferrin subsidiary are a portion of the instances of AMPs [34] that can likewise have the ability to double LPS-initiated cytokines in macrophages. Thus, controlling AMPs to inflammable reaction is a vital issue in the therapeutic field. In LPS, stimuli to upgrade the valuable and injurious impact of anti-microbial treatment that may cause over-response to the host resistant framework to perform do not have this kind of guideline on the fierce reaction. Similarly, in some resistant frameworks, this can lead to sepsis, extreme sepsis or septic shock [35].

Numerous contamination sicknesses are caused by pathogenic microorganisms that had dependably involved concern in numerous fields, especially in medications, therapeutic gadgets, dental rebuilding, clean applications, medical procedure hardware, nourishment building and capacity, social insurance, clinical surfaces, water cleansing frameworks, aerodynamics, residential machines, materials and so forth. Irresistible illnesses execute a larger number of individuals than other individual reasons. Many times, these ailments are of explicit significance in medical clinics where tremendous endeavours and fundamental overhead costs are devoured for attempting to use than against diseases. These occur in germs (microscopic organisms, parasites, infections and protozoa), which are brought by animals to various places, air, water and soil. For the most part, the diseases are caused by contacting, drinking or breathing, eating, which contain germs. Overall, these diseases fight with antimicrobial operators, which are impacted by their activity. Structure–action relationship examinations have yielded unmistakable data pertinent to the auxiliary prerequisites of antimicrobial peptides, showing that progressively powerful antimicrobial action depends on charge and hydrophobicity and that the underlying target is vigorously and adversely charged in bacterial external cell divider film due to electrocatalytic movement [37]. These investigations have been considered as potential endeavours to structure ultrashort, incredibly dynamic AMP platforms [38, 39], which are provided by easy and strong stage manufactured conventions, which can possibly lower costs as compared to the characteristic antimicrobial peptide partners [40, 41].

12.2 Cationic antimicrobial peptides

Similar to *S. epidermidis* PAMPs by the human safe framework, *S. epidermidis* has systems to detect the nearness of destructive particles. In particular, an AMP-detecting

framework has been distinguished, named aps, that is initiated by an assortment of AMPs and triggers staphylococcal AMP-cautious frameworks, including D-alanylation of teichoic acids, lysylation of phospholipids by the MprF protein and the VraFG proteins (Figure 12.2) The previous two components decline the anionic charge of the bacterial surface; hence, averting proficient fascination of cationic AMPs, while the last has the capacity of an AMP exporter, expelling AMPs from the cytoplasmic film.

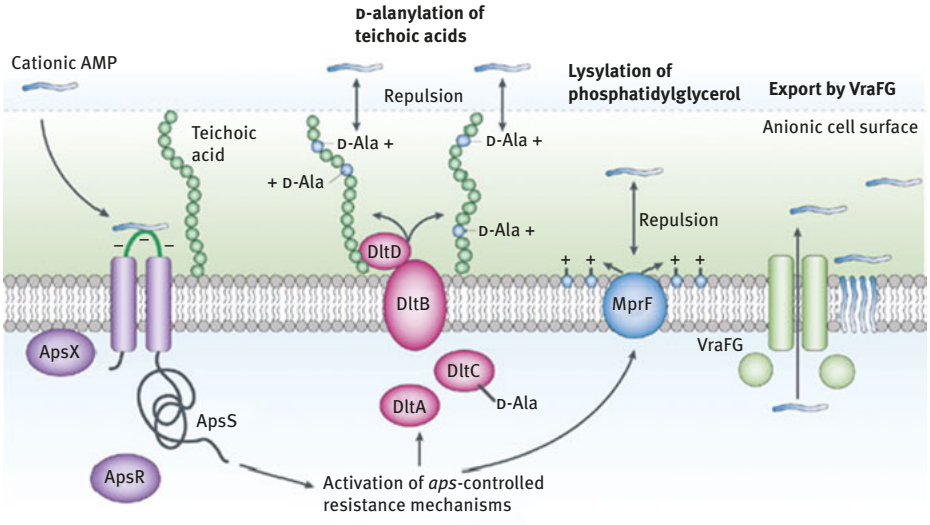

Figure 12.2: Cationic antimicrobial peptides (AMPs) attach to the negatively charged bacterial surface and membrane. (Adapted from).

The majority of AMPs is cationic AMPs inand are cationic antimicrobial peptides (Tops) [42]. Normally, inferred Tops regularly contain a net positive charge in the range of +2 and +9, as a result of their event of few or potentially no acidic deposits, that is glutamate or aspartate and a more noteworthy number of cationic amino acids, for example, arginine, lysine and histidine [43]. The hydrophobic build-ups have unique structure, including tryptophan and connected amino acids, for example in valine, half of the entire peptide structure help in permitting amphiphilic structure to form layers [44]. These trademark results show high positive charge that enable cationic AMPs to have an antimicrobial impact. Interestingly, changing this net charge to hydrophobic buildups can improve the movement and wide range of the peptides against attack of large group of microorganisms. The ascending action that can be restored is accomplished by a decrease in the lipophilic charge proportion to bear the cost of glycopeptides. All the more as of late,

glycopeptides proof that vancomycin prove to be increasingly powerful anti-infectious agents against Methicillin safe *Staphylococcus aureus*. Lipoglycopeptide basically identified as vancomycin can have a noteworthy effect in improving oritavancin and dalbavancin, which have side chains to expand the action against an accumulation of vancomycin-safe strains [45]. Adjustment of this alteration in the net charge of hydrophobic proportion and charge can fluctuate the action to clear changes in layer permeabilization cytotoxicity and antimicrobial selectivity/action [46]. Tops, considered as a mixed class, have a wide scope in auxiliary and practical assortment and in an expansive range of antimicrobial agents contain principally amino acids that build up the primary arrangement of the peptide [47].

Apart from the general comparison of auxiliary structures are only the optional structures that antimicrobial peptides may differ between classes; they share similar qualities of creating amphipathic particles with hydrophobic nature and being cationic amino acids under physiological conditions [48, 49]. Two fundamental types of optional structures incorporate amphiphilic β-sheet structures that cover a few disulfide structures often with a short α-helical portion or two-to-four β-strands. Proof of these peptides is likely in nature incorporates all classes of mammalian partners that have peptides, including α-defensin and β-defensin [50]. These outcomes prove that the straight peptides are disarranged in hydrophilic arrangements; however, structure of amphipathic α-helices primarily causes disturbance, and especially in the hydrophobic conditions of cell layers [51].

The revelation of peptides is of explicit significance as it has a rapid helical structure to gain a standard of hydrophobic deposits, while the contrary face involves generally polar amino acids allowing skilled solubilization of microbial cell layers [44]. Fundamental amphipathic α-helices need cysteine substitutions and in this way are unfit to shape into disulphide spans [52]. Precedents in regular cures incorporate mellitin obtained from bumble bee venom [53], magainin picked up from skin discharges of the frog species *Xenopus laevis* [54], creepy-crawly cecropins and some of the dipteran safeguard peptides [55]. Cyclic peptides are an energizing auxiliary class of Tops that contain β-turn and have been impacted by a singular disulphide bond and incorporate a dodecapeptide specifically, found in ox-like neutrophils [56].

Broadened structures with an extraordinary course of action of a couple of amino corrosive deposits, similar to glycine, proline, histidine, make up the rest of the four essential auxiliary assortments [57]. AMPs can have direct neutralizing effects on bacteria, for example, by membrane disruption through pore forming or by targeting internal structures (Figure 12.3) of bacteria. In addition to direct effects, AMPs may modulate cells of the adaptive immunity (neutrophils, T cells and macrophages) to control inflammation and/or to increase bacterial clearance. These peptides are normally delivered by living beings as a major aspect of their natural barrier framework to avert attacking organisms. Many have showed an expansive range of antimicrobial movement with low poison content towards mammalian

cells, and, critically, Figure 12.4 indicated decreased obstruction. This is believed to be because of their progressively summed up activities, whereby their high constituent convergence of cationic amino acids (lysine and arginine) drives an electrostatic force in the adversely charged phosphate head bunches present on the bacterial layers. The possibilities of these cationic peptides have not very much characterized or as unmistakable structure on account of the presence of novel folds. Tritrpticin obtained from nature include the porcine antecedent protein [58] and the indolicidin present in cow-like neutrophil [59]. Indolicidin (indol) is a direct cationic antimicrobial peptide made of 13 amino corrosive groupings in ~40% of tryptophan-rich deposits [60]. They both have a pontoon-like structure that is available at the spot of cell films as the rich tryptophan content associated with the hydrophobic surface of the layer with the presence of cationic lysine and arginine deposits orientated to the fluid condition [61]. The cationic AMP mimetic tyrocidin was principally the accessible anti-microbial. It was observed to have serious issues like being toxic to human blood and conceptive cells that lead to its withdrawal from the market [62]. The polypeptide bacitracin contains an increasingly productive presentation that clinically blend with both neomycin and polymyxin B, informally known as the skin protectant anti-toxin salve, Neosporin® arrangement authorized for the topical treatment of the assortment of confined skin and eye diseases [63].

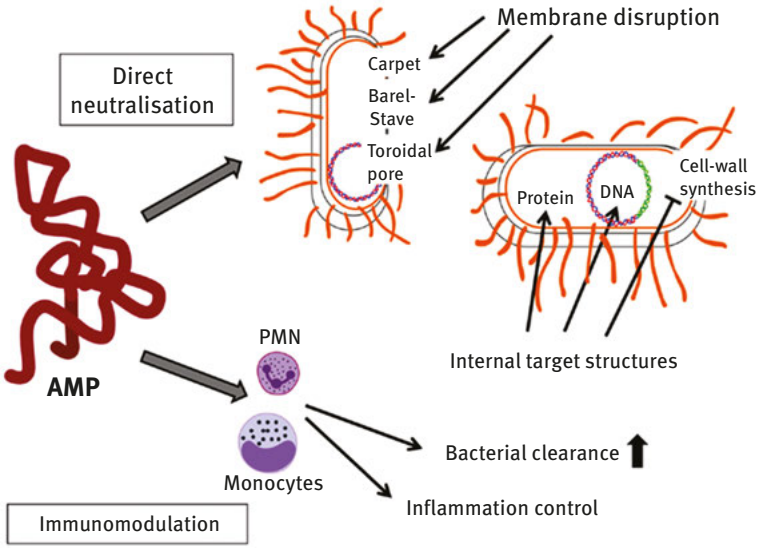

Figure 12.3: Different modes of action of antimicrobial peptides. (Adapted from Ref. [43]).

