The Role of 3D Printing for the Growth and Progress of Medical Healthcare Technology

Edited by

Dinesh Bhatia

The Role of 3D Printing for the Growth and Progress of Medical Healthcare Technology

The Role of 3D Printing for the Growth and Progress of Medical Healthcare Technology

Edited by

Dinesh Bhatia

Cambridge Scholars Publishing

The Role of 3D Printing for the Growth and Progress of Medical Healthcare Technology

Edited by Dinesh Bhatia

This book first published 2022

Cambridge Scholars Publishing

Lady Stephenson Library, Newcastle upon Tyne, NE6 2PA, UK

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

ISBN (10): 1-5275-8236-1
ISBN (13): 978-1-5275-8236-1

My sincere gratitude to my dear parents for their unconditional love, support and blessings in all my endeavours

Mr. Ashok and Mrs. Kanchan Bhatia

TABLE OF CONTENTS

CONTRIBUTORS

Ms. Kirti Agrawal, Amity School of Engineering & Technology, Amity University, Noida, UP, India

Dr. S. Bagyaraj, SSN Institute, Chennai, India

Dr. Dinesh Bhatia, North Eastern Hill University, Shillong, Meghalaya, India

Dr. Satarupa Biswas, Adamas University, Kolkata, India

Dr. Hirak Ranjan Das, Royal Global University, Guwahati Assam, India

Prof. K. K. Deepak, AIIMS, India

Dr Sameer Desai, SBSR, MIT-ADT University, Pune, India

Dr. B. Devi, SSN Institute, Chennai, India

Shubham Hajare, SBSR, MIT-ADT University, Pune, India

Dr. S. Arun Karthick, SSN Institute, Chennai, India

Dr. S.V. Kirthanashri, Amity School of Engineering & Technology, Amity University, Noida, UP, India

Mihir Kulkarni, SBSR, MIT-ADT University, Pune, India

Dr. Helen Mary M C, Rajiv Gandhi Institute of Technology, Bangalore, Karnataka, India

Dr. Moumita Mukherjee, Adamas University, Kolkata, India

Dr. Neelamshobha Nirala, National Institute of Technology, Ranchi, India

Rutwik Palaskar, SBSR, MIT-ADT University, Pune, India

Tanvi Parkhe, SBSR, MIT-ADT University, Pune, India

Shreyas Patil, SBSR, MIT-ADT University, Pune, India

Dr. Gauri M. Shukla, TSEC, Mumbai, India

Dr. Reema Shyamsunder Shukla, SBSR, MIT-ADT University, Pune, India

Swati Sikdar, JIS College of Engineering, Kolkatta, West Bengal, India

Dr. Dilbag Singh, NIT, Jalandhar, India

Dr AJ Vanisree, University of Madras, Chennai, India

Dr. S. Varadharajan, Amity School of Engineering & Technology, Amity University, Noida, UP, India

Diksha Zutshi, SBSR, MIT-ADT University, Pune, India

FOREWORD

I am pleased to write the Foreword for the book *The Role of 3D Printing for the Growth and Progress of Medical Healthcare Technology* by Cambridge Scholars Publishing, edited by Dr. Dinesh Bhatia. 3D printing came into existence in the early 1980s as additive manufacturing and over the years the technology has grown leaps and bounds. The technology gained huge popularity in the 2000s, when its importance came to the limelight and its role was explored to help improve the capability of humans. This additive technology is currently employed in different fields and affecting different aspects of human lives. It is also employed for industrial purposes, as well as in sophisticated fields such as nanotechnology. In the medical field, 3D-printing was seen as one of the biggest breakthroughs and it slowly became an integral part of mainstream medical practice. Different 3D-printed solutions were developed to benefit a large number of patients across different specialties.

I hope this book will help to explore the growth and advancement in 3D printing technology in different areas of healthcare and in improving patient care with better quality, customized designs and lower costs, thereby providing durability and patient comfort. I congratulate all the authors who have spared their valuable time and contributed to the book by sharing their knowledge and expertise in the field with potential readers and researchers to further enhance their skills. It will provide insight to budding researchers and students to explore the field further. I also acknowledge the role of the reviewers and publishers in providing their inputs to help in refining the quality and content of the book before final acceptance and production.

I extend best wishes to all potential users of this book.

Prof. S.K. Srivastava
Vice-Chancellor
North-Eastern Hill University
Shillong-793022, Meghalaya, India
29th May, 2020

PREFACE

This book explores explore the growth and advancement in 3D printing technology in different areas of healthcare and how it is being employed to help in improving patient care with better quality, customized designs and lower costs, thereby providing durability and patient comfort. Despite its existence for a number of years, not many books are yet available on this field, making this text unique. It incorporates knowledge and expertise from professionals who have worked in the field for a number of years to help establish the technology. It will provide insight to budding researchers and students to explore the field further.

In the medical field, 3D-printing is seen as one of the biggest recent breakthroughs and it slowly became an integral part of mainstream medical practice. 3D printing is currently employed in several industrial applications and in different medical fields specifically to replace human organ transplants, improve surgical procedures, develop surgical tools at lower costs, and improve the lives of disabled people reliant on prosthetic limbs. The field is creating huge interest among scientists and researchers and is revolutionizing the medical domain, and has benefits for varied patient populations. One example of this is the first 3D printed implant known as bioprinted airway splints for babies whose lives are at risk due to the collapse of tiny airways around the lungs. This is a special implant as it has the ability to grow with the baby and can be produced in less than an hour's time with minimal costs involved. 3D bioprinted tissues for burns victims, organ transplants, human organs, and liver, kidney and brain tissues can also be easily developed employing this technology.

I would like to extend my sincere gratitude to all contributing authors for their painstaking efforts and helping me in incorporating finer details into the present book. I am grateful to my parents and family members for their kind support in allowing me to complete the book in time. I also acknowledge the support of my present institution/University and Cambridge Scholars Publishing for allowing me to complete this challenging assignment in the prescribed time. I do hope the present book would serve the due purpose for which it was initiated and support people

working in the field to enhance their skills and guide budding researchers in the discipline.

Finally, I thank Almighty God for his kind blessings, wisdom and grace to enable me to complete this book.

Dr. Dinesh Bhatia, Editor
Associate Professor
Department of Biomedical Engineering
North-Eastern Hill University,
Shillong-793022, Meghalaya, India

CHAPTER ONE

INTRODUCTION TO 3D PRINTING

DR. NEELAMSHOBHA NIRALA

Abstract

3D printing began in the 1980s, when Charles Hull designed and printed a small eyewash cup. It has generated much interest since then. It is a cost-effective, localized, one-step manufacturing technique that can produce personalized, complex objects with minuscule precision. It has affected almost all industries, from edible cookies to jewellery; kids' toys to house designs; prosthetic limbs to humanoid robots (Kenshiro and Kengoro) that can mimic human activity; pharmaceuticals, and so on. Complicated structures that were impossible to produce with conventional manufacturing methods can now be easily produced by using 3D printing. Even an object which consists of several parts, such as the designing of a car, can be produced in a single operation. The flexibility of 3D printing allows for a reasonably quick and easy way to adjust the design without the need for additional tools or equipment. However, the practical application of, and potential for, 3D printing is to some extent limited because of its slow speed and the time required to fabricate the required 3D objects. In this chapter, we are going to discuss in detail the basic concept of 3D printing, looking briefly at its history, the advantages and limitation of this technique, and drawing a comparison with the subtractive method of manufacturing. We will also discuss various techniques presently being used for 3D printing, along with the materials used in various applications.

Introduction

One of the buzzwords of today's generation, which attracts many individuals ranging from technical researchers and scientists to homemakers, is 3D printing. With this technology it is possible to design customised chocolates at home while also enabling the design of complex physiological models,

like prosthetic limbs or fully functioning organs. Its wide application involves almost every industry, from automobile to aeronautics, food production to tissue engineering, and house-building to jewellery making. What is 3D Printing? It is a technology that supports the formation of an object by placing the contoured layers or slices on top of one another. Here, the parts are grown layer-wise from the base to the top until the object is formed.

3D printing began in the 1980s, when Charles Hull printed a small eye wash cup as the first 3D object and, since then, it has generated much interest. This technology is also known by many other names, like Additive Manufacturing (AM), Rapid Prototyping (RP), Solid Free-form Technology (SFF), Direct Digital Manufacturing, or Desktop Manufacturing, based on its specific abilities. When 3D printing was first proposed in the 1980s it was called Rapid Prototyping (RP) because it enabled the production of a prototype of an object without the use of tools, thereby saving both time and money. It provided the ability to evaluate the design in the computer before it went into the stage of actual production.[1] It was faster to generate an altered version of 3D printed designs. It is important to note that the time taken in creating a 3D object depends upon various factors, such as the size of object, the speed of the motor, the thickness of the slice, the type of 3D printer used, and so on. But with the use of computers and software, this has made the process of prototype designing much faster. This method is known as Additive Manufacturing (AM) because it develops an object by adding the layers of material instead of removing the material from the workpiece, as done in traditional Subtractive Manufacturing (SM).

Before comparing Additive Manufacturing with traditional Subtractive Manufacturing (SM) methods, let us see how the manufacturing cycle of the traditional method works. Mass manufacturing by traditional methods include various steps:

1. Collecting raw material or basic resources from various places. Depending on the distance between the source of the resource and the manufacturing plant, this factor consequently adds to the transportation charge.

[1] Andreas Gebhardt and Jan-Steffen Hotter, *Additive Manufacturing 3D Printing for Prototyping and Manufacturing*, ed. Cheryl Hamilton (Cincinnati: Hanser Publications, 2016); Thomas Birtchnell and William Hoyle, eds., *3D Printing for Development in the Global South: The 3D4D Challenge* (PALGRAVE MACMILLAN, 2014), https://doi.org/10.1057/9781137365668.0001.

2. Transportation of these collected raw materials to locations where refinement takes place.
3. Making initial products or components from refined materials in huge numbers using SM technique to reduce the unit cost. SM techniques include milling, turning, drilling, grinding, cutting moulding, beating, forging, machining, and so on.
4. Shipping of these initial products or components across the world.
5. In the case of components, the need to assemble them into the final products in huge numbers.
6. Transportation of final products into huge warehouses.
7. To reach the actual consumers, shipping is again needed to various distributors and standalone stores.

From the above, it is clear that the above-mentioned method has some areas of inefficiency and is most suitable for mass production, like wheels for four-wheelers of a similar kind, the standard size of clothes worn by a majority of people, or some kind of mobile phone for major needs. This method of manufacturing involves the consumption of huge resources in the form of raw materials, energy production, and distribution of goods among various places, often involving much labour-intensive activity, like an assembly line, warehouse or distributor. As an example, this kind of manufacturing process in the clothing industry works well while a defined mould is required and met, in terms of the cloth material required before the arrival of a new season, but as soon as trends or techniques change, the requirement of a new design group, human power, mould preparation, tools requirement, and fixture may lead to the discarding of obsolete tools and materials.

As the saying goes, necessity is the mother of invention. Society has sought to evolve from its more primitive stage towards a more civilised form, which has been made possible by the earlier industrial revolutions that have reshaped the way our societies function. Enthusiasts of AM or 3D printing see this as the third industrial revolution. Each industrial revolution transforms the world in some way. The first revolution transformed the means of production, whereby the world went from handmade production to automatic production which utilised machines which ran using steam or water. For example, tilling of the land by animals was replaced by tractors and wooden tools were swapped for metal ones. The second revolution raised the application of steam-driven engines for factories, and enabled mass production. The third revolution is expected to support the local production of greatly customized products on-demand at low cost while ensuring limited resource wastage. It is also expected to shorten the

traditional release cycles of products and design cycles. In view of the above points, let us observe a few advantages brought about by AM or 3D printing.

Advantages of AM

1. AM initiates the direct conversion of design to product. AM can produce a complete product, including finishing and processing within a timeframe (which may include interlocking of moving parts, holes, cavities, or bearings within the wheel) that is greatly reduced when compared to the SM method.
2. Approach towards zero waste manufacturing by reducing material wastage.
3. No tooling—3D printing eliminates the need for costly and time-consuming tools for the production of an object.
4. AM is especially suitable for the shortening and improvement of the product development process.
5. Since 3D records are available for access in the desired format, it eliminates the data exchange problems which arise with pre-processor in the subtractive manufacturing process.
6. Personalisation or customisation—with the availability of the internet and the ease with which data across the globe may be shared, AM allows the personalisation of goods at the time of fabrication by a consumer's choice in terms of design, colour, shape, and size. These initiate the manufacturing of a customised product.
7. Design flexibility—the unique feature of 3D printing is the layer-wise sequential fabrication approach that makes it promising to create complex geometrical structures. With the help of 3D printing, fewer load-bearing structures can be easily made with additional support material. In the case where weight is a considerable factor, where strength is required, the honeycomb-like structure can help to produce lightweight products, for example, the bones of birds. Overall, 3D printing provides the designer with the flexibility to selectively place the material at a precise location to obtain design functionality with lower material consumption.
8. Reduction in the cost of geometric complexity—3D printing provides freedom to the designer in the realisation of complex structures. In traditional manufacturing, there is a direct proportionality between the complexity of the shape and moulding cost, whereas in AM an increase in geometrical intricacy does not affect costs.
9. Sustainability—by altering the strength and flexibility in the design of an object, 3D printing can reduce the material utilisation and

conserves resources and money. Further utilisation of degradable material may reduce the consumption of petrochemical-based polymers.