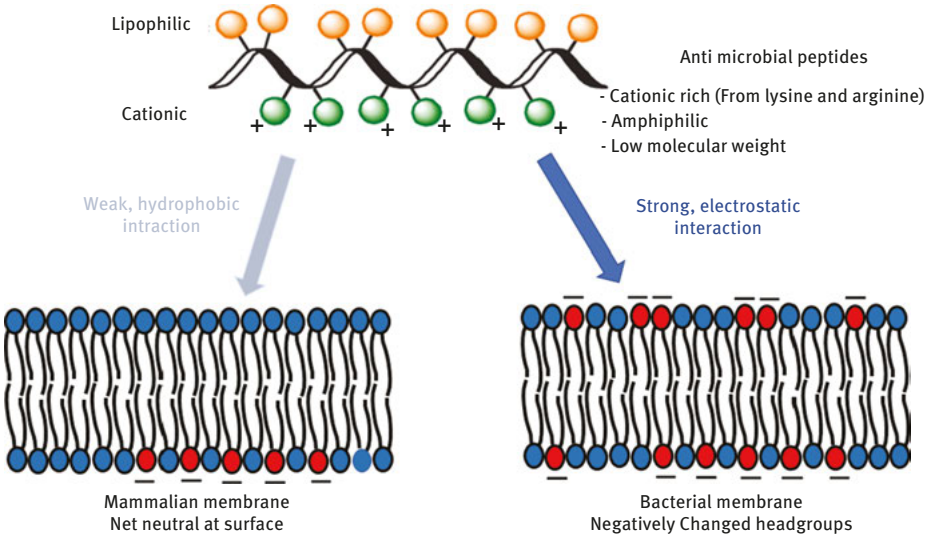

Figure 12.4: Schematic diagram showing the common structural properties and membrane targets of AMPs. Spheres on the AMP depict pendant amino acid side chains, orange for lipophilic groups and green for cationic groups. (Adapted from [73]).

12.3 Anionic antimicrobial peptides

Despite the disadvantages of antimicrobial peptides existing as cationic in nature, a generous number of anionic AMPs are helpful in the eukaryotes [64–66]. The dynamic peptides that are anionic in nature are biased towards glutamic corrosive then aspartic acids containing the solid land and water proficient peptide, for example, Maximin-H5 and Dermcidin, peptides that resulted after the human discharge of perspiration [67–68]. Commonly, the anionic antimicrobial peptides, as a rule, contain 5 to 70 amino corrosive gatherings, owing to a dispensable charge of –1 or –2 compared to physical arrangement confirmed that the abbreviated technique for the thick peptide B, named enkelytin, could possess a net charge as high as–7 [69]. While less mutual, major anionic peptides of 300 degree are present and the remaining charge at –20 have been expressed [70–72]. In a similar manner, to their cationic partners, anionic antimicrobial peptides can affirm variable amphiphilic gatherings, for example, the α-helix and β-sheet adaptation with coordinated effort through the microbial layer that is vital for activities.

A disadvantage of various anionic antimicrobial peptides is that they often need cations, in the case of zinc (Zn^{2+}), as cofactors for biocidal reactivity [73]. This may be generally situated at epithelial spots where commonly ionic outflow and bacterial presentation are maximum. Consequently, the anionic peptides, for example, surfactant related to a reactant of anionic peptides exist in respiratory tissues, have

been shown to possess increased strength against bothGram-negative and Gram-positive microbes in the presence of the moving cationic dynamic antimicrobial peptides and Zn^{2+} [74, 75]. These cationic moieties execute as a cationic association among the anionic antimicrobial peptides and furthermore the anionic bacteriological cell film. This case is proved by the transportation of the anionic peptides to intracellular objectives despite of the harm caused to the structure of microbial layer [76]. Thus, anionic peptides are objective ribosomes in the cell avoiding ribonuclease activity, consequent to bacteriological cell destruction [77, 78].

12.4 Land and water proficient antimicrobial peptides

Land and water proficient resultant peptides are remarkable instances of clearly occurring, physically changed activity of peptides through high antimicrobial adequacy. These are unconfined in layer outflows much of the time at high consideration and their development impersonates the advancement of creatures of land and water to their soggy home, an environment in a like manner pertinent for the development and rupture of clever pathogenic organisms and parasites [79]. Later the peptides are potential competitors against hostile tumour mixes, malignant growth [80] and similarly claim hostility towards viral development with plausible precaution against HIV [81]. They also delineate activity against the eukaryotic cells and consequently convey an asset by which creatures of land and water may be undermined after their predation [82]. The likelihood at that point happens that land and water proficiency that extract the real antimicrobial peptides may be cytotoxic to human beings. Such peptides are unlimited and cause harm or strain by decreasing myocytes that surround the organs [83] and might turn into a workroom over the use of minor electrical improvement [84] or embeddings of norepinephrine by the dorsal accomplishment [85].

The physical depiction has streamlined its fake creation by strong stage peptide mix (SPPS). In frogs, variations in the order accomplish a range of activities for these peptides that are broadened with several sorted dynamic peptides that provide resistance against an enormous arrangement of microbial and parasitic organisms [86, 87]. The fame of antimicrobial enthusiastic peptides in frogs is cationically attributable with high occurrence of lysine, though in many cases half of amino acids that are hydrophobic such as leucine was transcendent and an amphipathic α-helix thatlimits cell layering [88]. Pertinent types of frog that show this are Phyllomedusa [89], Litoria [90] Bombina [91], Xenopus [54] and Rana [92]. The *Bombina maxima*, which is mostly recognized as the Chinese red paunch amphibian devours as a factor for 40 hereditary components that undergo the development of divergent dynamic antimicrobial peptides [93]. Examination coordinated by Lai

et al. introduced that *Bombina maxima* formed a group of genuine peptides named as maximins that affirmed very low minimum inhibitory concentration (MIC) in the μg/mL quantity arrangement of bacteriological and contagious pathogens together with *Bacillus dysenteriae, Klebsiella pneumonia, Candida albicans, Staphylococcus aureus and Escherichia coli* [94]. Together maximin-3 and maximin-4 remained the best compelling peptides, these were checked with maximin-4 that demolish the bottommost MIC estimation of 2.7 μg/mL contrasted with *Staphylococcus aureus*. Maximin-4 contains 27 amino acids and has the possibilityto be formed unnaturally by SPPS. Dermaseptins are a lined lysine-rich cationic dynamic antimicrobial peptides because of the type of Phyllomedusa. They include 28–34 amino acid corrosive remains and ought to be presented to anticipate the improvement of a broad decent variety of protozoa, yeast, Gram-positive and Gram-negative microorganisms and organisms [89, 95].

12.5 Sane system and gathering of an antimicrobial peptide theme

The varying arrangement of distinguished antimicrobial peptides was open naturally and conveys a gigantic decision for the endeavour of creative and redesigned fake qualifications. Its buildup and results on the hydrophobic-charge connection are further large with regard to the driving antimicrobial activity compared to the size and the board of prime amino acid clusters, for example, explicitness, harmfulness and dauntlessness [96]. Ultrashort antimicrobial peptides contain around four or five amino acids, through amino corrosive get together remunerating the littlest arrangement of functionalities should require for dynamic antimicrobial development. These functionalities contain a charged moiety, for example, arginine and a lipophilic part, furthermost normally tryptophan, establishing an antimicrobial pharmacophore through the exact balance of lipophilicity and charge [97].

Strom et al. have shown that tryptophan conveys preferred mass and lipophilicity over tyrosine and at least two mass and two charged deposits and are important for providing movement against staphylococci, with *Escherichia coli* requiring an extra tryptophan. Arginine additionally exhibits an upgraded charge than lysine inside the most minimal theme [96]; however, arginine is difficult to work inside places of SPPS [98, 99]. The recognizable advantage to the utilization of ultrashort antimicrobial peptide is the tremendous decrease in rate in delivering these atoms near to engineered variations of normally occurring antimicrobial peptides. The association of an acyl chain to a functioning or dormant ultrashort cationic peptide likewise conceivably prompts an improved activity close to microorganisms in a comparative way to deal with local cationic antimicrobials [100]. Small varieties of methylene units present can change the range of movement of the antimicrobial,

the cell particulates and after that dimensions of hemolysis [101]. Makovsky et al. have announced that peptides containing four amino acids will be difficult to shape stable amphipathic structure, so questions still identify with their method of activity. The outcomes showed that the equalization of hydrophobicity and charge demonstrate a huge job similarly as in the higher antimicrobial peptides. Though differing, the amino corrosive stores and the acyl chain length was build for expanding hydrophobicity and additionally charge isn't uncovering to expanding antimicrobial capacity [102].

Host and microbial proteases and the unwanted pharmacokinetics convey existence of a key obstruction to the utilization of peptides as antimicrobials in vivo, with a few peptides limited to rehearse as fascinating employments. Human chymotrypsin-like catalysts work in amino corrosive buildups [103]; consequently, antimicrobial peptides are practical due to basic deposits available for antimicrobial action. The basic α-helical or straight structures in specific AMPs are powerless for proteolysis through an assortment of microbial proteases [104]. According to proteases, regular L-amino corrosive buildups, a change in the methodology of D-amino acids will separate the peptide to the different degree or absolutely impervious to proteolysis with no thrashing of antimicrobial action [105]. L-stereoisomers aremore hemolytic than their D partners [106]. Antimicrobial peptides remain orchestrated and are expanded further using D-enantiomers; in this manner, an extra supportable option is the decision of unnatural amino acids, for example, ornithine. The use of ornithine as a charged moiety is ideal for the utilization of a non-coded amino acid that conveys high efficiency next to proteases [96]. A straightforward case of fake tackling of ornithine's properties are set by Bisht et al. They affirmed the strong activity of ultrashort tetrapeptides with two ornithines exhibiting charge and two tryptophans that provided mass and hydrophobicity [39]. Adjustment occurs with the conjugation of cinnamic gatherings to the *N*-α-amino end of ornithine. The blend of ornithine into the lipopeptide platform has similarly been confirmed in the fake peptide MSI-843 [107]. Six ornithine deposits of the 10 and a conjugated octenyl end, phenomenal action was shown against Gram-positive *Staphylococcus aureus*; Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and the organism *Candida albicans*.

12.6 Ultrashort cationic antimicrobial peptides

The AMPs containing about four/five deposits of amino acids are for the most part ultrashort cationic antimicrobial peptides, with amino clusters accomplishing the minimal scope of properties basic for a genuine antimicrobial move to make dynamic spots. The use of cationic ultrashort antimicrobial peptides is valuable in creation of consolidated assembling costs and union periods. Their development

identifies with a propensity between peptides that developed these lesser peptides with respect to greater and further selected real peptides [108, 109]. Haug and partners with the lactoferrin results showed that the smallest subject occurred for antibacterial activity contrasted with a few frameworks of microscopic organisms with stressing of *Staphylococcus aureus* obstruction would share anti-toxin schedules [110]. The remaining activity is the finished balance in the midst of lipophilicity and charge through the presence of two sections in mass and twofold cationic charges required for film interface and their antimicrobial reactivity [96]. Clearly, antimicrobial peptides occur due to a vast lipophilic appeal through the main classification containing 30–50% extensive hydrophobicity, for example, tryptophan [44]. Primarily, the tetra- and pentapeptides content in pharmacophore with insufficient mass, therefore so as to gather a promising development in the lipophilic charge, in connection to the amino acids acylated by an assorted variety of hydrocarbon moieties [111]. With this data Haug et al. designed a successive minor dipeptides with all-inclusive detailing XRY; X implied a changeof cumbersome amino corrosive clusters through a lipophilic nearby link, in cyclohexylalanine; R indicated arginine to be the charged moiety. After this Y implied a C-terminal ester/lipophilic amide determined movement [97]. Subsequently, both X and Y give substance to fast pharmacophore. A sort of 2,5,7-tri-tert-butyl-tryptophan-arginine-benzyl amide was presented to the most efficient peptide for this examination. These cluster of bases are needed for paired pieces of mass, showing that tryptophan-inferred and benzene circle start the benzyl amide gathering and twofold units of charge passed on by arginine's guanidino together next to the free *N*α-amino station. Predominant of this, Bisht et al. showed a plot that has molded a succession of ornithine and tryptophan-containing tetrapeptides and evaluated them contrasted with planktonic frameworks of endless Gram-positive (G+) and Gram-negative (G–) organisms [39]. Towards the escalation of hydrophobicity of cinnamic corrosive, the outcomes are confused to the amino limit expected to peptide subjects. These terminal carboxylic acids included 4-hydroxycinnamic corrosive 3,4-dimethoxycinnamic corrosive, 3-(4-hydroxyphenyl) propionic corrosive and after that cinnamic corrosive.

12.7 Lipopeptides

The lipopeptides are primarily peptides required by lipid moiety. Such a peptide may hold a antimicrobial peptide. Innate lipopeptides are structured in microbes and organisms that are non-ribosomal by gaining reasons for carbon to adjust the multifaceted cyclic gatherings [112]. It normally includes 6, as well as 7 amino acid bunch with the addition of N-terminal unsaturated fat moiety. They are fiery rather than a progression of multi-safe organisms and microbes [113]. As needs are, the antimicrobial lipopeptides are primarily bacterial buildings produced through the

non-ribosomal biosynthetic ways and contain a peptidyl partition conjugated by methods for unsaturated fats to frame an acylated peptide [112]. Various normally occurring lipopeptides were being cyclized and in a similar way hold strong amino acids that oppose steadiness contrasted with proteolytic hardship. Thus, the lipopeptides may incorporate a cationic (e.g., polymyxin B, colistin), and anionic (e.g., Daptomycin, surfactin) peptide theme, which directs the wide scope of activity. At the point when the greasy amines, unsaturated fats, alcohols and glyceryl esters have presented to show variable evaluations of antimicrobial movement [114, 115], the acylation of peptide structures has been affirmed to expressively advance for the main antimicrobial activity [116]. For example, when the first mixing of polymyxin is joined to an unsaturated fat at the furthest point, rejection of this end primes to a decrease in antimicrobial endeavour [117]. The conjugation by an unsaturated fat moiety at the *N*-end could similarly causes harm to hydrophobicity inside the peptide chain established on amino corrosive leftover portions [118]. In this way, the amalgamation of an uplifted peptidyl structure and increase in *N*-terminal acyl (i.e., unsaturated fat) with basic antimicrobial activities were communicated through a strategy for the development of successful antimicrobials, whereby the scope of activity may be directed through change of *N*-terminal substituent, while maintaining a strategic distance from the feasible dividers going together with unique dynamic regular AMPs in high amounts.

12.7.1 Affirmed and economically open lipopeptides

An arrangement of cationic and anionic lipopeptides are used for attractive use, similarly in a manner of unmistakable quality of the therapeutic test of these antimicrobials. These grasp polymyxin B, polymyxin E (colistin) and daptomycin.

12.8 Chief classes of AMPs

12.8.1 Classification

Generally, the enzymatic mechanisms are not elaborate in the antimicrobial actions of AMPs [119]. For instance, however, the lysozyme was a monomeric peptide, and it is not confidential as an AMP since it is comparatively bulky (148 aa) and kills the bacteria over enzymatic occurrings via contravention for 1,4-β-linkages in peptidoglycan connectivities [120]. Also, in this chapter, we classify the actual AMPs based on their goal, and manner of exploitation. For normal AMPsthat we discuss, emphasis will be made on eukaryotes, expressed as the chief mammals.

12.8.2 Antiviral peptides

Antiviral AMPs kill illnesses by taking interest in the obsessive wrapping or the group cell layer one. Earlier modifications have been made that commonly encased RNA and DNA infections could be focused through the antiviral AMPs [121, 122]. The genuine AMPs could take an interest in viral wrappings and source film insecurity, deciphering the infection's lack of ability to pollute the host cells [123, 124]. The AMPs can comparably diminish the official of host cells among infections [125]. For example, defensins fix the infection-related glycoproteins in herpes simplex infections (HSV) unequipped for the scrape to the shallow of the host cell divider [126].

Likewise, the intrusion of viral covers and impeding viral receptors, which were around the antiviral dynamic AMPs could stay away from the solace viral units to approaching host cells by possessing exact receptors on mammalian cells [127, 128]. In this case, heparan sulfate has a big role as the impact of HSV viral particles to the the host cell [129]. The heparan sulfate parts are highky charged through glycosaminoglycan particles [130]. Subsequently, a portion of the α-helical cationic peptides, for example, lactoferrin [131], can stay away from HSV infections through the attachment to heparan atoms and obstruction for infectious receptor relations [132].

In Figure 12.5, mechanisms of activity of cationic antiviral peptides are shown. Cell surface targets: (1) Interaction of peptides with various glycosaminoglycan (e.g., HS) present on the cell surface opposing the infection for cell restricting destinations, (2) blocking of viral passage into the cell by restricting the peptide to viral CXCR4 co-receptor required for its entrancement [129], (3) suppression of cell combination by meddling with the action of ATPase protein, intracellular targets: (4) concealing viral quality articulation, (5) inhibition of peptide chain lengthening by inactivating the ribosome, (6) activation of a resistant modulatory pathway by acceptance of NK and IFN, viral protein targets: (7) binding of peptides to viral proteins causing hindrance of adsorption/infectious cell combination [131]. The viral section can be blocked through the collaboration of peptide with hemagglutinin (HA), ordinarily connecting with the buildup of corrosive sialic acid (Figure 12.6). This results in the change of HA capacities, and accordingly, flu virion cannot be connected to the layer of a host cell. The second antiviral activity of peptides might be completed intracellularly because of obstruction of HA compliance change that normally prompts opening of endosome, what is more, the spread of viral genome [132].

Identified as dynamic AMPs that have target neurotic receptors on the cell surface surrounding the AMPs do not challenge the infection-related glycoproteins that are vital to the heparan sulfate receptors on surfaces of the cell divider. Other than this, these vigorous antiviral AMPs can cross the cell packaging and restrict the cytoplasm with the organelles, stimulating varieties in the quality appearance layout of the host cells, which can profit for the host opposition grouping challenge contrasted with square popular quality/infections' appearance. In this case, NP-1, an AMP in rabbit neutrophils, turns away Vero and Caski cell positions and starts

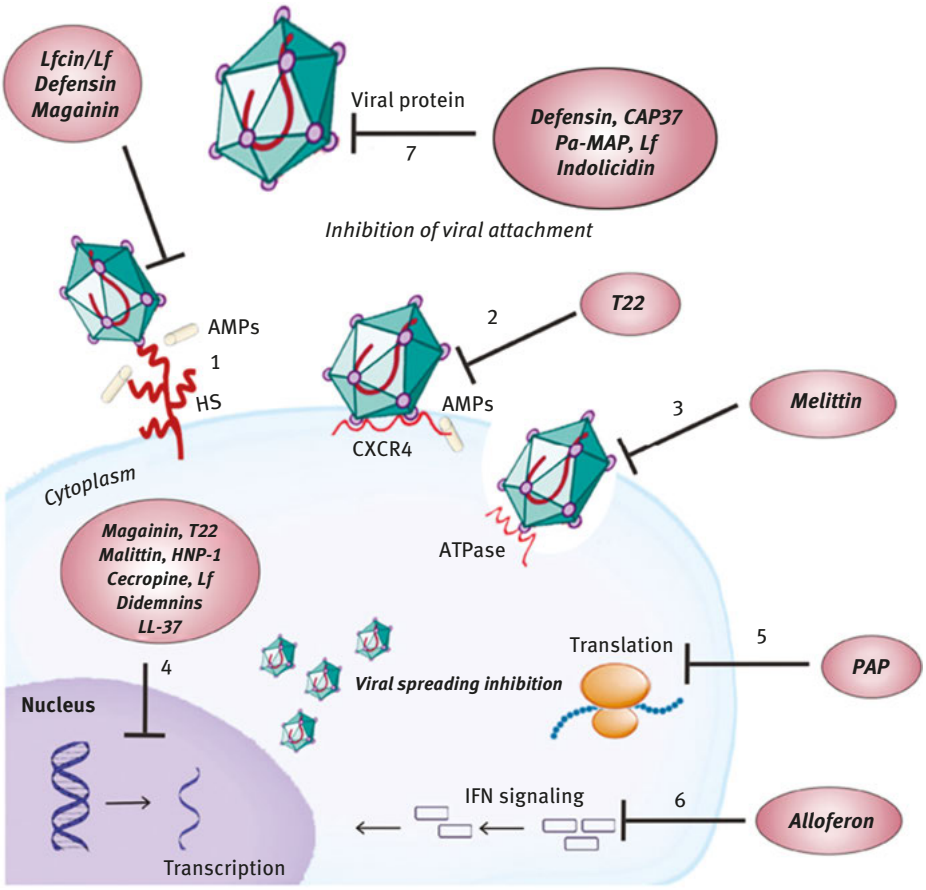

Figure 12.5: Mechanisms of action of cationic antiviral peptides. (Adapted from [129]).

damaging herpes simplex of sort 2 infections (HSV-2). This sort of AMP will break the infections by maintaining a strategic distance from migration for a premier viral protein, VP16, fixed through the core. This viral protein was fundamental to host transcriptional impacts to empower the presence of essential viral qualities, which are obligatory for the infection to overcome the underlying cell [133]. Thus, this AMP was not challenged by viral particles of the receptor cell that keeps away from cell-to-cell experience of viral particles [134].