10. Reduction in obsolescence—with the advance of technology, old products become obsolete and raise the inventory of old parts. With 3D printing, we can design the 3D models of obsolete parts of automobiles and machines which are no longer available.
11. Economies of scale—in traditional manufacturing, mass production is required to overcome the cost involved in the transportation of goods, long storage periods, distribution networks, manufacturing in low-cost areas, and labour costs, so that per product costs drop-down at the consumer end. In AM, however, the above-stated costs are not going to be as affected by the number of products manufactured. Moreover, it reduces the costs associated with manpower, consumption of raw material, human error, transportation, and inventory.
12. Avoids the assembling of parts—with the flexibility in the design of intricate shapes by 3D printing, it is possible to produce shapes that would otherwise need assembling of various parts by traditional methods. In the future, it will be possible to produce products with "single part assemblies", which means a single product will be fabricated in which the parts and joints will be present in their respective places with support material. During post-processing, these support materials can be easily removed.
13. Reduces the delivery time of the product. With local fabrication of 3D objects, the delivery time of the product can be reduced.

Since we are now familiar with extensive advantages of AM, let us see what a 3D printer is made up of, and how it works on the design part. The most widely used 3D printer is based on the Fused Deposition Modelling (FDM) technique. In FDM, thin filaments of melted plastic will be placed in layers to form a 3D part. Fig1.1 shows the basic component of the FDM-based 3D printer. It consists of a frame or chassis, build plate or area, a filament spool, an extruder, linear movement components, and a controller unit. The chassis is the frame of a 3D printer; it can be made up of wood or metal plates to support the movement and vibration during working, as well as to hold the printed object. The build area is a flat, levelled plate where the actual printing takes place. A thin filament of thermoplastic is coiled in the filament spool to feed the extruder. The extruder is the main part of the FDM technique which melts the filament supplied to it, and then pushes the melted filament out from the small diameter nozzle to place it in the desired location in the build area. The extruder can move in both x and y direction,

which means it can move from left to right and front to back or vice-versa to reach a particular x–y coordinate. It is essential to level the build plate perfectly parallel to the extruder so that the built part will be level. The linear movement component helps in the movement of the extruder in a defined direction through the stepper motor. End-stops are placed to limit the linear movement of the extruder in each axis. A controller unit guides the whole movement of the 3D printer. In 3D printing, we directly fabricate a physical model of an object from its digital design. So, let us consider the steps involved in the fabrication of a part by 3D printing.

1. To print any 3D object or its prototype, we first need a 3D model of an object, material to fabricate, and a 3D printer.
2. There are three possible sources for the 3D model of an object. First, the use of 3D modelling software, like CAD (Computer-Aided Design) software that allows you to create your model. Second, the use of a 3D scanner that allows you to construct and virtualise 3D models of the real object. Last, by downloading 3D files from many open websites.
3. The material used for the AM process includes a wide variety, ranging from photopolymers, thermoplastics, resins, metal powder, ceramics, sand, fibres and glass, even to biomaterials. However, the choice of material depends upon various factors, like the type of available 3D printer techniques, cost, and so on.
4. A 3D printer can use different ways to build up staggered layers of materials, like a fusion of liquid polymers by laser, binding minute granular particles by laser, or by using a binding material or the extruding of liquefied material.
5. Once all three things are obtained, fabrication starts with the 3D model of an object. The 3D model is the digital representation of an object which tells the printer what to print. The most common type of file format used in 3D printing is STL which stands for “Stereo lithography”. The STL file defines the special coordinates and connects those coordinates to form a series of triangles or mesh objects. The STL file is then sent to the slicer software.
6. The slicer software will cut the digital file into numerous thin layers or slices. It generates a printer-readable digital file, commonly called a G-code file. This code will direct the movement of the printer. Slicer software also creates a supporting structure to avoid the dropping of hanging parts.
7. The G-code is uploaded to the 3D printer so that printer can print an object.

8. Sometimes post-processing is required to clean and finish the printed object.

With the reduction in the cost of computers, CAD software, lasers, and inkjet printing, there is democratisation in the design process which initiates engineers as well as lay people into how to utilise this technology.[2] Furthermore, projects like "RepRap", which stands for "replicating-rapid-prototype", introduced in 2006, make this technology available to hobbyists and consumers by providing freely open code-bases.[3] However, apart from the ease of fabrication and various advantages, AM also has certain limitations. One of the biggest disadvantages is the high cost of a 3D printer. For small objects and prototype design it is good, but designing bigger objects is still challenging. The cost of materials is another limitation, especially in the case of moulds, as 3D printed moulds usually degrade with time and are sensitive to environmental exposure. Even though 3D printing can produce complex designs, they can lose their functionality and resistance over time. The material properties of the object are generated during the production of the 3D object and may vary with its orientation. AM is a computer-controlled technique which can significantly affect the workforce requirements in production. Certain other economic effects of AM include a reduction in economic imbalance due to the demand on local production, giving rise to job opportunities and industries related to 3D printing. The use of 3D printing for unchecked production of dangerous items raises intellectual property issues. Large distance transportation used for shipment of goods and resources will be affected.[4]

History of 3D printing

The history of 3D printing is quite interesting and was started almost 40 years ago, in May 1980, when a Japanese doctor, Dr Hideo Kodama, from

---

[2] Wei Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering," *Computer-Aided Design* 69 (2015): 65–89, https://doi.org/10.1016/j.cad.2015.04.001.

[3] Liza Wallach Kloski and Nick Kloski, *Make: Getting Started with 3D Printing*, ed. Roger Stewart, *Journal of Chemical Information and Modeling*, vol. 53 (Canada: MakerMedia, San Francisco, CA, 2019), https://doi.org/10.1017/CBO9781107415324.004.

[4] Alexandru Pîrjan and Dana-Mihaela Petroşanu, "The Impact of 3d Printing Technology on the Society and Economy," *Romanian-Economic Business Review, Romanian-American University* 7, no. 2 (2013): 360–70; Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."

the Nagoya Municipal Industrial Research Institute, filed for a patent for Rapid Prototyping technology, which is now known as 3D printing. He was the first to invent the curing of photopolymers by a single laser beam. Unfortunately, he was unable to submit the full patent requirements by the deadline. After that, this technology was picked up by a French team of engineers (which consisted of Alain Le Méhauté, Olivier de Witte and Jean-Claude André), and filed the patent for the stereo lithography process in 1984. Due to a lack of proper funding, along with the absence of a well-thought-out business perspective, they had to abandon the patent. Meanwhile, an American named Charles Hull, who held a Bachelor's degree in Engineering Physics, and who was working for a company that made a tough coating for table tops and furniture using an ultraviolet lamp, suggested a new way of using ultraviolet technology for placing many thin layers of plastic on each other. After 2 months of experiments, he submitted a patent for the stereo lithography technique, which was issued in 1986. After obtaining the patent, he founded the company called 3D Systems Corporation, in 1988, and released its first commercial 3D printer, called Stereo lithography apparatus (SLA-1), which was delivered to only a few selected customers. Later on, with the feedback obtained from those customers, an improved version called SLA-250 was released by the company. The first 3D object made by Charles Hull from his 3D printer was an eyewash cup.

In the same year, 1988, another 3D printing technology, called Selective Laser Sintering (SLS), was submitted for patent by an undergraduate, Carl Deckard, from the University of Texas. Whilst the SLS patent was waiting for approval, another 3D printing technology, called Fused Deposition Modelling (FDM), was filed for patent by Scott Crump. A year later Scott Crump co-founded a company called Stratasys with his wife Lisa Crump. Both 3D Systems Corporation and Stratasys are currently the leading companies in the 3D printing industry. In 1993, the Massachusetts Institute of Technology patented another technology called 3-Dimensional Printing (3D Printing) Techniques, and that is how the term 3D printing originated. Another milestone in the popularity of 3D printing was in 2006, when Dr. Gordan initiated an open-source project called RepRap to develop a self-replicating 3D printer (a 3D printer which is capable of producing another 3D printer similar to itself).[5]

[5] C Lee Ventola, "Medical Applications for 3D Printing : Current and Projected Uses," *P&T* 39, no. 10 (2014): 704–11; Elizabeth Matias and Bharat Rao, "3D Printing : On Its Historical Evolution and the Implications for Business," in *2015*

Even in the field of Biomedical Engineering, the breakthrough of 3D Printing can be seen when a scientist from the Wake Forest Institute for Regenerative Medicine prepared a 3D-printed synthetic scaffold of a human bladder, in 1993, which was later coated with the patient's cells and successfully implanted in a patient who was undergoing a urinary bladder augmentation. In 2002, a working miniature kidney was developed by 3D printing that could filter blood and dilute urine. Later, in 2008, a first prosthetic leg, which included a knee, a foot and a socket, was 3D printed and successfully implanted. Many other 3D printed inventions have been made. Successful 3D prints include an implantable 3D prosthetic jaw; a 3D printed prototype of the car, Urbee; 3D printed food; and a 3D printed house of 1022 square feet into which a family moved.

Different techniques of 3D printing

The first three AM technologies that emerged were Stereo lithography, Selective Laser Sintering (SLS), and Fused Deposition Modelling (FDM). Later on, they were broadly classified into four categories, which include extrusion, granular, laminated, and light polymerised. However, the American Society for Testing and Materials (ASTM) and International standard organisation have divided the AM technologies into seven categories.[6] Fig.1.2 shows the categorisation, and names of all AM techniques. The working principle, advantages, and limitations of each of these technologies are set out below.

Stereo lithography (SLA) – Stereo lithography is the first technique proposed to build a 3D object, by Charles Hull. This works on the principle of photopolymerisation of photopolymers or light-activated resins, by application of a UV light source. Light-activated resins or photopolymers are light-sensitive polymers that harden when exposed to a defined energy of UV light. The interesting part is that only the exposed polymer undergoes solidification, while the rest remains in the liquid phase. Some of the known photopolymers are polyamide, polyacrylate, polyisoprene, epoxies, and

---

Portland International Conference on Management of Engineering and Technology (PICMET) (Portland International Conference on Management of, 2015), 551–58, https://doi.org/10.1109/PICMET.2015.7273052; Swati B Nale and Prof A G Kalbande, "A Review on 3D Printing Technology," *International Journal of Innovative and Emerging Research in Engineering* 2, no. 9 (2015): 33–36; Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."

[6] Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."

polyimides. Resins initially used in this process were polymers with low molecular weight, which have now been replaced by hybrid polymers to overcome limitations, like high-temperature resistance, shrinkage and high absorption of moisture.[7] Fig.1.3 shows the arrangement made in SLA.

It consists of a vat that holds the polymer, an elevator that holds the working platform used for 3D object making, a UV-laser source for the emission of focused UV light, and an x-y scanning mirror that guides the movement of the laser beam in the defined x-y direction. The movement of the laser beam depends on the 2D pattern given by the STL file for each layer. Initially, the platform is placed in such a way that a thin layer of resins will be available for light exposure. Based on the 2D design of a single layer, the laser beam will be focused on resins. As soon as light falls on the resins, they will solidify and form the first layer, while the rest of the unexposed part will be in liquid form. Now, the platform will go down by the length equal to the thickness decided for each layer (approximately 0.02mm to 0.15mm), allowing the fresh liquid resin to pour on top of the solidifying resin. Once again, the light will expose and help to solidify a new layer of resin which combines with the initial layer. This process will continue until the entire object is ready, and the platform is then raised out of the vat for removal. Support parts are manually removed from the final object, which is then submerged into the chemical bath for removal of excess resins, and finally dried in the UV oven.

The major advantages of this technology include the ability to fabricate objects with high accuracy, and this technology also enables the production of excellent smooth surfaces. This made the application of SLA in biomedical engineering and jewellery design a very viable technique to be utilised. It is usually used for prototype fabrication because it is not as time-consuming. Prototypes made by SLA are strong enough to withstand machining, and may be used as a master pattern for various casting and moulding processes. SLA also needs the support part to hold hanging features that can be manually removed. However, the material used for SLA is still limited to polymers and a few ceramics and is quite expensive.[8]

---

[7] Shiwpursad Jasveer and Xue Jianbin, "Comparison of Different Types of 3D Printing Technologies," *International Journal of Scientific and Research Publications* 8, no. 4 (2018): 1–9, https://doi.org/10.29322/IJSRP.8.4.2018.p7602.

[8] Sandeep and Deepak Chhabra, "Comparison And Analysis Of Different 3d Printing," *International Journal of Latest Trends in Engineering and Technology* 8, no. 4 (n.d.): 264–72; Richard Horne and Kalani Kirk Hausman, *3D Printing for*

Some of the manufacturers of 3D printers using SLA technology are: Envision technology GmbH, Laser solutions, Objet Geometries, and 3D Systems.

Material extrusion – This category of additive manufacturing designs 3D objects by extrusion of materials. It is of two types: Fused Deposition Modelling (FDM) and Contour crafting.

Contour crafting is one of the more recently developed 3D printing methods, and has been exclusively created for the construction sector. This method was researched by Dr. Behrokh Khoshnevis, at the University of Southern California's Information Science Institute. He is also the founder of the company, Counter Crafting Cooperation. Here, they build buildings using cranes and a robotic arm in a layer-by-layer manner. The materials involved are quick setting concrete and sand. This method has great potential in building government offices, as well as houses for people during migration or facing disasters, at a fast rate.[9]

Fused Deposition Modelling (FDM) or Fused Filament Fabrication (FFF)

Scott Crump was the one who proposed this technique of AM; soon after obtaining the patent for this technique, in 1989, he co-founded the company, "Stratasys". Crump invented this technology to prepare a toy frog for his daughter using glue gum and a mixture of polyethylene and candle wax.[10] This is one of the most widely used AM techniques and is prominently used for model preparation, rapid prototyping, and rapid manufacturing. Fig.1.4 shows the setup for FDM.

Here, we have spools holding thin filaments of plastic or metal. These filaments are uncoiled and supplied to the extrusion nozzle. The nozzle is heated to the specific temperature required to bring the filament in a semi-liquid state and the flow is then turned on. The 3D object or part is produced by extruding small droplets of material to form layers. The thermoplastic

Dummies, ed. Richard Horne and Kalani Kirk Hausman, 2nd ed. (New Jersey: John Wiley & Sons, Inc., Hoboken, New Jersey, 2017).

[9] Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."

[10] Vinod G Gokhare, "A Review Paper on 3D-Printing Aspects and Various Processes Used in the 3D-Printing," *International Journal of Engineering Research & Technology* 6, no. 06 (2017): 953–58.

nature of the material allows the filament to fuse during printing and solidify at room temperature immediately after extrusion from the nozzle. The nozzle can move in both a horizontal and vertical direction, where its movement is guided by a controlled mechanism directly through the CAD software. Stepper or servo motors are used for the movement of the nozzle head. Once a layer of material is extruded at the desired location, the extruder head will move up or the platform can push down for placing the next layer. Each new layer hardens as it extrudes and bonds with the previous layer until the final object is prepared.

Compared to SLA, FDM is simple and cost-effective due to the use of thermoplastics, but less accurate. Moreover, the process can be time-consuming for designs having a complex geometry. Commonly used thermoplastics include polylactic acid (PLA), polyvinyl alcohol (PVA), acrylonitrile butadiene styrene (ABS), nylon, or composite materials. These kinds of thermoplastics are usually sold in spools of thin filament with a diameter of 1.75mm and 3.00mm. After the expiration of the patent for this technology, a huge number of open-source communities developed, and many commercial variations of this type of 3D printer have appeared on the market. Moreover, the low-cost and flexible extrusion system of this technique has contributed to its rising popularity among people interested in this technology.

Some of the limitations of this technique include the need for high operating temperatures, as well as the fact that the finished products exhibit high porosity and warping. Poor surface finish, weak mechanical properties, and limited resolution are other drawbacks. The layer thickness, air gap in and between layers, width, and orientation of filament are major factors in deciding the mechanical properties of the printed object. The removal of supporting parts leaves marks that need removing and sanding. The use of water-soluble supporting material can be worked without leaving marks. Only limited testing is possible in thermoplastic material. Some examples of 3D printers that used FDM technology are the Cube, the Mojo, the Buccaneer®, and the MakerBot Replicator 2X.[11]

Powder bed fusion – In general, the powder bed fusion technique contains a thin layer of fine powder spread on the packed platform. The fine powder in each layer is fused with the use of an energy beam, which can be a laser

[11] Horne and Hausman, *3D Printing for Dummies*; Jasveer and Jianbin, "Comparison of Different Types of 3D Printing Technologies"; Sandeep and Chhabra, "Comparison And Analysis Of Different 3d Printing."

or an electron beam. The application of an energy beam causes selective melting and fusion of the powder bed as per the cross-section design generated by digital data. Once a layer is scanned, the powder bed thickness is reduced by one layer. Subsequent layers of powder are spread on top of the prior layer through a rolling mechanism and scanned until all the layers are fused, and the final 3D part is prepared. Finally, the printed part is removed and allowed to cool down, and excess powder can be easily removed. One of the major requirements in this technique is the need to maintain the temperature of the sealed chamber equal to the melting point of the powder material.

Based on the type of energy source used, this category is further divided into 2 groups. The first group contains Electron Beam Melting (EBM), where melting of powder takes place with an electron beam of energy up to 60kV. The second group contains a list of three techniques, called Selective Laser Sintering (SLS), Selective laser melting (SLM), and Direct Metal Laser Sintering (DMLS). In these groups, a high-powered laser is a source of energy. The main advantages of this method include the fact that it can work with fine resolutions: it does not need support material, and can produce printing of a high quality. Such qualities increase its use in biomedical engineering, aerospace, and electronics. However, its limitations include the fact that it is a slow process, is high in cost and the challenges in powder size distribution and packing which ultimately determine the density of the final printed part. What follows is an overview of each of these techniques.[12]

Selective Laser Sintering (SLS) – SLS is a very economical and time-efficient technology. The overall process in this technique is the same as mentioned for powder bed fusion. Fig.1.5 shows the working principle of the SLS technique. SLS supports a wide range of material utilisation for processing, like plastic, ceramic metals, or glass. The laser used in SLS is a high energy CO2 laser. In SLS, laser scanning does not fully melt the powder but raises its surface temperature so that fine particles of powder can fuse at a lower molecular level. The speed of scanning and power of the laser used are major factors to be considered in SLS. In SLS, the quality of the surface and the strength of printed parts are greatly affected by the power of the laser, the temperature achieved, and the part orientation. The major

[12] Jasveer and Jianbin, “Comparison of Different Types of 3D Printing Technologies”; Gao et al., “The Status, Challenges, and Future of Additive Manufacturing in Engineering”; Sandeep and Chhabra, “Comparison And Analysis Of Different 3d Printing”; Horne and Hausman, *3D Printing for Dummies*.

advantage of SLS is that it does not need any structural support for the design of complicated parts, as the part itself lies on the bed of the powder material. As a result, this technique prevents material loss, and eliminates the post-processing which would otherwise be required for supporting material removal, thereby reducing the production cost and saving on assembly time. In case a metallic support structure is made, then its removal is highly time-consuming and even its proper removal needs the same level of accuracy as an SLS process. Based on the material used, 100% density can be achieved with comparable material properties. SLS permits rigorous testing of the prototype. SLS makes it possible to design a complex internal structure and passage that would be otherwise difficult to cast or machine. Due to the use of metals, functional prototypes can be made from the same material as that used for product design. Parts printed by SLS are robust and durable compared to traditional production methods, like injection moulding. This is one of the AM techniques used for tailor-made production in the dental, prosthetic, and aerospace sectors. A few factors that need consideration while printing include feature details, particle size distribution, and printing through an error in the z-axis. To achieve an extremely smooth surface, polishing can be done.

Selective laser melting (SLM) – SLM is an advancement over SLS with a similar method used for layer deposition. While SLS may be used for a wide variety of materials, SLM is used for limited metals, like steel, cobalt, chromium, titanium, tungsten, and aluminium. Unlike in SLS, in SLM metallic powder is heated till complete melting is achieved, which results in superior mechanical properties. To avoid metal oxidation, the whole printing process takes place in a vacuum or an inert gas environment. Complete melting of metal in SLM helps in the reduction of porosity, thereby achieving greater control over the crystal structure to prevent part failure.

Direct metal laser sinister (DMLS) – Also known as the Laser Powder Bed Fusion (LPBF) technique, it is mainly used for making extremely difficult metallic geometries, with accuracy within a reduced timeframe that is not otherwise feasible using other metal manufacturing methods. Similar to SLM, DMLS supports many metals and alloys, like Monel®k500, stainless steel 316L, stainless steel 17-4, nickel alloy 178, and so on. Here, the temperature is raised to heat the metal but not completely melt it. DMLS printed parts are much stronger, corrosive resistant, and denser than traditional casted metal parts. To get a better finish, post-processing may include machining and heat treatment. Apart from the above-mentioned advantages, DMSL suffers from the high price of the printer; it is more

porous than SLM, and is limited to printers having a small build volume which end up designing only small structures. The major application of DMLS can be seen in making medical and dental implants and in the aerospace industry.

Laminated object manufacturing (LOM) – The LOM technique was developed by California-based, Helisys Inc. in 1986, and was patented in 1987. Cubic Technologies is now the successor organisation of Helisys Inc. LOM uses a continuous sheet of materials, like paper, plastic, fibre, or even metals. The material is initially coated with an adhesive material. The feed roller mechanism helps to spread the material sheet onto a build platform. Then, by passing the heated laminating roller over the material surface, the adhesive will melt and pressure is applied by the roller pressing the material layer onto the platform. After this, a computer-controlled laser or sharp blade will cut the material as per the design given by the slicer. Excess material that is left after cutting provides support to the structure and can be removed after printing. As soon as the first layer is formed, the build platform will go down, depending upon layer thickness. Then the successive layer will similarly be spread out unless the whole object is printed. When the final object is formed, the printed material is removed from the build platform and excess materials are removed. Finally, post-processing, like sanding, painting and varnishing can be done, based on the type of material and the desired features. Advantages of LOM include low material, machine and process cost, material availability, high surface finished details, low internal tension, and fragility. This technique does not require any support structure due to the use of solid sheets which are left out after cutting. Unlike other techniques, LOM does not depend on solidification of materials but depends only on cutting the sheets to the desired contour, which can be done by multiple layers at a time, increasing the speed of this technique. A few drawbacks of this technique include the limitation of the strength of the printed object as it needs to be similar to the sheet material used, and poor resolution in the z-axis. Removal, recycling, and describing of waste material in LOM still needs to be considered as well. Non-involvement of any chemical reaction and no maintenance of the enclosed chamber, and availability of material make this technique suitable for printing big models and rapid prototyping.[13]

[13] Gokhare, "A Review Paper on 3D-Printing Aspects and Various Processes Used in the 3D-Printing"; Tuan D Ngo et al., "Additive Manufacturing (3D Printing): A Review of Materials, Methods, Applications, and Challenges," *Composites Part B* 143, no. December 2017 (2018): 172–96,

Material Jetting (MJ) – The MJ technique was patented as PolyJet by Object Ltd. in 1999, and merged with Stratasys in 2012. This technique combines the features of inkjet technology with photopolymers, to design multicolour and multi-material 3D printed objects. The working principle of the MJ technique is elaborated in Fig.1.6. The MJ printer needs a build platform, multiple movable nozzle print heads filled with different UV sensitive thermoset photopolymers, and a UV light source. The process starts with the heating of a photopolymer from 30ºC to 60ºC to achieve the required viscosity for printing. The print head then jets numerous micro droplets of photopolymers at the desired locations. With the exposure of UV light, photopolymer cures and forms the first layer. The rest of the process is similar to other techniques, which involves moving down the build platform spraying successive layers on top of the previous layer until the model is printed. This technique provides excellent resolution of up to 0.016mm, with no staircase effect, enabling high dimensional accuracy and surface finish. It also provides a varied choice of colours and materials at a relatively lower cost, and with a reduced printing time. MJ is most suitable for preparing realistic visual and haptic prototypes with a smooth surface finish. MJ provides the option to choose a matte or glossy finish. Other than the above-mentioned advantages, it does, however, suffer from a smaller build volume, post-processing step (due to the need of support structure), and needs printing media. The support structure can be easily removed by immersion in an ultrasonic bath or by cleaning with water at high pressure. Sometimes warping may occur. The material cost of photopolymers is high, and they also suffer from poor mechanical properties that degrade with time. This technique has wide applications in the production of realistic prototypes, especially in the medical and jewellery making fields. The main market players for the MJ printer are Stratasys and 3D Systems and XJet.[14]

Binder Jetting (BJ) – Binder jetting is also known as powder bed inkjet printing. Here, a thin layer of powder is first spread on the build platform. The powder is either of ceramic (like glass or gypsum) or metal (stainless steel). There are two ways to spread the powder: either through the roller shaft, or through the jetting reservoir or blade. The roller shaft method is more efficient in spreading the low mobility dense powder, with consistent layer thickness. The adhesive material is then selectively deposited onto the powder layer to bind with it. When a single layer is completed, the powder bed moves down slightly, and once again a new layer of powder is spread,

https://doi.org/10.1016/j.compositesb.2018.02.012.