12.8.3 Dynamic antibacterial peptides

Dynamic antibacterial AMPs are the most purposeful AMPs till date and the majority of them are cationic AMPs, bacterial cell films and source fracture of the lipid

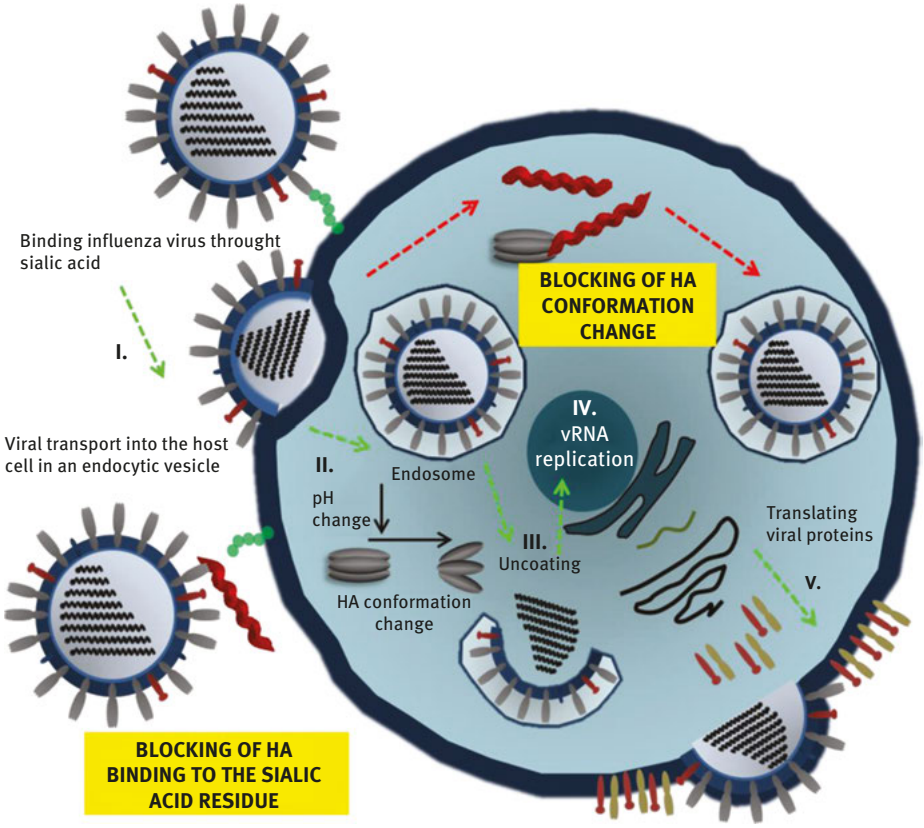

Figure 12.6: Mechanisms of inhibition of virus entry by peptides. (Adapted from [131]).

bilayer [135, 136]. These dynamic AMPs are relatively amphipathic by both hydrophobic and hydrophilic regions. Such developments have been conveyed difficulty to the AMPs to phospholipid congregations (hydrophilic area) and lipid devices (hydrophobic segment) [137].

Surprisingly, in ongoing decades the specialists have built up that a few AMPs at low assimilations can demolish the microorganisms that are denied of inconsistent film. Through this film, these real AMPs demolish the microorganisms by avoiding increasing ways that classify the cell, for example, protein arrangement and DNA reiteration [138]. Buforin II can diffuse into cell dividers by fixing into RNA and DNA that do not harm the cell layers [139]. Drosocin, apidaecin and pyrrhocoricin are the other cases of such AMPs. These AMPs consume 18–20 corrosive amino acid clusters with an enthusiastic site for their intracellular objective [140, 141].

As needed, the firm AMPs have been uncovered to obliterate the anti-toxin safe microscopic organisms. By delineation, commonly vancomycin (an anti-microbial) and nisin (an AMP), can hinder the cell divider arrangement. By and by, a methicillin

versatile *Staphylococcus aureus* (MRSA) strain was portrayed to be strong to vancomycin, in spite of the fact that it was still sensitive to nisin [142].

12.8.4 Antifungal peptides

Indistinguishable to genuine antibacterial AMPs, antifungal peptides can devastate the organisms through coordinating through the cell dividers of their points of interest [143, 144] or intracellular mechanical assemblies [145]. However, the bacterial film with their cell mass of growths consume the inner areas. As an example, chitin is one of a kind of primary mechanical assemblies of parasitic development of cell dividers for antifungal peptides are practised by chitin [146–148]. Such restricting performance was made for AMPs to the objective to parasitic cells. Considering these, the cell divider knocking down antifungal AMPs break the objective cells through films [149, 150] by aggregate permeabilization of the plasma layer [151], or by means of making pores in a straight way [152].

While the regular antifungal AMPs are polar and dynamic amino acid bunches for their congregations, [137] these do not appear to be a solid relationship among the development of a functioning AMP and the classification of cell dividers for this situation, antifungal peptides have partners from unique structure modules, for example, P18 [153]), α-helical (D-V13K [154], β-sheet (defensins [155]) and reaching out to indolic acid [156]).

12.8.5 Antiparasitic peptides

Fundamentals of antiparasitic peptides are a minor set identified with further three AMP modules. The first antiparasitic peptide assigned is magainin, which is demolishes *Paramecium caudatum* [157]. In cutting edge, a counterfeit peptide was set up against Leishmania parasite [158]. In another example, the antiparasitic peptide is cathelicidin, which destroys *Caenorhabditis elegans* through making openings in the cell divider films [159]. In any case, parasitic microorganisms stay towards multicellular organisms, the technique for misuse of antiparasitic peptides was indistinguishable as further dynamic AMPs. They devastate cells through straight interrelation with the cell layer [159].

12.9 Component of endeavour

As the standard of antimicrobials, the interface of the cell layer for organisms is basic to the technique for the endeavour of cationic antimicrobial peptides. The

significant modification in the designs of the main eukaryotic films is an consideration to prokaryotic layers that highlights the basic precision for the cationic antimicrobials through the bacterial cell divider. Phosphatidylethanolamine and Phosphatidylcholine were the comparabilities of phosphatidylcholine, with sphingomyelin sorted out with the sterols, ergosterol and cholesterol mostly begin in eukaryotes other than by and large devour for no staying as a chief charge towards a comprehensive fair-mindedly charged phospholipid bilayer. By the assessment, prokaryotic microbial cytoplasmic layers are adversely charged through a high electrical probable inclination given in the section of the presence of acidic hydroxylated actioned phospholipids, for example, cardiolipin, phosphatidylserine and phosphatidylglycerol [15]. From the cation, genuine antimicrobial peptides will become positive by adversely charged phospholipid bilayer of main bacterial cells [160]. This is valuable through regard to falling harmfulness into some degree of the potential remedial for the present circumstance. The nonattendance of precise receptors will also make it dangerous for microscopic organisms to give obstruction towards the peptides. Microscopic organisms would devour to change the assets of their film as a whole generally than precise receptors. Since a total positive charge associated through cationic real antimicrobial peptides assets that essential electrostatic interface through the bacterial cell film resolve to incorporate regions of consolidated anionic charge. For the most part, the acidic polymers, for example, teichoic acids in Gram-positive (G+) while [161] phosphate bunches existing on LPS in Gram-negative (G–) microorganisms [162] was allowed embellishment of the peptide previous to the improvement of transmembrane openings and in the long run film permeabilization.

The parasitic cell layers belong to the zwitterionic eukaryotic layers [163]. Subsequently, peptides that mostly claim antifungal activity grade to involve the independent amino acids by territories of high division signifying that a selective basic development association has occurred [164]. Impervious to this development, the activity and the ill repute of lipophilic moieties in the peptide structure were given by Lopez-Garcia et al. [165]. They showed that there was a straight relationship among the antifungal movement of receptive peptides and the fitness of peptides to the course of action of aggregative improvements through lipid innovations. Four key premises could happen with respect to how antimicrobial dynamic peptides will achieve access into the bacterial cell divider (Figure 1: Section A–D). These are the toroidal pore area, total, barrel fight and floor covering portrayals.

In Figure 12.7, components of antimicrobial peptides are shown. In a similar manner, antimicrobial peptides (barrels) through the charged hydrophilic territories (red) and hydrophobic zones (blue). (A) Has a place with the ‘total’ commonplace: the antimicrobial peptides reorient to a technique that removes the layer, gather peptide and lipid micelle advancements, incidentally no particular situation takes place; (B) the ‘toroidal pore’ will emerge when the peptides supplement vertical to plane for the lipid bilayer, through hydrophilic gatherings on the peptides

interrelating to methods for membranous phospholipid head congregations and the hydrophobic territories connecting through the lipid centre. A lipid bilayer smooth pore was moulded as the layer circular segments here; (C) the 'barrel-fight' types, in a similarmanner includes expansion of real peptides at a straight arrangement starts the plane for bilayer; however, boards are structured in a barrel that form a gathering attributable to hydrophilic helpings of the predictable peptide interrelating with the lumen of pore and hydrophobic segments of the peptide corresponding through the lipid bilayer; (D) the 'cover' run of the mill contains the build-up of peptides at a parallel heading, which includes lipid bilayer containing tangling of zones for the film. Along these lines, the micelles are structured straight at the genuine edge, considering first to a cleanser-like activity and the development of gaps in the cell layer, which was adjusted from Jenssen, Hamill and Hancock 2006 [166] related topic.