[14] Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."

which is bonded with the earlier layer by spraying adhesive material. This process continues until the final 3D print is ready.[15] The schematic diagram of BJ is shown in Fig. 1.7. After completion of printing, the green parts are removed and sent for additional drying to eliminate residual moisture to achieve the highest mechanical properties. It is common to add infiltrates like cyanoacrylate, wax, or resins to improve the mechanical properties of the printed object. Several parameters affect the BJ process, like the diameter of the nozzle, speed of printing and spreading droplet spacing, and heater temperature. Binder concentration can considerably affect the mechanical strength of the printed object, as high concentration causes lower porosity and reduced strength. BJ provides high roughness and porosity compared to other methods. However, an increase in porosity leads to poor mechanical strength and high friability. The major application of BJ involves tissue engineering and pharmaceuticals.[16]

Directed energy deposition (DED) – DED is a direct fabrication technique, where highly focused thermal energy is used to melt the material while depositing it on the surface. The source of thermal energy can be a laser, electron beam, plasma, or an electric arc. The materials usually employed in DED are metallic powder or wires and ceramics. DED can achieve a high theoretical density of the material. The main application of this technique is remanufacturing or repairing of existing parts by deposition of material. Based on the different types of energy sources, DED is divided into **Laser Engineered Net Shaping (LENS)** and **Electron Beam Additive Manufacture (EBAM)**.[17]

In LENS, the material is fed directly to the focal point of energy to build a molten pool. The powder material is fed along with an inert gas, which also acts as a coolant to solidify the molten material as soon as the powder is

[15] Ankit Chourasia, Pravin Kumar, and A Cad, "Analysis and Review of Rapid Prototyping Technology, & Study of Material Used in Process of 3D Printing," *International Research Journal of Engineering and Technology* 6, no. 10 (2019): 573–79.

[16] Jia-chang Wang, Hitesh Dommati, and Sheng-Jen Hsieh, "Review of Additive Manufacturing Methods for High-Performance Ceramic Materials," *The International Journal of Advanced Manufacturing Technology* 103, no. 2019 (2019): 2627–47, https://doi.org/https://doi.org/10.1007/s00170-019-03669-3; Kapilkumar Vithani et al., "An Overview of 3D Printing Technologies for Soft Materials and Potential Opportunities for Lipid-Based Drug Delivery Systems," *Pharm Res* 36, no. 4 (2019): 20, https://doi.org/https://doi.org/10.1007/s11095-018-2531-1.

[17] Wang, Dommati, and Hsieh, "Review of Additive Manufacturing Methods for High-Performance Ceramic Materials."

deposited onto the surface. The working principle of LENS is shown in Fig.1.8. Printed materials are generally carried grains that need to be post-processed by grinding, polishing, and gliding.[18]

In EBAM, an electron beam is used to weld the material powder or wire. The printing process of EBAM is similar to LENS except that the electron beam is more efficient than laser and works under vacuum.[19]

Applications of 3D Printing

In recent years, 3D printing has gained wide popularity in various fields. According to one survey reported by Allied Market and Research (3D Printing Market Technology-Global Opportunity Analysis and industry Forecast, 2019–2025), the worldwide 3D printing market will reach $44.3 billion by 2025, with the compound annual growth rate of 21.8%, where North America is the highest contributor. Although the major use of 3D printers and print services can be seen for medical and dental devices, as well as in the aerospace industries, the major share was still held by Industrial machining, Automotive, and Consumer products. A few fields where 3D has wide applications are mentioned below.

3D printing in aircraft and aeronautics

With the advent of commercial manufacturing, industries are trying to subjugate various fundamental problems, such as material cost, product quality, manufacturing speed, required raw material, and wastage of left-over substances. 3D printing, which is also known as Additive Manufacturing (AM), is presently evolving in such a manner that it is in a position where it can resolve most of the problems mentioned above.[20] AM technology allows engineers to utilise computer models to design and produce various metallic or non-metallic (usually polymer) parts of an aircraft in a very precise manner. Commercially, 3D printing came into existence in the 1980s, when

[18] Gao et al., "The Status, Challenges, and Future of Additive Manufacturing in Engineering."
[19] Chourasia, Kumar, and Cad, "Analysis and Review of Rapid Prototyping Technology, & Study of Material Used in Process of 3D Printing."
[20] Bernhard Mueller, "Additive Manufacturing Technologies – Rapid Prototyping to Direct Digital Manufacturing," *Assembly Automation* 32, no. 2 (April 2012): aa.2012.03332baa.010, https://doi.org/10.1108/aa.2012.03332baa.010.

it was introduced by a company named 3D Systems.[21] Subsequently, AM or 3D printing has been utilised for diverse scientific and industrial purposes. However, one of the fields where use of AM has dominated is in aeronautics and aircraft manufacturing. In the early 1990s, AM was used by the Boeing and Bell companies to manufacture specific polymer parts for a helicopter.[22] Another step towards AM technology was taken by MIT labs when they developed a laser-beam melting process.[23] They also developed the ProMetal technique, and made it marketable with the help of the Extrude Hone Corporation. Between 2000 and 2005, Extrude Hone Corporation commercially provided 7075 aluminium helicopter parts developed by 3D printing.[24] In 2015, it was mentioned in a few articles that Russian manufacturing companies had developed parts of an aircraft engine with the help of a 3D model prototyping process.[25] Studies have also shown that the aircraft industry has played a major role in the consumption of AM products for the last 10 years, and is currently generating 18.2% of the overall market capacity in the AM industry.[26]

The aircraft repair and manufacturing unit deals with high-grade metallic parts, which holds extra strength, is lightweight, and is intended to exhibit a quality of permanence which would ensure that it could always resist extreme conditions. It also enables the production of an extremely complex structure that cannot be done by conventional manufacturing technologies. AM offers a wide range of manufacturing methodologies where techniques, such as selective laser melting, direct metal laser sintering, laser metal

---

[21] R. Liu et al., *Aerospace Applications of Laser Additive Manufacturing*, *Laser Additive Manufacturing: Materials, Design, Technologies, and Applications* (Elsevier Ltd, 2017), https://doi.org/10.1016/B978-0-08-100433-3.00013-0.

[22] L. Jyothish Kumar and C.G. Krishnadas Nair, "Current Trends of Additive Manufacturing in the Aerospace Industry," in *Advances in 3D Printing and Additive Manufacturing Technologies*, 2016, 1–186, https://doi.org/10.1007/978-981-10-0812-2.

[23] Mary Kathryn Thompson et al., "Design for Additive Manufacturing: Trends, Opportunities, Considerations, and Constraints," *CIRP Annals - Manufacturing Technology* 65, no. 2 (2016): 737–60, https://doi.org/10.1016/j.cirp.2016.05.004.

[24] R. Shivpuri et al., "Evaluation of 3D Printing for Dies in Low Volume Forging of 7075 Aluminum Helicopter Parts," *Rapid Prototyping Journal* 11, no. 5 (2005): 272–77, https://doi.org/10.1108/13552540510623576.

[25] Anton V. Agapovichev et al., "Application of Additive Technologies in the Production of Aircraft Engine Parts," *Modern Applied Science* 9, no. 4 (2015): 151–59, https://doi.org/10.5539/mas.v9n4p151.

[26] Kumar and Nair, "Current Trends of Additive Manufacturing in the Aerospace Industry."

deposition and wire arcing laser sintering are extensively used for the production and development of long-lasting mechanical parts.[27] Laser metal deposition is a technique in which metal wire is deposited over the metallic or deposition surface. A high-energy laser beam is used to heat the metallic wire, which will ensure it is accurately deposited over the targeted area. This technique is preferably used for aircraft servicing and allows for rapid solutions to critical issues.[28] Another form of AM methodology is selective laser sintering, where metal powder is used to build the model layer by layer. Single or multiple lasers can be utilised to get the sintering process done, which enables the rapid manufacturing of mechanical parts right from the computer to a real 3D object.[29] These days, an array of the laser beam has been used by manufacturers, which offers a more accurate structure along with layer thickness minimisation. In aircraft manufacturing, 3D printing is currently used for rapid prototyping, rapid tooling, and rapid manufacturing processing. It covers the development of testing equipment, generating a prototype model, production of spare parts and UAVs (unmanned aerial vehicles).[30] There are a few companies, among which are Boeing and GE Aviation, that are collectively manufacturing more than 200 spare parts, including nozzles and engine equipment.[31] As AM technology is evolving, it is making it possible to generate the model as quickly as possible with an

---

[27] Xi Zhang and Enquan Liang, "Metal Additive Manufacturing in Aircraft: Current Application, Opportunities, and Challenges," *IOP Conference Series: Materials Science and Engineering* 493, no. 1 (2019), https://doi.org/10.1088/1757-899X/493/1/012032.

[28] Ali Gökhan Demir, "Micro Laser Metal Wire Deposition for Additive Manufacturing of Thin-Walled Structures," *Optics and Lasers in Engineering* 100, no. July 2017 (2018): 9–17, https://doi.org/10.1016/j.optlaseng.2017.07.003.

[29] P. A. Mikhalev, A. S. Filimonov, and A. N. Korolev, "Experimental Study of Gas Permeability of Polyamide 12 Additive Laser Sintering Additive Method Details," *XLIII ACADEMIC SPACE CONFERENCE: Dedicated to the Memory of Academician S.P. Korolev and Other Outstanding Russian Scientists – Pioneers of Space Exploration* 2171, no. November (2019): 170013, https://doi.org/10.1063/1.5133324.

[30] Yu Cheng Wang, Toly Chen, and Yung Lan Yeh, "Advanced 3D Printing Technologies for the Aircraft Industry: A Fuzzy Systematic Approach for Assessing the Critical Factors," *International Journal of Advanced Manufacturing Technology*, no. 1 (2018): 1–11, https://doi.org/10.1007/s00170-018-1927-8.

[31] Vadalmannati Sriram, Vipin Shukla, and Soumitra Biswas, "Metal Powder Based Additive Manufacturing Technologies-Business Forecast," in *3D Printing and Additive Manufacturing Technologies*, ed. L. Jyothish Kumar, Pulak M. Pandey, and David Ian Wimpenny (Singapore: Springer Singapore, 2019), 1–311, https://doi.org/10.1007/978-981-13-0305-0.

enormous range of applications and their prototyping. It will be beneficial for aeronautics and space aviation technology to use the overall model, made by additive manufacturing. It produces almost zero waste material and provides the desired product while consuming a lesser amount of production energy.[32] However, there is a need to clear all the security levels related to AM, but as this technology improves it may become one of the biggest achievements towards sustainable growth and industrial development.[33]

3D Printing in Healthcare

The 3D printer can produce 3D geometry of various sizes, shapes, directions, cross-sections, and relevant physiological thickness with fibres ordered precisely. Tissue engineering is a field involving the development of tissues and organs which can be used to heal, reintroduce and reconstruct damaged parts.

The 3D printer has the potential to create such organs performing the same biological functions. While the focus has been on 3D printed implants and medical devices used by patients, one of the largest areas of application is the fabrication of anatomical replicas. Doctors are currently utilising models produced by 3D printing from patient scan data to enhance the diagnosis of illnesses, clarify treatment decisions, plan and have, in some cases, chosen surgical interventions in preparation for the actual treatments. The models enable doctors to understand patient anatomy which would otherwise be difficult to visualise, especially when using minimally invasive techniques. Models also assist in precisely sizing medical devices.[34]

3D printing has a great impact on healthcare. It provides opportunities for the design of patient-specific implants, prostheses, and surgical tools. The design of an anatomical model for study and demonstration can overcome the need for dissecting a cadaver. Through 3D printing, better visualisation

---

[32] Sunpreet Singh, Seeram Ramakrishna, and Munish Kumar Gupta, "Towards Zero Waste Manufacturing: A Multidisciplinary Review," *Journal of Cleaner Production* 168 (2017): 1230–43, https://doi.org/10.1016/j.jclepro.2017.09.108.

[33] Richard Russell et al., *Qualification and Certification of Metal Additive Manufactured Hardware for Aerospace Applications*, *Additive Manufacturing for the Aerospace Industry* (Elsevier Inc., 2019), https://doi.org/10.1016/b978-0-12-814062-8.00003-0.

[34] Sharda Gupta, Akalabya Bissoyi, and Arindam Bit, "A Review on 3D Printable Techniques for Tissue Engineering," *BioNanoScience* 8, no. 3 (2018): 868–83, https://doi.org/10.1007/s12668-018-0525-4.

of disease, location identification, and examination can be done. Even the visual model of a specific case study can be printed and saved for future reference. Surgeons can have a better preoperative idea before dealing with patients, and can improve their communication by discussing with patients using the anatomical model for reference. A lot of research is required for bio-inks, vascularisation, preparing a model that can mimic human tissues, like bone and cartilage, as well as anatomically relevant model design. Flaws or degradation in anatomical models can be disastrous.[35]

History of 3D Bio Printing in Healthcare

1839 – Formulation of cell theory, and the realisation that cells are the building blocks of life.

1930 – First computing devices are created.

1978s – Stem cells are discovered.

The 1980s – Proliferation of personal computers and the invention of microprocessors.

1984 – Charles Hull invents the first 3D printer, allowing tangible 3D objects to be created from digital data.

1990s – Proliferation of supercomputers many times faster than personal computers.

1996 – Discovery that individual cellular aggregates can be arranged and that they could fuse—self-organising and self-assembling to form new combined structures.

1998 – Biologist, James Thompson, developed the first human stem cell lines.

1999 – First lab-grown implanted organ.

2000 – Medical field begins using 3D printing.

[35] Anna Aimar, Augusto Palermo, and Bernardo Innocenti, "The Role of 3D Printing in Medical Applications : A State of the Art," *Journal of Healthcare Engineering* 2019 (2019): 10.

The 2000s – Proliferation of quantum computers, markedly faster than supercomputers.

2003 – Thomas Boland creates the first bio printer.

2003– Completion of computer mapping of the human genome from both physical and functional standpoints.

2006 – Dr Shinya Yamanaka makes a ground-breaking discovery which wins the Nobel Prize: he showed that mature adult specialised cells can be reprogrammed back into a stem cell state, confirming that cellular differentiation is not unidirectional.

2006 – Lab-grown human bladder is implanted.

2009 – First blood vessels are 3D bio printed.

2010 to present – Additional advancements in 3D printing technology enable the production of bones, ears, exoskeletons, windpipes, blood vessels, vascular networks, tissues, and even simple organs.

Application of 3D Bio Printing in Healthcare

There are four core uses of 3D printing in the medical field that are associated with recent innovations—creating tissues and organoids, surgical tools, patient-specific surgical models, and custom-made prosthetics. With the help of 3D printing, we can improve medical education.

The 3D printing of drugs consists of printing out the powdered drug layer to make it dissolve faster than average pills. 3D printing also allows the modelling of implantable tissue. One example is the 3D printing of synthetic skin for transplanting to patients who have suffered burn injuries. It may also be used for testing of cosmetic, chemical, and pharmaceutical products. Another example is the replicating of heart valves using a combination of cells and biomaterials to control the valve's stiffness, or the replicating of human ears using moulds filled with a gel containing bovine cartilage cells suspended in collagen.

Bio printing can be combined with CAD technology to make 3D models based on the medical images of a patient. Images are typically collected from CT and MRI scanning, but other imaging modalities, like ultrasound or angiography have also been used. Prosthetic replacement heart valves are associated with high rates of complications, such as mechanical failure and

calcification. One approach to heart valve replacement uses extrusion-based bio printing to build a heart valve conduit composed of hydrogel and human aortic valve interstitial cells. The results showed that the valve was viable, and subsequent studies proved that a complex living aortic valve conduit could be constructed with bio printing. Bio printing technology can be used to regenerate bone and cartilage. Cells are delivered via extrusion-based bio printing. Cells printed in this way were shown to maintain viability and showed oestrogenic differentiation. Researchers have fabricated scaffold-free nerve grafts for the regeneration of peripheral nerve injury. The resulting graft was implanted into a rat, and axons at the proximal stump grew to reach the distal segment of the sciatic nerve in the printing-based bio printing, which has been used to create biomimetic multi-layered skin tissue composed of human skin fibroblasts and keratinocytes. In this application, cells and collagen hydrogel were printed separately. The resulting tissue showed the formation of layers, resembling dermal, and epidermal skin layers. And an *in-situ* skin printer has been developed to print skin cells directly onto the body for burn wounds.

Materials Used

An ample range of therapeutic biomaterials are used in 3D printing for the fabrication of scaffolds.[36,37] The selection of materials for scaffold preparation depends upon the fabrication technique and its application. For laser-based printing, photosensitive polymers are used, whereas thermoplastics are used in the Fused Deposition Model (FDM).[38,39,40] Moreover, bio-inks

[36] Fatemeh Asghari et al., "Biodegradable and Biocompatible Polymers for Tissue Engineering Application: A Review," *Artificial Cells, Nanomedicine and Biotechnology* 45, no. 2 (2017): 185–92, https://doi.org/10.3109/21691401.2016.1146731.

[37] Piyush Bajaj et al., "3D Biofabrication Strategies for Tissue Engineering and Regenerative Medicine," *Annual Review of Biomedical Engineering* 16, no. 1 (2014): 247–76, https://doi.org/10.1146/annurev-bioeng-071813-105155.

[38] Uwe Scheithauer et al., "Thermoplastic 3D Printing - An Additive Manufacturing Method for Producing Dense Ceramics," *International Journal of Applied Ceramic Technology* 12, no. 1 (2015): 26–31, https://doi.org/10.1111/ijac.12306.

[39] Fuda Ning et al., "Additive Manufacturing of Carbon Fiber Reinforced Thermoplastic Composites Using Fused Deposition Modeling," *Composites Part B: Engineering* 80 (2015): 369–78, https://doi.org/10.1016/j.compositesb.2015.06.013.

[40] H. Bikas, P. Stavropoulos, and G. Chryssolouris, "Additive Manufacturing Methods and Modeling Approaches: A Critical Review," *International Journal of Advanced Manufacturing Technology* 83, no. 1–4 (2016): 389–405, https://doi.org/10.1007/s00170-015-7576-2.

are the materials used for the one-way fabrication technique.[41] To fabricate the scaffold for 3D printing, remarkable advances have been made in improving the materials which are being used.

Using a broader classification, the use of a 3D printing system can be classified as passive or active. In the passive 3D bio printing techniques, the scaffold is being prepared using either natural or synthetic polymer, and then the cells are grown on the scaffold. The effectiveness of this technology depends upon the number of feasible cells proliferated on the scaffold. In the active 3D bio printing technique, however, the positioning of cells and their numbers are controlled using bio-ink containing live cells.[42]

Synthetic Polymers

Synthetic polymers are synthesised bio-material used in 3D printing, and they are used primarily because of their high mechanical strength, and controlled degradation rate. The different synthetic polymers are given below:

Polycaprolactone – It is one the most biodegradable and biocompatible polymers, and it is totally removed from the body after 4–5 years.[43]

Acrylonitrile Butadiene Styrene – Rezayat et al. explored the use of acrylonitrile butadiene styrene (ABS) various FDM parts (with different raster angles) because of its mechanical property (strain field, strain energy density, effective young's modulus).[44]

Polylactic Acid – Polylactic acid (PLA)'s surface is altered by ablation using direct laser writing and the scaffold is printed by using 3D technology known as fused filament fabrication.

Polyamide – Polyamides are polymer with repeated amide, -CO-NH-, linkage.

---

[41] H. Rezayat et al., "Structure-Mechanical Property Relationship in Fused Deposition Modelling," *Materials Science and Technology (United Kingdom)* 31, no. 8 (2015): 895–903, https://doi.org/10.1179/1743284715Y.0000000010.
[42] Asghari et al., "Biodegradable and Biocompatible Polymers for Tissue Engineering Application: A Review."
[43] Asghari et al.
[44] Rezayat et al., "Structure-Mechanical Property Relationship in Fused Deposition Modelling."

Polydimethylsiloxane – Elastomeric material polydimethylsiloxane (PDMS) is the most common non-biodegradable polymer.[45]

Polyetheretherketone – An improvement in bone regeneration with the use of ceramic materials helped to develop bone grafts for the particular bone defect.

PLGA – Zhao et al. employed a two-stage fabrication method to prepare the synthetic polymer PLGA, which supports the adipose-derived stem cell/fibrinogen/cellulose using low-temperature 3D bio-printing method.[46]

PVC – Zhang et al. fabricated a disposable microfluidic device by using a laser to cut the thermoplastic polymer PVC to form the base and cover of the microchannel, and control layers are made up of pressure-sensitive PET.[47]

Natural Polymers

Natural polymers are also biomaterial and are used for printing cells by avoiding coarse conditions, like temperature, but the number of natural polymers is limited. It includes gelatine, collagen, chitosan, alginate, collagen, etc. These natural polymers are used to repair bone, cartilage, nerves, and skin.

Some of the current and future applications of 3D printing are focused on the testing of cosmetic products and other consumer goods, drug screening, personalised medicine, regenerative medicine, cell-based biosensors, food and other animal products, education, academic research, and bionics. Some of the limitations of a 3D printer include the challenge of dealing with bio-inks and bio printing as it can only work with limited resolutions in tissue engineering; the fact that it requires a costly setup to fabricate the scaffold; and it also requires a large build area for the scaffold. It is also difficult to get documentary guidelines for designing a 3D Printer.

---

[45] Asghari et al., "Biodegradable and Biocompatible Polymers for Tissue Engineering Application: A Review."

[46] Yu Zhao et al., "Three-Dimensional Printing of Hela Cells for Cervical Tumor Model in Vitro," *Biofabrication* 6, no. 3 (2014), https://doi.org/10.1088/1758-5082/6/3/035001.

[47] Xinjie Zhang et al., "Inexpensive, Rapid Fabrication of Polymer-Film Microfluidic Autoregulatory Valve for Disposable Microfluidics," *Biomedical Microdevices* 19, no. 2 (2017): 1–9, https://doi.org/10.1007/s10544-017-0169-0.

3D printing in the medical field and design needs to think outside the current norms in order to be in a position to revolutionise health care. The three main pillars of this new technology are its ability to treat more people where it was previously not feasible; to obtain better outcomes for patients; and that it helps to save time when used under the direct supervision of medical specialists. In short, 3D printing consists of "enabling doctors to treat more patients, without sacrificing results".

3D printing in the Jewellery industry

In recent years, 3D printing has been used to generate the pattern for expensive casting, and to print jewellery. Very complex designs can be created using 3D printing. The introduction of 3D printers allowed for the first time for the creation of rapid prototypes. The acute functions of 3D printing have opened up many inventive prospects as well. One of its major applications is in 3D jewellery printing. Gold ornaments are highly prized by Indians, and the potential for 3D jewellery printing is huge in India. In the past, jewellery casting designs were cut from wax, utilising CNC machines. With 3D printing, the various patterns can be made immediately and in an exceptionally brief period of time. Multiple designing can be created in a single print by using 3D printing. This implies that it is exceptionally cost effective, despite low volumes of creation. To create jewellery, the 3D printing uses two methods—investment casting and direct printing. The investment casting includes 8 steps: pattern formation, moulds assembly, shell building, burnout, pouring, knock off, cut off, and finishing. Directly printing parts from metal powder are a less popular method for producing 3D printing. Sections can be printed via silver, platinum, and gold alloys but, for the finishing process, a significant amount of post-processing is required. The direct printing method is more costly compared to the investment method, even if it is only for one portion. The direct method needs precious power management at a high level.

In 3D printing, there are two types of printers used for jewellery making—FDM or Fused Deposition Modelling and SLA or Stereo lithography device. The sustained filament of thermoplastic material, which is fed by a movable and heated printer extruder head, is used in the FDM method. The FDM method can produce semi-metallic jewellery which is the combination of metallic filaments and plastic. For the production of prototypes with a simple design and the actual manufacturing of jewellery, the FDM is a cost-effective method. The SLA method uses photo polymerisation for producing parts in a layering process. The SLA method is also known as an

optical fabrication photo-solidification, or resin printing. In this method, link-forming polymers and molecules are generated by light. The 3D solid object of the body is created by polymers. This method can produce prototypes with elaborate designs with precise and precious stone as per the required jewellery settings. The steps involved in 3D printing of jewellery are as follows:

Specific CAD software is used for designing the desired jewellery model. This enables the craftsman to investigate different plans. The finished structure is traded as an STL or SLC document. This plan is then printed utilising exceptional 3D jewellery printers. The 3D jewellery printing process empowers the printing of numerous models of various geometries simultaneously. This not only saves time in the wax configuration step, but also the expense of printing. Then, in the next step, a casting tree is generated by dewaxing/removing supports from the 3D printed jewellery models. These models are then cast utilising the investment casting strategy. This includes pouring the investment material over the jewellery models in a steel flask. These flasks are then put in a kiln and heated at a temperature that burns out the wax. The jewellery models are then vacuum cast, replacing the centrifuge casting method. The last step includes setting precious stones (if needed) and polishing each piece.

The merits of 3D printing jewellery.

1. The 3D printed jewellery does not need further manual finishing as it will already have a smooth finish. In India, it is the manual finishing that increases the cost of the jewellery.
2. High accuracy and resolution.
3. Individuals can structure their jewellery accordingly and may examine the design that they wish to have by utilising cheap plastic models as references. They can then print the jewellery when they are satisfied with the prototype.

There are three basic types of 3D jewellery printers:

1. Drop on Demand – these printers work on piezoelectric jet technology.
2. Digital Light Processing (DLP) – uses a projector to define individual layers at a time of a projected image.
3. Stereo lithography (SLA) – uses a laser to “draw” each layer of a CAD file.

Major benefits of using 3D Printed Jewellery:

1. Shorter time to market.
2. Automation will lead to increased precision, complexity and significantly reduces cost.
3. Improved surface finish, less wastage of precious metals.
4. Inventory-less retail.