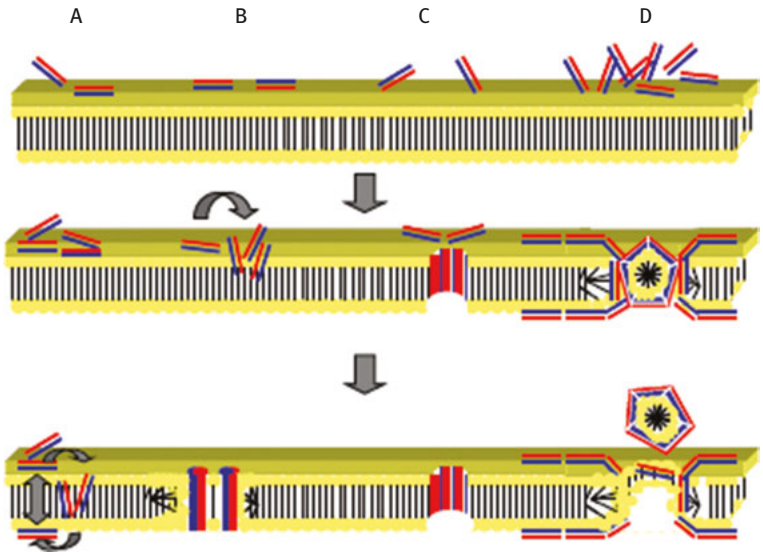

Figure 12.7: General system of antimicrobial peptides. (Adapted from [164]).

Since the toroidal pore types, which include the peptide infusing vertical into the cell layer through electrostatic relations among the hydrophilic regions of the peptide and to the phospholipid head since the bilayer. In the hydrophobic areas of the peptide curve, the lipid monolayers makeprevailing water fundamental wrinkled by lipid head congregations and infused with dynamic peptides [167]. By embracing, this real film bends inside, so that the bilayer layouts of the channel as fine as the peptides. As of now, the toroidal pore is in this manner planned by positive bending, conceding to access the extra antimicrobial peptide [168]. In the

consolidated kinds, a movement emerges to that of toroidal pore morals. However, peptides have not affirmed any exact situation, upon expansion to the cell layer they secure the film as a group of peptide and lipid micelles reactants [169].

Moreover, the channels that do framework separate fundamentally, so copious fragmented film expansion may be prime to the development of negative curve and peptide collection inside the bilayer [170]. Opposite enhancement of the peptides was establishing barrel-like gatherings or boards that would emerge in the barrel-fight types [171]. Pores can emerge since as minor as three peptide particles and speculatively to allow these openings to shape the dynamic peptides that are needed to share an amphipathic or hydrophobic α-helix, β-sheet development or together [172]. Ordinarily, the barrel-fight results in a transmembrane pore of free size. In general, this predictable pore for hydrophilic segments of the dynamic peptide restricts the lumen, beginning at the interior was likewise Van der Waal's attraction mixed among the hydrophobic peptide activities and the lipid focal [173]. The sprinter types recommend that peptides accumulate in the film by the hydrophobic areas of the peptide associating through the anionic phospholipid head collections on the principle of shalllow layer and hydrophilic locales to the polar dissolvable/medium [174]. By the restriction of receptive boss peptides, it can make a floor-like covering on the layer till first light mindfulness is developed. At this point, an indistinguishable cleaner movement will occur with a definitive improvement of micelles and passing gaps. Unsettling influence of the cell layers prompts film discontinuity [175]. It has been assumed that the further outside lipid layer existing on Gram-negative animals involving LPS coincides with the self-elevated endorsement approach to occur for cationic peptides [176]. With the regular key jobs, peptides have a gigantic liking for LPS, they are problematic to them, aggressively changing divalent cations by activities of magnesium and calcium particles since their certified restricting spots [177]. Commonly, the magnesium and calcium particles are obligatory for cell's shallow determined quality by cross-connecting specialists of carboxylates and phosphorylated head gatherings in their lipids [178]. The end of these divalent cations primes to the modification of the outside film making openings into extra peptides and further minor atoms (for example, topical customary anti-toxins). As a result of this, an explanation was conveyed by a method as to why in Gram-negative organisms would prompt various cationic antimicrobial peptides execution in association with moderate anti-infection agents [179].

Communication of cationic dynamic antimicrobial peptides through the run of the mill antimicrobials is not defective to simply Gram-negative microorganisms; however, shown for both [180] parasites and Gram-positive microorganisms additionally [181]. A parallel improvement of the central cell film interference has been set up for antimicrobial peptides in the logical inconsistency of a progression of parasitic organisms. The α-defensin NP-2, magainin-2 and thick lactoferrin demolish energetic film permeabilization for the phone divider hurt in *Candida albicans* responsive subject [182]. As indicated by antimicrobial lipopeptides picked up since

the estimations of *Bacillus subtilis*, is its fungicidal activity on cell films [183]. Lipopeptide accumulations and lipopeptide/phospholipid improvements framework at the peptide-layer limit the following to the ionic sound pores that allow the enlarged attack of potassium particles and parasitic cell passing [184]. These pores may likewise convey that ATP is unconfined from harm to the cell divider. The layer damage results in getaway and confined upsurges in extracellular ATP as the bacterial film is ruined. Eventually, intracellular metabolic methodology proceeded towards the advancement, Vylkova et al. accepted this may give rise to cell passing in the *Candida albicans* and different microorganisms by disentangling more peptide endorsement or invigorating the group's unmistakable ensured answer through ATP remains in as a chemoattractant at the situation of infection [185].

12.10 Summary and conclusion

For the most part, the generous dire necessity to accomplish new dynamic antimicrobial peptides has been overwhelmingly certifiable for AMPs investigation. Through the quick development in connected data and main mixes, further AMPs may arrive at restorative tests and handle in the contiguous things. Eventually, the infection controls through AMP and was as yet to defer by various tests checking low explicitness, likely noxiousness to creature cells, high constructer cost and nonappearance of overwhelming guidance for the typical endeavour. For instance, we have acknowledged since the counterfeit and improved AMPs updates, it is loose to change highlights of an AMP with consistency by the little adjustments. All things considered, expecting the results of these varieties is as yet animating. In a similar manner, there is a basis to value the assets of physical modifications on the physiochemical appearances of AMPs just as their objective range and their dynamic development. Of late, these sorts of updates were developing and computational strategies have associated with AMP examination. These efforts will profit for high values of the activity strategy of AMPs to anticipate their doings. In addition, to understudy its part is devouring in AMPs to switch anti-toxin safe microorganisms, persisters and biofilms. These sort of objectives are amazingly flexible to the novel customary anti-infectious agents and demonstrate the noteworthy parts in viruses. Then, the dynamic AMPs has focused on the cell film, they have moral potential in such propelled applications. On the other hand, since the AMPs devours that it is not been all around determined for persister control and biofilm, they may be tied in with characteristics that are dynamic to AMPs, that are really against these objectives through the potential cooperation by anti-infection agents relatives. By applying the AMPs with biofilm network debasing to proteins may too be a better than average way to deal with evacuating the biofilms. Likewise, the more extension in this degree of AMP inquire about in wide going advantage starts the

contiguous relationship of different adjustments and creative instruments could decode the structure-work affiliation and they are further expected to produce and change the AMP particle movement.

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13 Biocidal polymers: Future perspective

Abstract: Biocidal polymers like cationic polymers, amphiphilic hyperbranched, natural and synthetic rubber, polylactic acid, polyethylene glycol, polyurethane, acrylate based polymers, biocidal plastic and composites based on polymer and nano sized metals and antimicrobial peptides have shown broad-spectrum antimicrobial activity. In this chapter, emerging opportunities of antitimicrobial polymers are discussed.

Keywords: cationic, amphiphilic, biodegradable, nanoparticles, polyurethane, polylactic acid, poly(2-methyloxazoline).

The modification of polymers and fibrous surfaces to alter the porosity, wettability and other characteristics of polymeric substrates will enable the production of implants and biomedical devices, which exhibit greater resistance to microbial adhesion and biofilm formation. A number of polymers have been developed that can be incorporated into cellulose and other materials, which will provide significant advances in many fields such as food packaging, textiles, wound dressings, coating of catheter tubes and sterile surfaces.

Cationic polymers undergo self-assembly in aqueous conditions and impart biological activity by efficiently interacting with bacterial cell walls; hence, they can be used in chemical disinfectants and biocide formulations. The cationic charge and hydrophobic segments facilitate interactions with the bacterial cell surface and initiate its disruption. The perturbation of transmembrane potential causes leakage of cytosolic contents followed by cell death; hence, the development of polymer-based antimicrobials may help to address the challenges posed by drug-resistant bacterial infections.

Cationic nanostructured particles self-assembled from cationic bilayer fragments and polyelectrolytes are effective against four multidrug-resistant (MDR) strains of clinical importance. In addition, a non-haemolytic [poly(diallyldimethyl ammonium chloride) (PDDA) chloride] polymer as the outer nanoparticle (NP) layer shows remarkable activity against these organisms. The mechanism of cell death involves bacterial membrane lysis, as determined from the leakage of inner phosphorylated compounds and possible disassembly of the NP, with the appearance of multilayered fibres made of the NP components and biopolymers withdrawn from

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the cell wall. NP display broad-spectrum activity against MDR microorganisms, including Gram-negative and Gram-positive bacteria and yeast.

Quaternary ammonium and phosphonium salts have been extensively studied and applied to a variety of antimicrobial agents, for example, quaternised vinyl pyridine of varying alkyl chain length may be used as a powerful antibacterial and antifungal agent. The alkyl chain length of quaternary ammonium/polyethylene glycol (PEG) copolyoxetanes can be varied for greater antimicrobial efficacy, haemolytic activity and cytotoxicity. The biocidal properties of this polymer are due to polycationic interactions with bacterial membranes. Moreover, polyethylenimine (PEI)-grafted butyltriphenylphosphonium bromide polymers can exert antimicrobial activity against various Gram-positive and Gram-negative bacteria. Polymethyl methacrylates (PMMA) bearing cationic quaternary ammonium cations (QUAT) are future biocidal materials and the biocidal properties of these type of polymers are due to the polycations.