One of the most significant ways in which 3D printing has affected the jewellery-making industry is through the technique of prototyping and tooling. 3D printing is utilised to make the essential model or an immediate wax design from which to achieve an elastic or silicon shape. By eliminating some of the most time-consuming steps of the traditional process, these applications have shown big improvements in several areas of the jewellery sector. Kale et al. proposed an integrated approach for future e-commerce jewellery business using 3D printing, assisted by smart technologies.[48] In 2018, the Envision TEC became a leading choice among goldsmith jewellers, and allowed for custom and large manufacturers of 3D printing jewellery patterns for casting. Roxana et al. proposed a modelling method that increased flexibility in modifying and reusing existing models, and is alleviating the difficulties related to mastering advanced mathematical mechanisms by providing improved visual feedback to the user. The 3D modelling software application was implemented to surpass known drawbacks affecting the performance of current 3D modelling software applications.[49]

3D printing and its application on automobile

There are various approaches to shaping and creating the products around us. Creation can be done by employing two considerable operations: addition and elimination. In the early days of the Industrial Revolution, product manufacturing companies were utilising the elimination process to create the desired object, where a fine product can be obtained by removing unwanted portions of a big solid structure. This process was prolonged, poor, and delicate as it left a huge number of residual parts. Industrial development made a significant breakthrough when 3D printing came into existence in the 1980s. This new methodology is known as Additive

[48] Sandip A Kale, "Smart Integrated Approach for Future E-Commerce Jewelry Business Using 3D Printing," no. April (2019).
[49] Roxana Draganoiu et al., "Interactive Visual Modeling with Applications in Jewelry Design," *Proceedings - 2019 22nd International Conference on Control Systems and Computer Science, CSCS 2019*, 2019, 272–79, https://doi.org/10.1109/CSCS.2019.00051.

Manufacturing (AM) because it creates the required product by adding various fine layers on top of one another. This is the technique in which the manufacturing material process is in the form of various sequential layers. It shows its effectiveness by producing almost no residual parts after the completion of the whole printed model. Initially, a fully developed 3D printer was first established by Charles W. Hull in 1984.[50] At the time, stereo lithography apparatus (SLA) was on course to creating 3D printed models. The growth of 3D printing also provides a substantial push to upscale other manufacturing markets, such as automobile manufacturing. In the early stages of 3D printing, the technique had been used to develop polymer prototype models as it was many times faster than earlier prototyping mechanisms.[51] There are diverse forms of 3D printing, but in the early days selective laser sintering (SLS) and binder jetting were used to create automotive parts. From the 1990s to 2010, the 3D technique was extensively used to develop polymer models of automobiles. Afterward, ORNL and Cincinnati Inc., became the first fully-developed automobile manufacturer.[52] In 2014, a complete working model of the electric car was designed using a computer-simulated model and a 3D printing facility.[53] This car was developed by combining fewer than 50 different parts, compared to traditional cars, which are made up of more than 30,000 parts. BMW, a leading vehicle manufacturer, says that it can reduce the overall weight of the vehicle by 72%.[54] 3D printing may also be favourable in terms of manufacturing costs and speed of production. As per the survey conducted by Siemens (a manufacturing company) in 2017, production cost may decrease by 50% and speed may rise by 400% due to the application of additive manufacturing technologies in the automobile industries.[55]

---

[50] Mohsen Attaran, "The Rise of 3-D Printing: The Advantages of Additive Manufacturing over Traditional Manufacturing," *Business Horizons* 60, no. 5 (2017): 677–88, https://doi.org/10.1016/j.bushor.2017.05.011.

[51] David Bak, "Rapid Prototyping or Rapid Production? 3D Printing Processes Move Industry towards the Latter," *Assembly Automation* 23, no. 4 (2003): 340–45, https://doi.org/10.1108/01445150310501190.

[52] M R Talagani et al., "Numerical Simulation of Big Area Additive Manufacturing (3D Printing) of a Full-Size Car," no. July (2015): 27–36.

[53] V Sreehitha, "Impact of 3D Printing in Automotive Industries," *International Journal of Mechanical And Production Engineering*, no. 5 (2017): 2320–2092.

[54] Sreehitha.

[55] Attaran, "The Rise of 3-D Printing: The Advantages of Additive Manufacturing over Traditional Manufacturing."

Conclusion

Indeed, 3D printing and its related techniques, software, applications have immense potential to revolutionise the world. Its capability to ensure fast, personalised and on-demand, one-step local production makes it unique. It can greatly affect the economy and workforce if implemented properly. It offers vast research scope as it encourages the search for less costly materials such as bio-inks. 3D printing seeks to make improvements in the mechanical and physical properties of the printed objects, thereby creating the right balance between design complications and production of functional prototypes and products.

CHAPTER TWO

THE ROLE OF 3D PRINTING IN THE GROWTH AND PROGRESS OF MEDICAL HEALTHCARE TECHNOLOGY

DR. DINESH BHATIA, HIRAK RANJAN DAS

Abstract

3D printing came into existence in the early 1980s as additive manufacturing and, over the years, the technology has grown by leaps and bounds. It gained huge popularity in the 2000s when its importance came to the fore, and its role in helping to improve the capability of humans was explored. The additive technology is being employed in different fields and is affecting many different aspects of human life. 3D printing, in various healthcare fields, represents the opportunity to develop pharmaceutical and medical devices for personalised treatment to patients with 3D printed patient-specific drugs, equipment, and anatomical models. In the medical field, 3D printing was seen as one of the biggest breakthroughs, and it slowly became an integral part of mainstream medical practice. Biomedical 3D printing techniques include fused deposition modelling, hot-melt extrusion of filament, stereolithography, inkjet printing, and selective laser sintering. The present chapter aims to give an overview of the growth, advancement, and future scope of 3D bioprinting in different areas of healthcare; how it is being employed to improve and develop patient-specific care with greater quality, customisation; and how it lowers the cost of products, while providing durability and patient ease. Personalised treatments would be easier with the employment of 3D bioprinting by enabling the possibility of making anatomical replicas of the patients, in order to be able to study them before proceeding with the operation. 3D bioprinting also helps in producing bioprinted implants and surgical devices, patient-specific customised drugs, and is also invaluable in the dental field for its ability to make braces and teeth. The chapter also explores

the challenges and limitations that lie ahead in the adaptation of this technology for mass usage in the healthcare sector.

Introduction

Three-dimensional (3D) printing technology was first introduced in the 1980s, known as stereolithography, also referred to as additive manufacturing. With the introduction of additive manufacturing, 3D printing technology came into existence. Dr Chuck Hall is referred to as the father of 3D printing technology for developing the first 3D printer for printing an eyewash cup. It gained tremendous popularity in the 2000s when its role came to prominence as it helped to improve human capabilities and performance outcomes. This technology can design and construct complex anatomical structures as a guide for complex anatomical studies, recreate complex organs with complex 3D microarchitecture, and fabricate frameworks for stem cell differentiation. Medical uses of 3D-printing technology are ideally suited because they are built on this inimitable quality of additive manufacturing, whereby it can make optimised and customised products.

The technology is being employed in several fields, and is projected to grow at a CAGR of about 20%, with revenues expected to cross the $4 billion mark by 2026, as per the Grand View Research report, published in 2019. The swift growth in the status of the 3D printed material is because it can be customised and personalised as per an individual's needs, and not as a mass-manufactured, one-size-fits-all concept. Additionally, it has precision and speed, and there are major savings in costs with the help of this technology.

3D printing is the process of creating physical objects from computer-aided digital (CAD) models by the fusion of materials over successive layers, with the help of light, heat, and chemicals. 3D printing has both enabled and led to tremendous innovations in the medical field, and it is becoming an integral part of it. It is generating huge interest among scientists and researchers and is revolutionising the medical domain. It is helping in human organ transplants by making customised replacements possible; in improving surgical procedures; in developing surgical tools at reduced costs; and is improving the lives of disabled people reliant on prosthetic limbs. A life-saving device for infants, the first 3D printed implant is known as a bioprinted airway splint. This implant prevents the risk to life which may be caused by the sudden collapse of tiny airways around the lungs. This implant can grow with the child and can be produced easily, within an hour,

with minimum costs involved. 3D bioprinted tissues, human tissues for burns victims, organ transplants, and human organs, such as liver, kidney, and brain tissues can be easily developed by employing this emerging technology.

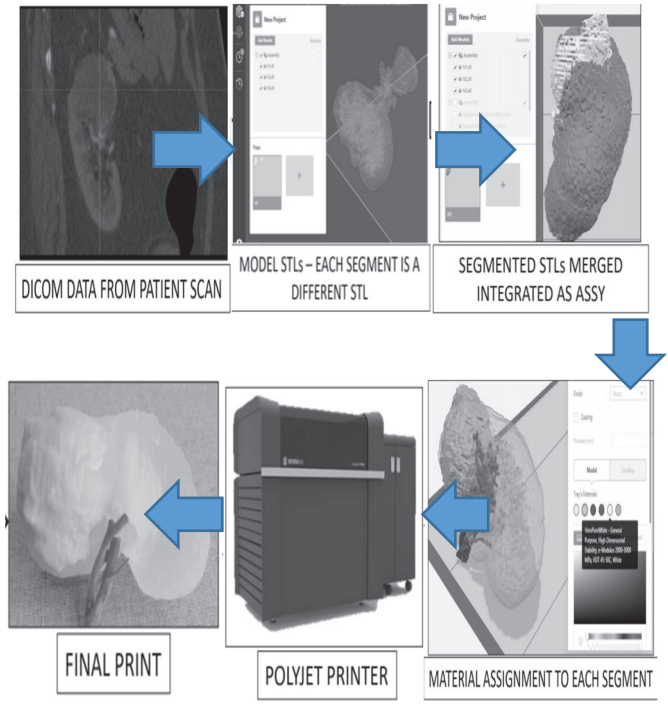

Figure 1: Steps in 3D bioprinting
Source: Altem Technologies

3D printers can be classified as solid, which employ the Fused Deposition Modelling technique; liquid, which employs the Stereolithography (SLA)-based technique; or powder, which uses the Selective Laser Sintering (SLS)-based technique. By employing thermoplastic or material wire, FDM technology produces objects layer-by-layer with the help of a nozzle spray. In the SLA technique, a vat packed with liquid plastic or photopolymer is used, which produces a perforated platform on the top of the tank, leaving a thin layer on the surface. With the help of a light beam, each layer is hardened or solidified, and the platform is lowered to insert the next layer. The SLS technique is similar to the SLA technique but employs polymer,

metal, or ceramic powder instead of plastic in liquid form. The laser beam selectively sinters the powered raw material, spread in the form of a thin layer, and, at the end of each set, the powder bed is pulled down and layer-by-layer of powder is added for laser sintering.

What follows is an exploration of the development of 3D bioprinting technology and its growth as the most favourable technology in healthcare. Furthermore, the chapter highlights certain key challenges that need to be overcome before it is possible to completely reap the benefits of this technology.

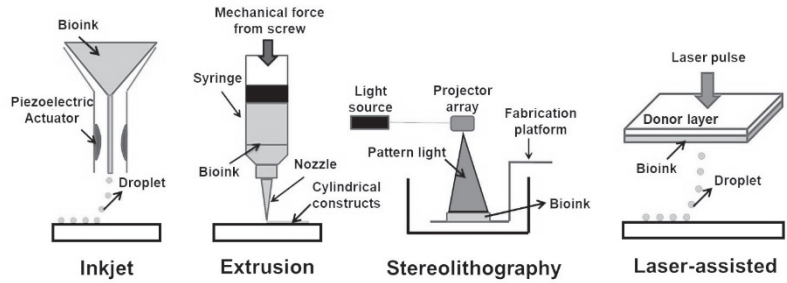

Figure 2: Various 3D bioprinting Techniques

3D Bioprinting in the Medical World

The role of 3D bioprinted products has grown tremendously over the years and they have extensive applications in several clinical healthcare domains, such as organ replacement, implants, dental, pharmaceuticals, etc. For the success of the product, a suitable 3D bioprinter, along with appropriate modelling software must be available. The model is developed precisely, layer-by-layer, with the help of bio-inks made up of hydrogel biomaterials that allow adhesion and proliferation of living cells, to provide support during natural extracellular matrix growth. With the use of various biomaterials, the complex characteristics of tissues and organs can be reconstructed. 3D printing can be employed in genetic engineering for stem cell development to be used in studies. It can be employed to produce customised on-demand drugs with patient-specific dosage forms with various shapes and sizes and immediate-release tablets, offering flexible treatment methods to patients. Also, multiple drugs can be made into a single tablet, layer-by-layer, from the customised dosage that has been made

possible through this technology, as each patient would require different dosages of the drug.

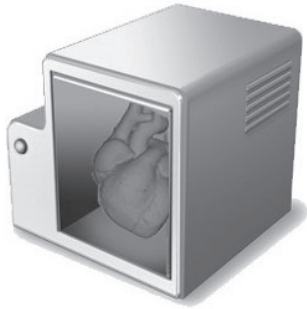

Figure 3: Representation of a 3D biomedical printer

The following sections will discuss the growth of 3D bioprinting in different domains in the area of healthcare.

Bioprinted organs

3D technology is being rapidly employed in bioprinting and tissue engineering for developing tiny organs or organoids, such as the kidney or liver, to take over the function of the organic organ if it fails to function as it normally should. This technology employs the use of digital models to create a replica of the desired organ by printing layers of matrix until the final prototype is ready. The 3D printed skin for burn victims is extensively used as it has wide applications, in addition to cost saving, and providing immense technological benefits. As burn victims have limited options for healing disfigured skin, the 3D bioprinter prototype can produce human skin, which can be used for their rehabilitation. The human skin contains several layers and is, therefore, considered to be a perfect case in order to investigate and employ the 3D bioprinting technology. A team of scientists from Madrid have employed the method of transplanting bioprinted skin in mice having burn injuries, an experiment which was later extended to humans. The biological ink is made from human plasma and material extracts taken from skin biopsies.

Additionally, scientists have created 3D-printed ovaries that were successfully implanted in mice to help manage infertility, a traumatic condition for many women. Human organ printing, such as of the kidneys, the liver, and the heart, is in the development phase. However, Organovo, one of the biggest

bioprinting companies, has created bioprinted human liver tissue that can be used for carrying out studies in toxicology, metabolic and drug interaction tests. The availability of such organs would reduce the long patient waiting time, which may range from between 2 and 5 years (at a minimum), before a patient is matched with the right transplant, thereby helping save many lives every year. This would also help in reducing the costs of patient treatment, care, and hospitalisation. Soon, bioprinted organs may also be employed by different pharmaceutical industries to test the toxicity of new drugs, replacing the animal models currently being used.

There are presently many methods in the 3D culture that include scaffold-based models (hydrogels or solid biomaterials) and scaffold-free ones for spheroid development: that would provide more dependable cellular models and it would also decrease the use of animals in drug toxicity tests and efficacy tests. The individual differences between patients make it difficult to predict the outcomes of human disease treatments, and because drug testing is very time-consuming there is a need to develop new medical treatments in order to overcome some of these limitations. Personalised medicine is presently gaining attention and is becoming applicable to medical practices as well. Precision treatment is expected to develop, which will be based on disease-specific and even patient-specific treatment, something that will be made possible from 3D organoid culture.

Bioprinted anatomical models

3D printing provides various applications that allow surgeons to print anatomical models of the patient's body parts, such as the ear, skull, and lungs, etc., which are required to be operated on. This helps surgeons to better prepare themselves before the actual surgery, and enables them to plan procedures before the actual operation to reduce time and error during surgery by approximately 33%. This helps to decrease the risk of infections, and helps improve the recovery rate of patients, thereby reducing the length of hospital stays. This technology is widely employed in preoperative planning by clinical and trauma orthopaedic surgeons and other healthcare specialties, such as gastroenterology, neurology, cardiology, etc. 3D printed orthopaedic models can be generated with the help of CAD models from MRI scans that represent specific anatomy, and are readily available to help in better understanding the surgical procedures, reduction in procedure time to fix fractures, and help in complex surgeries related to the acetabular joints in the hip region which require precise procedures to heal faster.

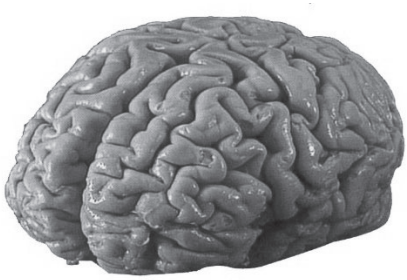

Figure 4: 3D printed brain model

The technology is also being employed in major heart surgeries with the help of cardiac mapping of catheters. It is being employed for the treatment of different lung diseases, such as chronic obstructive pulmonary disease (COPD), where the patient experiences increased symptoms of breathlessness. It helps in the removal of obstructive pathways and improves airflow to the lungs. The technology may also be employed for testing cosmetics, chemical, and pharmaceutical products. It can be employed for replacing heart valves or to control their stiffness by employing a combination of human tissues and biomaterials.

Bioprinted bones

3D printing technology is widely employed in carrying out complex vertebra or bone replacement surgeries. Due to the unavailability of implant material for carrying out bone replacement surgeries, doctors use 3D-printed replicas made up of titanium alloys as the ink. This technology is found to be highly successful in the treatment of some of the most complex cancer cases, where the surgeons intend to employ innovative solutions to save the lives of their patients. This technology was employed in 2017, in China, for cancer treatment, and in 2018, in the Netherlands, where the use of 3D bioprinting made possible the creation of “smart scaffolds” to combine with the patient’s natural stem cells and growth factors to produce natural cartilage-like tissue structures.

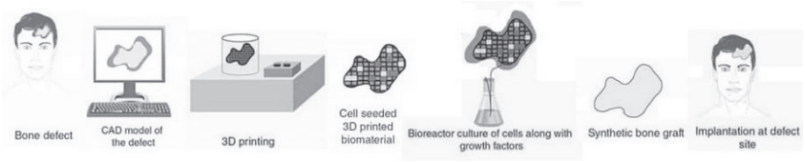

Figure 5: Representation of 3D printing of bones

To overcome the frequent breakdown of shock absorber fibrocartilage material in the knee due to normal bodily wear and tear, this promising clinical trial shows that the 3D bioprinted material, combined with the patient's stem cells, may provide long term relief. The scaffolds can be planned and designed accordingly, to apply the physical and mechanical forces required to differentiate them into the specific types of cells. With the assistance of the right growth factors, it may cause stem cells to form cartilage, bones, muscles, or other types of living tissues. The basis behind this technique is to provide hierarchical mechanical support to different cells during the process of tissue regeneration, and help in engineering the surface properties of materials with which the cells come into contact, to improve their biological response. 3D printed technology is being employed in treating long term spinal injuries or joint damage, such as arthritis of the knee. The scaffolding silicon acts as a platform for placing specialised 3D printed biomaterial on the top surface. By employing CT and MRI imaging techniques in making a CAD model, a scaffold may be built that would help to fill and repair the damaged regions. It is important to check the softness, stiffness, pore size, and porosity of the cells in the body environment to determine their survivability. Patients using the 3D bioprinted products have reported an improved quality of life based on their mobility and pain levels.

Bioprinted pills

The importance of employing 3D bioprinted pills is growing rapidly, and healthcare and life sciences companies are presently investing huge sums of money in this area. 3D printed pills can house multiple drugs and release them into the body at different intervals, as per the prescribed dosage and a patient's requirement, which is popularly known as the "polypill concept". Soon, it may allow patients to print the required drugs at home by using an affordable 3D-printer. In future, employing the 3D bioprinting technique would make it possible for patients to buy the drugs online by showing their medical prescription, procure the digital ink with blueprint, and print the drug using their bioprinters at home. The development of this concept was

stimulated after approval by the Food and Drug Administration (FDA) in 2015 for the first 3D-printed drug, known as Spritam (levetiracetam), an oral drug for epileptic patients suffering from seizures. It is also being widely used by patients with diabetes. The 3D printing process for Spritam binds layers of powered medications together with an aqueous fluid instead of relying on compressive forces to bind the drugs. The solid bioprinted material, which is porous in nature, rapidly disintegrates, and is suitable for patients who struggle to take pills or medicines regularly.

It is widely believed that the customised medicine solution as per a patient's requirements will soon revolutionise patient care, although some challenges still need to be overcome. 3D printing of drugs has presented the opportunity to produce customised on-demand drugs with patient-specific dosage forms with various shapes and sizes, and immediate-release tablets, offering flexible treatment methods to patients. Biomedical 3D printing technologies include fused deposition modelling, hot-melt extrusion of filament, stereolithography, inkjet printing, and selective laser sintering. 3D printing technique can be employed to produce personalised drugs with the customisation of shape and size of the tablet and dosage as required. Multiple drugs can also be made into a single tablet with layer-by-layer printing of those drugs as per the proportions required by the patient, thereby benefitting the patient as their medications will be customised according to their individual capacities, with regard to how their bodies can accept the drug. GSK healthcare is investigating the benefits of 3D printing technology to provide customised-manufactured pills thus enhancing the field of personalised medicine to provide patient-specific solutions.

Bioprinted implants

3D bioprinting technology is becoming extremely popular with clinicians to treat an injury or to repair the human body, alongside medical devices being presently employed. This technology provides a perfect fit as it is designed according to the patient's anatomy, which also helps in contributing to the fast recovery time of the patients. Bioengineers are using 3D printers in a variety of ways to develop flexible and long-lasting hip and knee joints, prosthetic limbs, and to produce living tissues attached to the printed scaffold material. Researchers printed small soft gel implant materials by filling them up with the neural stem cells employing a 3D printer. These implants were surgically placed inside a tiny gap in a rat's spinal cord which led to the growth of new nerve cells and axons after a certain time interval that helped in connecting with the rat's existing

circulatory system. This ensured their survival in the body and allowed precision fixing of the soft gel material into the cellular matrix at the wound site.

In the United Kingdom, more than 10 million people suffer from hearing loss, and the trend is similar on a global scale as well. This necessitates the availability of a large number of cochlear implantations and hearing assistance to those with auditory issues. Scientists globally are employing medical 3D bioprinted patient-specific implants and prosthetic products to treat and manage patients with hearing loss. Moreover, the 3D prosthesis and implants are being employed for diverse purposes, such as the reconstruction of the face and breasts; hip and ankle replacement; tissue generation; reconstruction of the acetabular and maxillofacial joints post-injury and trauma; respiratory disorders; and for bone infection and cancer. The bioprinted tissues can be utilised to test the effects of the drug treatments to grow complete organs for transplantation in humans at a later stage. A group from the University of California, San Diego, has bioprinted a spinal cord that can be custom-fitted into the site showing injury.

3D printing of surgical instruments

3D bioprinting is being used to create patient-specific organ replicas and anatomical models that can be used by surgeons for investigation of the complexity of a medical problem, thereby enabling surgeons to practice before undertaking complicated operations. This technique is beneficial as it helps to speed up medical procedures, eliminate errors, and reduce patient trauma. The procedure is routinely being employed in surgeries ranging from a full-face transplant to spinal procedures. Doctors have successfully operated on a patient who suffered from a cerebral aneurysm in the veins, by employing a 3D printed model of the patient's arteries to develop maps in order to safely navigate the blood vessels, and reduce the chance of causing injury.

Medical bioprinting is seen as an innovative technology by surgeons, and with the support of the 3D printed anatomical representations, enabling a more detailed and closer look inside a patient's body before performing necessary complex procedures. Furthermore, these representations can benefit trauma surgeons as they may deliberate over the best possible approach on how to fix intricate fractures. However, the accuracy of these 3D printed representations, identical to the look of human bone, needs to be scrutinised. Accuracy is significant when it comes to the fitting of surgical guides and plates, as it is challenging to describe and analyse these fractures

prematurely, even with the assistance of CT scans. A joint assembly of researchers from the Netherlands carried out an authentication study to test the accuracy of 3D printed anatomical replicas for surgical preparation purposes.

Surgeons usually require years of preparation to convert a two-dimensional (2D) appearance into a three-dimensional (3D) appearance in their mind in order to gain an accurate understanding of the fracture patterns. CT software, however, effortlessly assists volume rendering of 2D CT into a 3D reconstruction. Nevertheless, it remains inconclusive how a 3D-printed model relates to a human bone. From an extensive literature survey, no studies can confirm the accuracy of 3D-printed replicas in a preoperative planning plan, when applied to real human bones.

Surgeons in Belfast last year successfully performed kidney transplants with the help of a 3D printed model of the donor's (father's) kidney. The surgery was quite complicated as the donor had an incompatible blood group and the kidney had a cancerous cyst. By employing the 3D printed model of the donor's kidney, the surgeons were able to assess the size and placement of the tumour and cyst. Moreover, sterile surgical instruments, like forceps, clamps, and scalpels can be developed using 3D printers. The size of these instruments can be made as precise as possible, and in such minute sizes that they can be employed in the operation within the narrow regions of the human body without causing damage to the nearby surrounding tissues. The main benefit of employing 3D printed technology is that the production costs are much lower than traditionally employed devices.

Custom-made prosthetics using 3D printing

3D printing could possibly be employed to develop customised prosthetic limbs that fit and suit the user. This allows users to have 3D printed products at much cheaper prices with similar functionality as conventionally manufactured prosthetics already available on the market. This is highly beneficial where children are concerned as they quickly outgrow their prosthetic limbs, and it thereby also helps in saving costs. The 3D printed prosthetics allow patients to design their limbs and sockets as per their requirements. This affords the patients an easier experience and leads to less patient discomfort.