Cationic polymer-functionalised gold NP have large concentrations of positive charge, which promote their adsorption onto negatively charged bacterial membranes via electrostatic interactions. This kind of antimicrobial material, with effective antibacterial activity and better biocompatibility, can promote the healing of microbially infected wounds and could have promising applications in the biomedical field.

The development of environmentally friendly antimicrobial cationic polymers has gained increasing attention from researchers due to their low manufacturing cost and ease of synthesis compared with small antimicrobial peptides (AMP). These polymers are designed to mimic amphiphilic structures of AMP, which can disrupt negatively charged bacterial membranes, leading to potential antibiotic agents that can fight emerging drug resistance. However, reports of biodegradable antimicrobial polymer NP are rare; hence, there is a pressing demand to develop PEG-polyamino acid-based polymers, which exhibit antimicrobial properties. The synthesis of biodegradable cationic polycarbonates containing propyl and hexyl side chains quaternised with various nitrogen-containing heterocycles, such as imidazoles and pyridines, and their in vitro antimicrobial application is an important area of further research.

The non-covalent immobilisation of water-insoluble and organo-soluble cationic polymers onto a surface is a facile approach to prevent microbial contamination. Therefore, the synthesis of water-insoluble and organo-soluble polymeric materials, and their structure–activity relationships against various human pathogenic bacteria and fungi, is crucial for the development of future antimicrobial materials. One way to achieve this is by the use of cationic hydrophobic polymer coatings, which easily disrupt the lipid membrane of both bacteria and fungi, and thus exhibit a membrane-active mode of action; these polymers may hold great promise as coating materials for developing permanent antimicrobial paints.

The preparation of waterborne multifunctional polyvinyl amines (PVAm) can be achieved via the postpolymerisation modification of PVAm with a bifunctional coupler and functional cationic couplers, reactive azetidinium groups and alkyl chains; these polymers could provide excellent antimicrobial surfaces.

The use of polymeric ammonium salts and a sulfonic acid salt of sodium is an easy and efficient way of coating fabrics; furthermore, employing the layer-by-layer (LbL) deposition technique can broaden the application of *N*-halamine biocides in other polar substances for use as antimicrobial coatings. Branched PEI, polypropylene (PP) and styrene maleic anhydride copolymers are a very good coating for food packaging materials, possibly due to the presence of both cationic- and *N*-halamine-forming structures. *N*-halamine cationic antimicrobial polymers based on (acrylamidopropyl)trimethylammonium chloride, PDDA chloride and poly(2-acrylamido-2-methylpropane sulfonic acid sodium salt) have been synthesised and coated onto cotton fabrics via an LbL deposition technique.

Ammonium ethyl methacrylate-based polymers show increased inhibitory effects against various pathogenic bacteria. The interaction of synthetic random copolymers, based on methacrylates, with prototypical bacterial membranes can lead to a micellar aggregate in the water phase and the aggregate, when interacting with the bacterial membrane, induces clustering of oppositely charged anionic lipid molecules and disrupts the ordering of lipid chains.

Antimicrobial and cell-penetrating peptide chains can be tailored to achieve the following important properties: (i) high biocidal efficiencies and selectivity for bacteria (Gram-positive/Gram-negative) (ii) the stable and mild encapsulation of viable isolated embryonic and larval cells to escape immune systems and (iii) pH-, temperature- or light-triggered interaction with cells. The charge density, species of cationic and guanidinium side groups and hydrophobicity (including polarity of stimuli-responsive moieties) will guide the design of new copolymers, which interact with microbial cell membranes.

The application of porous polymeric monolith (PPM) columns are an effective tool to cause bacterial cell lysis. By taking advantage of the large surface area and controllable pore size inherent to PPM, a dual mechanism cell lysis technique can be employed. The bacterial cell wall is mechanically sheared by flowing through the porous medium of the PPM column and is also damaged and disintegrated by physical contact with the antibacterial polymeric biocide, which covers the porous surface. PPM columns can be obtained from *n*-butyl methacrylate and *N*-(*tert*-butyloxycarbonyl)aminoethyl methacrylate via photopolymerisation. The porous network can be prepared directly inside a microfluidic channel, fabricated in a crosslinked-PMMA substrate by laser micromachining.

Cationic amphiphilic polymers are very useful, especially as antibacterial agents. These kinds of polymers can easily undergo self-assembly in aqueous conditions and impart biological activity by efficiently interacting with the bacterial cell wall; hence, these can be used in chemical disinfectants and biocide

formulations. Both the cationic charge, as well as hydrophobic segments facilitate interactions with the bacterial cell surface and initiate its disruption. The perturbation in transmembrane potential causes leakage of cytosolic contents followed by cell death. The development of polymer-based antimicrobrials will help to address the challenges posed by drug-resistant bacterial infections and may provide a new platform to efficiently combat such infections.

NP of the conducting polymer poly(*N*-ethylaniline) and poly(*N*-methylaniline) can be prepared using a green approach, that is, photocatalytic oxidative polymerisation. These polymeric nanomaterials exhibit enhanced antimicrobial activity against various pathogenic bacteria, and therefore find potential applications in biomedical sciences.

The antibacterial activity of copolymers of nanostructured polynaphthylamine with polyaniline (PANI) and *o*-toluidine exhibit greater antibacterial activity than pristine PANI. The antimicrobial effects of these kinds of copolymers make them useful ingredients for biomaterials used in food packaging and medical devices.

Recent studies have explored different mechanisms of antimicrobial action by designing hybrid nanomaterials that provide a new approach to killing microbes. One option employs a low-cost methodology to prepare nanosized copper–PANI nanocomposite materials with enhanced antibacterial and antifungal properties. Copper oxide nanocrystals immobilised in a PANI matrix coated onto fabrics for the improvement of antimicrobial activity could be an excellent application in coating technology.

Natural biodegradable polymers with tailor-made properties offer excellent opportunities for advanced functional materials, for example, biodegradable conductive nanocomposites based on polypyrrole (Ppy)/dextrin or PANI/dextrin provide enhanced conductive and antibacterial activities.

Furthermore, composite films of nanofibrillated cellulose (NFC)/Ppy and NFC/PPy–silver NP are a suitable candidate for use in biomedical applications. Because of electrical conductivity and strong antimicrobial activity of these silver composites, they can be used in various applications, in particular, biomedical treatments and diagnostics.

A number of investigations have indicated that the introduction of zinc oxide can improve ultraviolet resistance and endow antimicrobial properties to PP materials. Hence, PP composites containing zinc oxide along with silver can further enhance the antimicrobial properties of this polymer. Antibacterial elastomer composites of silver zeolite/silicone could be a useful material to satisfy a range of requirements, including good mechanical properties due to the incorporation of zeolite, and good antibacterial properties.

An antimicrobial coating for food packaging materials is one of the best ways to prevent microbial growth, for example, copolymers from branched PEI and styrene maleic anhydride can be applied onto the surface of PP. The resulting coatings exhibit low surface energy and better antimicrobial characteristics due to the

presence of both cationic- and *N*-halamine-forming structures. Such antimicrobial coatings could improve food safety by reducing the microbial contamination of food processing equipment.

The addition of azo dyes into polymer matrices, such as polylactic acid (PLA) and PEG can result in very interesting antibacterial and antifungal materials, which have the capability to inhibit biofilm formation. The antimicrobial properties of such compounds are probably due to the release of azo groups, which can be determined via spectrophotometric analyses. These materials can be used in several fields, including biomedical tools, biodegradable antibacterial coatings and films for active packaging. Another approach to obtaining improved antimicrobial properties is the synthesis of nanocomposites based on PLA and zinc oxide, which exhibit better thermomechanical properties and could be a promising antimicrobial agent against bacteria.

Functionalised antimicrobial polymers can be developed by chain transfer (reversible addition-fragmentation transfer) polymerisation involving amino derivatives of methacrylate containing polydimethylsiloxane, which are further quarternised to yield new cationic polymers and are useful as antimicrobial coatings.

In many applications, microbial contamination is the primary risk of using conventional petroleum-based plastics, potentially causing infection when used in medical applications and contamination when used in food packaging. Non-traditional materials, such as protein, are being examined for their potential use in the production of bioplastics for applications that require uncontaminated materials. In terms of antibacterial activity, albumin–glycerol and whey–glycerol are the best bioplastics, as no bacterial growth was observed on these plastics after 24 h of inoculation. This material will be employed for the increased production of bioplastics, which are used in food packaging, as well as biomedical applications.

Polyurethanes (PU) and PEG-containing PU with and without free isocyanate end group-coated silica particles can be fabricated and show good potential for use in practical antibacterial applications.

A new methodology to fabricate antimicrobial polymeric surfaces, which exhibit antiadhesive properties has been developed using PP modified by air plasma and has the potential to be an excellent coating surface with activity against several pathogenic bacteria.

It is quite challenging to bind any polymer onto a wool fibre surface because of the lack of any available functional groups on its surface. Hence, a new approach of introducing anionic sulfonate groups onto a wool fibre surface by grafting with polystyrene (PS) sulfonate has enabled the binding of cationic quaternised chitosan (CS) via ionic bonding. The grafting of wool fabric with PS sulfonate improved not only its tensile strength and elongation, but coating with quaternised CS further increased its strength and reduced the elongation at break. The PS sulfonate-grafted wool fabric coated with quaternised CS retained its antibacterial activity.

CS, which is a natural polymer containing active groups, such as $-NH_2$, can be functionalised to introduce new positively charged *N*-atoms. The CS–iodine complexes exhibit better antimicrobial properties.

The antimicrobial, oxygen barrier and mechanical properties of certain nanocomposites were all improved due to the localised aggregation of cellulose nanowhiskers (CNW) and CS, driven by electrostatic interactions, thereby making the nanocomposites potentially useful for many applications, including food and antimicrobial packaging. Green hybrid films based on nanocomposites of polyvinyl alcohol (PVA), CNW and CS have been prepared by an environmentally friendly water-evaporation process. The electrostatic interaction between CNW and CS, as well as the hydrogen bonds between the three materials, play a very important role in determining the properties of the composites.

The chemical modification of CS biopolymers via reductive amination, to yield alkylated CS derivatives, and further quaternisation result in very efficient antibacterial materials; the degree of activity is correlated to the length of the alkyl chain and bacterial strain. The most active CS derivatives are more selective at killing bacteria than the quaternary ammonium disinfectants, cetylpyridinium chloride and benzalkonium chloride and AMP. Vanillin can be used as a crosslinker of CS; using this approach, functionalised antimicrobial polymers based on CS, vanillin, Tween® 60 and so on may be easily prepared. Imino–CS biopolymer films, prepared by the acid condensation of the amino groups of CS with various aldehydes, can be used as functional biodynamic materials.

Curcumin-loaded CS/cellulose microcrystal composite films can be prepared by the 'vapour-induced phase inversion' method and show good antimicrobial action against bacteria and fungi. Cellulose fibres made of CS/lignosulfonate (LS) multilayers exhibit antimicrobial and antioxidant activities and can be constructed on fibre surfaces via the LbL deposition technique. Antimicrobial testing determines its suitability for certain applications. Herbal essential oils, such as *Cuminum cyminum*, are natural antifungal agents consisting of many different volatile compounds; hence, materials based on CS and caffeic acid, a natural antifungal agent, can be used to improve antimicrobial activity. This finding could be of great significance in medicinal plant remedies to achieve an enhanced performance of herbal essential oils.

CS-based derivatives, such as *O*-quaternary ammonium *N*-acetyl thiourea CS, *O*-quaternary ammonium CS and so on, with enhanced antibacterial activity and good water-solubility, compared with natural CS, are interesting candidates for antibacterial coating applications. The positive charge introduced by the quaternary ammonium and thiourea groups further increase the overall positive charge of the CS derivative, thereby enhancing its antibacterial activity. Antimicrobial testing demonstrated that the CS/LS multilayer fibres, modified with CS in the outermost layer, exhibited higher antimicrobial activity against *Escherichia coli*. Antioxidant testing showed that the antioxidant activity of the CS/LS multilayer modified fibres was better than that of unmodified fibres under the same oxidation conditions.

It has been previously reported that all nanoformulations exhibit high antimicrobial activity against various microorganisms; hence, CS–metal complexes could be promising candidates for antimicrobial agents in the cosmetic, food and textile industries. Furthermore, enhancing the adsorption efficiency of CS toward arsenic (III), a highly toxic and predominant arsenic species in groundwater, may be useful, especially as there is an increase in antimicrobial activity at neutral pH values (pH of natural waters). Therefore, copper–CS/alumina nanocomposites may also be used as antimicrobial materials. Carboxymethylated chitosan (CMC)-stabilised copper NP can be synthesised via the chemical reduction of a copper (II)–CMC complex in an aqueous medium, which could be used as an antifungal agent for external coating applications.

Antimicrobial CS–silver nanocomposites are also potentially new and exciting antimicrobial agents against various pathogens. CS–silver nanocomposites, either in the form of silver NP or as ionic dendritic structures (Ag^+) can be easily synthesised by a simple and environmentally friendly in situ chemical reduction process and could be efficient antimicrobial agents. Below specific critical concentrations, the collective action of silver NP and Ag^+ ions facilitate enhanced and synergistic antibacterial activity.

Magnetic NP coated with CS to produce CS-magnetic NP also exhibited antimicrobial activity against tested microorganisms (with a more pronounced effect against Gram-negative than Gram-positive bacteria), and thus, should be further studied as a novel nanoantibiotic for numerous antimicrobial and antituberculosis applications.

Over recent years, the number of patients infected by drug-resistant pathogenic microbes has increased remarkably worldwide, and a number of studies have reported new antibiotics from natural sources. Among them, CS, which has a high molecular weight (MW) and α-conformation, exhibits potent antimicrobial activity, but its application as an antibiotic is limited by its cytotoxicity and insolubility at physiological pH; however, this problem can be solved by using low MW β-CS. The active target of β-CS is the bacterial membrane, where calcein leakage is induced by artificial PE/PEG vesicles, that is, a bacterial mimetic membrane. β-CS could potentially be used in anti-infective or wound-healing therapeutic applications. Bionanocomposite coatings comprising PVA-capped silver NP embedded in a CS matrix also exhibit excellent antibacterial activities against pathogenic bacteria.

There is a large gap in the field of microbiological fibre testing and the successful establishment of appropriate techniques is essential. Antimicrobials prevent bacterial cell division by damaging the cell wall or affecting the permeability of cell membranes; in addition, they denature proteins, block enzyme activity, resulting in microbial death. Intracellular potassium cations are released by the inhibition of pathogenic microorganisms and their quantitative determination enables monitoring of the bactericidal effect of antimicrobial agents. CS, a biodegradable natural polymer, exhibits antimicrobial characteristics that depend on a number of factors,

including the quantity of protonated amino groups, degree of acetylation, MW, solvents and so on. Over recent years, CS has increasingly used for fibre functionalisation. By employing such an approach, daptomycin may offer an antibacterial alternative for the treatment of endophthalmitis, caused by methicillin-resistant *Staphylococcus aureus* (MRSA), and mucoadhesive CS-coated alginate (ALG) NP are an effective delivery system for daptomycin permeation across ocular epithelia. CS–ALG NP can be prepared via the ionotropic pregelation of an ALG core followed by CS polyelectrolyte complexation and are characterised by particle size, polydispersity, zeta potential and encapsulation efficiency.

Microbial attachment and subsequent surface colonisation lead to the spread of deadly community acquired and hospital-acquired infections; hence, the non-covalent immobilisation of water-insoluble and organo-soluble cationic polymers onto a surface is a logical approach to prevent microbial contamination. Linear polymers are more active and result in a higher killing rate than branched polymers and therefore, the preparation of water-insoluble and organo-soluble polymeric materials and determination of their structure–activity relationship against various human pathogenic bacteria and drug-resistant strains, such as MRSA, vancomycin-resistant *Enterococcus faecalis* and β-lactam-resistant *Klebsiella pneumoniae*, as well as pathogenic fungi such as *Candida* spp. and *Cryptococcus* spp., may be an important strategy. These polymers have excellent flow levelling and adhesion compatibility with other medically relevant polymers, such as PLA, and can also be used to develop permanent antimicrobial paint.

Food packaging materials containing antimicrobial substances are used to limit the microbial surface contamination of foods to enhance product safety and extend the shelf life, which is of increasing interest to the packaging industry. Several biocidal polymers can be used for different types of packaging materials. Over the last 5 years, consumer demand for natural food ingredients has increased due to safety issues and increased food availability, hence natural polymers are beginning to replace chemical additives in foods and help alleviate safety concerns. Recent research has mainly focused on the application of natural antimicrobials in food packaging systems. Biologically derived compounds, such as bacteriocins, phytochemicals and enzymes, can safely be used in antimicrobial food packaging.

The future of therapeutic CS and poly(γ-glutamic acid) (γ-PGA) composites, which can also be used as AMP and chemical delivery systems, involves using ionic complexation between the positively charged peptides and negatively charged polyelectrolyte γ-PGA to obtain microparticles with negative zeta potentials, and is an important step in the development of the next generation antimicrobial agents.

CS and polyethylene oxide blended nanofibres within nanofibrous scaffolds can be prepared by electrospinning at ambient conditions, which further increases the effectiveness of their antimicrobial properties.

Until now, the treatment of non-healing wounds, as a result of repeated tissue injuries, bacterial contamination and altered physiological conditions, has represented a severe problem, which has led to huge costs for the healthcare industry

worldwide. In order to alleviate this challenging issue, innovative biomaterials have been developed, which are capable of preventing bacterial infection, draining exudates and favour wound healing, for example, a novel technique is based on the slow diffusion of tripolyphosphate to prepare macroscopic CS hydrogels in the form of soft pliable membranes, which contain antimicrobial silver NP stabilised by a lactose-modified CS (chitlac).

Bacterial infection associated with medical devices remains a challenge to modern medicine as they provide the surface and environment for bacterial colonisation. In particular, bacteria commonly adhere more preferably to hydrophobic materials, which are commonly used to make medical devices. Bacteria are also becoming increasingly resistant to common antibiotic treatments as a result of the misuse and abuse of antibiotics. There is an urgent need to find alternatives to antibiotics in the global prevention and treatment of device-associated infections. Silver NP have emerged as a promising non-drug antimicrobial agent and have shown effectiveness against a wide range of both Gram-negative and Gram-positive pathogens. Therefore, a new approach may involve the incorporation of silver NP into a hydrophobic polycaprolactone matrix, which will improve the release of silver ions from the matrix leading to enhanced antimicrobial efficacy.

Polymers containing QUAT are well-known antimicrobial and disinfectant agents. In contrast, longer *N*-alkyl analogues lead to reduced antimicrobial activity, showing a general structure–activity relationship that depends on the amphiphilic balance of the polycation. These polymeric families may provide scope for developing a new class of versatile antimicrobial QUAT with promising biomedical applications.

The synthesis of functional polymers containing silver NP is environmentally friendly, experimentally simple and extremely quick. It opens up new possibilities for the development of antimicrobial coatings with medical and sanitation applications.

Antibacterial and antifungal PLA-based films containing antimicrobial azo dyes are interesting candidates for biomedical tools, biodegradable antibacterial coatings and films for active packaging. Recently, well-defined polymeric antimicrobial agent carriers have been developed, for example, drug loading/release, particle stability and other characteristics. The in vitro antimicrobial activity of silver-bearing functionalised phosphoester and L-lactide polymeric NP will be important in the prevention of bacterial skin and urinary tract infections.

Surface wettability trends and the blood component adhesion of some cellulose acetate phthalate/hydroxypropyl cellulose blend films have been analysed to allow their adaption to biomedical applications. It is important to ascertain the antimicrobial activity of the studied blends to develop new applications in the biomedical field.

Dental repair materials face the problem that the dentin below the composite fillings is actively decomposed by secondary caries and extracellular proteases. To

address this problem, poly(2-methyloxazoline), which contains a biocidal and polymerisable terminal, will be explored as an additive for a commercial dental adhesive.

Air-ozonolysis has already been revealed to be an accessible and effective approach for surface activation and the further functionalisation of hydrocarbon polymers. Antimicrobial contact-active polyethylene and PS can be designed by the surface generation of OH-functional groups and the covalent grafting of a dimethyloctadecyl [3-(trimethoxysilyl) propyl] ammonium chloride quaternary ammonium salt. This strategy might establish a new general and safe platform for the future development and applications of contact-active antimicrobial polymers.

13.1 Concluding remarks

Biocidal polymers are considered to be the next generation of antibiotics, which can be effectively used against microbial infections. The antimicrobial activities of silver-bearing functionalised polymers and L-lactide polymeric NP are an important aspect in the prevention of bacterial skin and urinary tract infections. Biologically derived compounds, such as bacteriocins, phytochemicals and enzymes, can be used in antimicrobial food packaging.

CS–metal/alumina complexed nanocomposites could be promising candidates for antimicrobial agents in the cosmetic, food and textile industries. Quaternised CS and CS–iodine could be future materials with enhanced biocidal properties. Improving our understanding of antimicrobial mechanisms will enable the continued design of new biocidal polymers, which is necessary to combat the development of microbial resistance.

Abbreviations

3D	Three-dimensional
AA	Acrylic acid
Ac-DEX	Acetyl-dextran
ACHT	2-Amino-4-chloro-6-hydroxy-s-triazine
AC-nAg	Alginate-chitlac silver nanoparticle hydrogel
AEO	*Allium sativum* essence oil
AIBN	Azobisisobutylonitrile
ALG	Alginate
AMP	Antimicrobial peptide
APMA	*N*-(3-aminopropyl)methacrylamide
APTES	(3-Aminopropyl)triethoxysilane
ARGET	Activator regenerated by electron transfer
AST	Antimicrobial susceptibility test(ing)
ASTM	American Society for Testing and Materials
ATP	Adenosine triphosphate
ATRP	Atom transfer radical polymerisation
BBB	Blood–brain barrier
BF	Bilayer fragment(s)
BSA	Bovine serum albumin
BuI	Butyl iodide
BV	Bacterial vaginosis
CAP	Cationic antimicrobial peptide(s)
CFU	Colony forming unit(s)
CHPTA	3-Chloro-2-hydroxypropyltrimethylammonium chloride
CLSI	Clinical and Laboratory Standards Institute
CLSM	Confocal laser scanning microscopy
Cl-TMPM	*N*-chloro-2,2,6,6-tetramethyl-4-pipridinyl methacrylate
CMC	Carboxymethyl cellulose
CNW	Cellulose nanowhisker(s)
ConA	Concanavalin A
CP	Conducting polymer(s)
CPE	Conjugated polyelectrolyte(s)
CS	Chitosan
CSA-13	Cationic steroid antimicrobial-13
CTAB	Cetyltrimethylammonium bromide
DA	*N*-(1,1-dimethyl-3-oxobutyl)acrylamide
DABCO	1,4-Diazabicyclo-[2.2.2]-octane
DCC	*N,N′*-dicyclohexylcarbodiimide
DEAPMA	*N*-[3-(diethylamino)propyl]methacrylamide
DMAEMA	*N*-[3-(dimethylamino)ethyl]methacrylate
DMAPMA	*N*-[3-(dimethylamino)propyl]methacrylamide
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DODAB	Dioctadecyldimethylammonium bromide
DOPG	1,2-Dioleoyl-*sn*-glycero-3[phospho-rac-(1-glycerol)]
DOX	Doxorubicin

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DPPH	1,1-Diphenyl-2-picrylhydrazyl
DS	Disuccinimidyl
DSC	*N,N'*-disuccinimidyl carbonate
DSS	Dicyclohexyl sulfosuccinate
DTX	Docetaxel
eATRP	Electron-atom transfer radical polymerisation
ECM	Extracellular matrix
EDA	Ethylenediamine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EPR	Electron paramagnetic resonance
FDA	US Food and Drug Administration
Fmoc	Fluorenylmethyloxycarbonyl
f-PANI	Functionalised-polyaniline
FTIR	Fourier-Transform infrared
GMA	Glycidyl methacrylate
GTMAC	Glycidyltrimethylammonium chloride
HA	Hydantoin acrylamide
hBM-MSC	Human bone marrow-derived mesenchymal
stem cell(s)	
HBP	Hyperbranched polymer(s)
HDP	Host-defence peptide(s)
HEC	Hydroxyethyl cellulose
HMDI	Hexamethylene diisocyanate
HMQ	2-Hydroxy-5-methylquinone
HMS	Hollow microsphere(s)
HPC	Hydroxypropyl cellulose
ICAR	Initiators for continuous activator regeneration
ICP	Intrinsically conducting polymer(s)
IEP	Isoelectric point
IGF-1	Insulin-like growth factor-1
ImS	Imidazolium salt
ISO	International Organization for Standardization
JIS	Japanese Industrial Standard
LB	Lysogeny broth
LbL	Layer-by-layer
LDH	Lactate dehydrogenase
LDPE	Low-density polyethylene
LEAP	Liver-expressed antimicrobial peptide(s)
LLDPE	Linear low-density polyethylene
LS	Lignosulfonates
MA	Maleic anhydride
MBC	Minimum bactericidal concentration(s)
mcl	Medium chain length
MDPB	Methacryloyloxydodecyl pyrimidinium bromide
MDR	Multidrug-resistant
MFC	Minimum fungicidal concentration
MHA	Müeller–Hinton agar
MHB	Müeller–Hinton broth
MIC	Minimum inhibitory concentration(s)

MMA	Methyl methacrylate
MMT	Montmorillonite
*M*n	Number average molecular weight
MNP	Metal nanoparticle(s)
M-NR	Polymeric micelles incorporating nile red
mPE	*m*-Phenylene ethynylene(s)
mPEG	Methoxy polyethylene glycol
MRSA	Methicillin-resistant *Staphylococcus aureus*
MTC	5-Methyl-5-(α-tocopheryl)carboxyl-1,3-dioxan-2- one
MW	Molecular weight
NFC	Nanofibrillated cellulose
NHS	*N*-hydroxysuccinimide
NMR	Nuclear magnetic resonance
NP	Nanoparticle(s)
NSP	Nanosilicates platelet
o-PDA	*o*-Phenylenediamine
OD	Optical density
OPE	Oligomeric *p*-phenylene ethynylene(s)
p-DNA	Plasmid-deoxyribonucleic acid
p-HEMA	Poly(2-hydroxyethyl methacrylate)
PAA	Polyacrylic acid
PAN	Polyacrylonitrile
PANI	Polyaniline(s)
PAS	Poly-amido-saccharide(s)
PBS	Phosphate buffered saline
PC	Polycarbonate(s)
PC-4	Polyquaternium-4-cellulose
PC-10	Polyquaternium-10-cellulose
PCL	Poly(ε-caprolactone)
PDA	Potato dextrose agar
PDDA	Poly(diallyldimethylammonium chloride)
PDI	Polydispersity index
pDMAEMA	Poly[2-(dimethylaminoethyl)methacrylate]
PDMS	Polydimethylsiloxane
PE	Polyethylene
PEG	Polyethylene glycol
PEI	Polyethylenimine(s)
PEI_+	Alkylated polyethylenimine(s)
PEO	Polyethylene oxide
PET	Polyethylene terephthalate
PEUA	Carboxylated polyurethane
PG-1	Protegrin-1
PGON	Polyguanidinium oxanorbornene
Pgp	P-glycoprotein
PHA	Polyhydroxyalkanoate(s)
PHBV	Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
PLA	Polylactic acid
PLGA	Poly(D,L-lactide-*co*-glycolide)
PLLA	Poly(L-lactic acid)

PLL-CA	Cholate grafted poly(L-lysine)
PMA	Polymethacrylate(s)
PMDETA	*N,N,N′,N″,N‴*-pentamethyldiethylene triamine
PMMA	Polymethyl methacrylate
PMPI	*N*-(*p*-maleimidophenyl)isocyanate
PMX	Polymyxin
PO	Polyolefin(s)
poly(1)	4-(Dimethylaminomethyl)-styrene
poly(2)	4-Octylstyrene
poly(3)	Poly[trialkyl-3-(and 4-)vinylbenzylammonium chloride]
PolyHMQ	Poly(2-hydroxy-5-methylquinone)
poly(HS-*co*-MMA)	Poly(hydroxystyrene-*co*-methyl methacrylate)
poly(MEO_2MA-*co*-HOEGMA)	Poly[2-(2-methoxyethoxy)] ethyl methacrylate-*co*-hydroxyl-terminated oligoethylene glycol methacrylate
POSS	Polyhedral oligosilsesquioxane
PP	Polypropylene
PPE	Polyphenylene ethynylene(s)
PPM	Porous polymeric monolith
Ppy	Polypyrrole
PS	Polystyrene
PSSNa	Poly(sodium styrene sulfonate)
PT	Peptic-tryptic
PtBA	Poly(*tert*-butyl acrylate)
PTFE	Polytetrafluorethylene
PTh	Polythiophene
PU	Polyurethane(s)
PVA	Polyvinyl alcohol
PVAm	Polyvinyl amine(s)
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
PVMK	Polyvinylmethyl ketone
QAMA	Quaternary amine methacrylate
QAS	Quaternary tetraalkylammonium
QUAT	Quaternary ammonium cation(s)
RAFT	Reversible addition-fragmentation transfer
RBC	Red blood cell(s)
RIF	Rifampicin
RNA	Ribonucleic acid
ROMP	Ring-opening metathesis polymerisation
ROP	Ring-opening polymerisation
RSV	Resveratrol
RT	Room temperature
SAM	Self-assembled monolayer(s)
SARA	Supplemental activator and reducing agent
SCDLP	Soybean casein lecithin polysorbate
scl	Short chain length
SDA	Suborn dextrose agar
SEC	Size exclusion chromatography
SEM	Scanning electron microscopy

SMA	Poly(styrene-alt-maleic anhydride)
SMAMP	Synthetic mimics of antimicrobial peptide(s)
SOPC	Stearoyl-oleoyl-phosphatidylcholine
SOPS	Stearoyl-oleoyl-phosphatidylserine
SSNa	Sodium styrene sulfonate
TB	Tuberculosis
TBAM	Poly(2-*tert*-butylaminoethyl) methacrylate
TBAP	Tetrabutylammonium perchlorate
TBT	Tributyltin
TEM	Transmission electron microscopy
Tf	Transferrin
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIS	Triisopropylsilane
Tob	Tobramycin
TPU	Thermoplastic polyurethane(s)
TSB	Tryptic soy broth
UV	Ultraviolet
VE	Vitamin E
w/o	Water-in-oil
WPC	Wood–plastic composites
XPS	X-ray photoelectron spectroscopy
ZOI	Zone of inhibition(s)
ZSP	Zirconium (IV) silicophosphate
γ-PGA	Poly(γ-glutamic acid)

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