Prosthetics stood out as one of the first biomedical areas to be revolutionised by 3D printing, and continues to grow as the technology becomes more democratised, making replacing limbs easier and cheaper. A few years ago,

ASME.org introduced the RoboHand, a device made up from a 3D printer, using advanced technology by a South African carpenter, Richard Van As, and Ivan Owen from Bellevue, WA. Nowadays, with readily available open-source designs, individuals are 3D printing custom-made prosthetics for children, adults, and even dogs like Derby, who was seen at the 2015 White House Science Fair. 3D bioprinted choices are providing implements for those who have previously required access to practicable, inexpensive and well-timed solutions. 3D printed, customised prosthetic hands and arms are, of course, not found in all places. Such high standards of bionics are usually only accessible in higher socio-economic environments, but this needs to change as it should be available to anyone whose life might benefit from this technology, wherever they may be. Having these options is truly a game-changer and should be so for all, regardless of one's socio-economic standing.

3D-Bioprinting Challenges

New challenges that arise are an important part in the process of reaching a point of sustainability, and enabling the growth of any new medical technology. These challenges allow scientists and researchers to find new solutions using the technology of 3D-bioprinting to eliminate problems and improve the present technology. Although 3D medical bioprinting is a cheaper technology in comparison to available alternatives, the lack of expertise for its installation, and putting it to optimum use continue to remain persistent challenges for the healthcare industry. Additionally, more time is required to undergo different clinical trials and regulatory clearances before a final solution is made available to the consumers so that it can become established. Getting regulatory approval for 3D printing technology depends on the two biggest challenges namely, quality assurance and production control; these factors severely impact the scalability and the long-term adoption of this technology.

Nevertheless, medical 3D-printing offers tremendous opportunities to the healthcare industry to improve human health, thereby providing immense benefits to both the user and the practitioner. This technology is highly affordable, accessible and attainable, and encourages researchers and the clinical fraternity globally to explore this technology further. It is worthwhile imagining the transformation that 3D-bioprinting would bring in the healthcare business sector. But, to understand the maximum potential of 3D printing technology, several barriers must be overcome to make this technology fully implementable in the healthcare arena. Moreover, the

regulatory environment has not kept pace with rapid technological advancements, and the health sector is not fully prepared or equipped to approve 3D-printed products. Furthermore, the risk of patent and copyright infringement remains a concern, along with the availability of the desired level of technical expertise in the healthcare sector.

The relatively high costs of 3D printers remains an issue that limits their widespread usage, although the emergence of a large number of small and medium-sized 3D-printing technology companies creates a competitive business environment. The market is witnessing a rapid surge in joint associated initiatives as well-known healthcare companies are concentrating on integrating innovative technologies in their processes to attain sustainable profits and continue competing in the market. Leading healthcare companies are signing agreements with different 3D-printing medical device companies which seek to improve their technical expertise, and provide better patient care to further strengthen their clinical experience. A few hospitals in the USA have established their 3D-printing laboratory to overcome increased patient demand for 3D services in the area of head and neck cancer patients requiring reconstruction, and in the development of bone grafts to reconstruct the upper or lower jaw.

The technological developments in 3D-printing materials and mutual participation of industry players could position it as one of the most rapidly developing technologies in health research, with the potential to reshape the healthcare landscape in the near future. It could lead to the establishment of 3D printing technology for day-to-day routine healthcare activities, by helping surgeons in complicated medical procedures and producing human organs for transplantation, as well as pills being produced in the desired shapes, with variable drug release rates. Hence, the outlook for 3D printing techniques looks secure.

Scientists from Princeton University engaged 3D-printing technology to produce a bionic ear, capable of hearing radio frequencies not within the audible range of a normal human ear. This was made possible by combining electronics with live human tissue. A US-based company, Organovo, in San Diego, developed a novel bioprinting method that obtains cells from donor organs and transforms them into a printable bio-ink. Experts believe that bioprinting could create functioning organs for implantation within the next decade or so, but this could raise certain ethical issues as it may risk reducing human life to a manufacturing process. Moreover, any new technology that has higher production costs when introduced in the market enhances the risk of increasing health inequalities as it may make such

transplants available only to those who could afford it. Moreover, 3D printing technology has not yet attained the level required to bioprint an entire organ. The future possibilities of this technology, along with the enhanced awareness of the 3D printing capabilities by the public, could lead to further ethical concerns and queries. Hence, it can be stated that 3D printing technology is a rapidly emerging technology, but it is still in the early stages and requires further research and development.

It is essential to realise the basic boundaries of this method. Anatomical features and tissue construction may have details on the scale of hundreds of microns, which is currently a challenge to achieve with the use of the typical 3D printers that are presently available. Furthermore, this difficulty can limit the capability to create small features that survive the fabrication procedure, as powder particles must be bound together firmly. Another difficulty is the restricted number of biodegradables and biocompatible resins available. In 3D printing for biomedical uses there are limited materials obtainable, like collagen, gelatine, fibrin, ceramics, thermoplastics, or light-curable composites. To overcome these restrictions, the enhancement of different biomaterials that can be printed in combination with cells is necessary. These biomaterials must be biocompatible, effortlessly manufactured, and have adequate mechanical properties for cell support, and a safe 3D structure.

Case Study

A college student 3D printed his own braces

For under ₹5000, a student, based in New Jersey, 3D printed his own braces instead of getting a set of clear orthodontic aligners that usually sell for many thousands of rupees. Amos Dudley, a twenty-three-year-old college student, had braces as a child but could not maintain them. He had a kinked smile that he used to hate. He could not afford more work by orthodontists as his financial position did not allow him to fully complete the treatment.

He made 12 models using a 3D printer, Stratus Dimension 1200es, and with a vacuum-forming machine he created plastic aligners using special dental plastic that was bought online. He did a lot of research into orthodontic procedures and started with a mould of his teeth which was made using alginate powder and filled it with liquid permastone to set it. Afterwards, he scanned the cast and animated the advancement of his teeth digitally by using software to achieve his goal. Then, the correct position was animated by measuring the total distance it could travel, and then dividing it with the

recommended maximum travel distance of tooth per aligner. STL models were prepared from each animation frame.

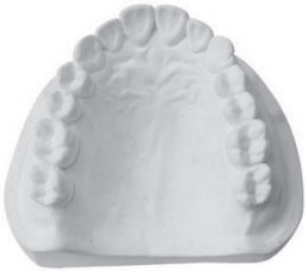

Figure 6: Mould model

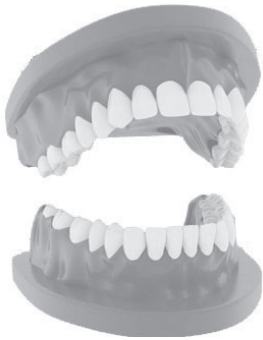

Figure 7: Digital model

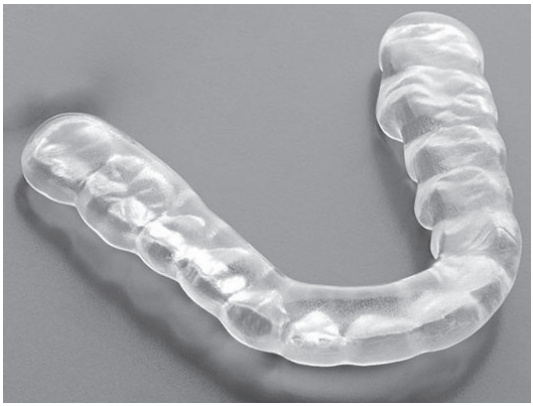

Figure 8: 3D printed braces

Future of 3D printing in healthcare

With the growing impact and advantages of 3D printing technology, several hospitals and clinicians are encouraging its usage in the healthcare domain. To evaluate the growth of 3D printing technology, a study by Visiongain found that its market more than doubled in 5 years, from $1.2 billion in 2013 to approximately $4 billion by 2018. The reasons for this tremendous growth can be attributed to the fact there has been widespread awareness and usage of 3D printed products, including devices, living tissues, and

prosthetics. With the help of bioprinting technology and employing edible bio-ink, the human structure can be built layer-by-layer to develop the required human tissue. Different research groups are testing this technology for use in creating skin grafts, knee cartilage, and small heart valves. Further work is ongoing which seeks to create organs, such as kidneys, pancreas, or a heart for the human body. If successful, this will have an enormous impact by reducing the lengthy wait time for donors or transplants, improving the chances of recovery, and possibly even controlling or curing chronic ailments, such as diabetes. As per the findings of researchers, the printing of cells or tissues from body parts is still in the nascent stage with lengthy ethical and regulatory approval processes that need to be addressed. However, the ability to have a perfect match and not wait for a donor would save countless lives and significantly improve present medical technology. It is expected that this sector will hit the market in the next decade or so, and the impact will be huge.

The external wearable prosthetic device market is projected to continue leading the 3D printing healthcare market due to the increased number of amputees, patients with auditory loss and dental problems, and the increasing availability of advanced biocompatible materials. The customised wearable devices as per individual requirements allow better comfort during use thereby driving market growth. The technology is also being employed in the field of dentistry to provide precise, less invasive, and localised treatment as per the requirements of the patient.

The present-day 3D bioprinting challenges for cell/tissue bioprinting may be overcome by the use of bioprinters that are well-suited to the physiologically appropriate materials and cells; increasing the firmness and speed of these mechanisms and their commercial uses; improving biomaterial composition and cell sources; developing vascular and nerve models; and understanding maturation models for every material. Therefore, it is important to also understand the future role robotics and nanomedicine will have in the development of this technology. There is a necessity to develop the biocompatibility and mechanical properties for cell support, which will assist in the formation of soft tissue and organs that could be transferred into the human body.

The final question surrounds the possibilities of a bio-fabrication line. A newly-published study addressed this question and concluded that the only economic and reasonable way to commercialise organ-printing technology is to methodically employ scalable, automated robotic technology, and to build an assimilated organ fabrication line. The bioproduction of a human

organ will need the improvement of a series of assimilated automated robotic devices. Currently, 3D printing manufacturing only signifies a small section of the market; yet, in the next 10 years, this section is expected to grow. 3D bioprinting has some obstacles to overcome, like the advancement of different biomaterials and refining printers' technology to advance results.

3D Printing Healthcare Market

Global 3D printing in the pharmaceuticals and medical devices market is projected to grow due to the increasing initiatives taken up by governments in the healthcare sector; growing technological advancements in 3D printing; easy development of medical devices; and the widening use of the 3D printing method in the healthcare industry. 3D printing already plays a vital role in the medical and dental manufacturing industry. It is cost-effective and is a favoured option by medical device companies, comprising all types of medical devices, such as hearing aids, prosthetics, and custom-made knee and hip implants. The 3D printing medical method is projected to show major growth throughout the forecast period due to several technological improvements in this area. Besides, some other influences, such as R&D investment and a rapidly expanding customer base, along with the increasing scope of biomedical application, have fuelled market growth. There are certain factors, however, that may obstruct the growth of the market in the future, which include adverse reimbursement policies, the high price tag of printers, biocompatibility issues of 3D printed medical devices, and limited technical expertise in medical devices.

As per a report published by Allied Market Research, the global market predictions allow 3D printing technology to grow around 26.2% between 2015 and 2020. The 3D bioprinting market would display major progress in the Asia-Pacific and LAMEA zones, owing to the improved number of start-up firms and growing awareness. The external wearable devices use market is projected to increase the 3D bioprinting healthcare market to about $2.3 billion, by 2020. Out of this, the hospital and medical centres are the major end-users of the 3D printing technique as they constitute around three-fourths of the share of the total 3D printing healthcare market. The global market for 3D bioprinted products is mainly driven by several technological breakthroughs, such as multi-material, full-colour 3D printers, powered by natural or renewable energy sources, like solar energy, that are portable and easy to use. Additionally, different public and private organisations are investing massively in R&D activities, thereby driving

market growth. Furthermore, the availability of customised, biocompatible and personalised medical devices, made possible by 3D printing technology, is providing the impetus for the large-scale growth of these products worldwide; as are an increasingly elderly population, patients with the early onset of osteoarthritis symptoms, enhancement in the amputee population, and patients with hearing loss and dental problems. The adoption of this technology in hospitals would reduce the duration of surgery, anaesthesia exposure and surgery risks by helping in pre-surgery planning, and improve a surgeon's practice before operative procedures, thereby promoting faster healing. Additionally, the 3D printed organs have provided alternative methods to animal testing, thereby reducing the period for clinical trials, associated costs, and inherent risks associated with the testing of new drugs, leading to market growth. However, the higher costs associated with advanced 3D printers, lack of structured regulatory frameworks, and reimbursement policies, along with copyright and patent issues of 3D printed products could impede market growth.

References

"3D Printing in the Design of Pharmaceutical Dosage Forms", Pharmaceutical Development and Technology 24(8):1-28 · June 2019

"Ethical Sourcing: An Analysis of the Literature and Implications for Future Research", Journal of Business Ethics 152(4) · November 2018

"Predicting the future of additive manufacturing: A Delphi study on economic and societal implications of 3D printing for 2030", Technological Forecasting and Social Change, Volume 117, April 2017

"Application areas of 3D bioprinting", Literature Review in Drug discovery today 21(8) · April 2016

"Additive manufacturing (3D printing): A review of materials, methods, applications, and challenges", Composites Part B: Engineering, Volume 143, 15 June 2018

"3D Pharming: Direct Printing of Personalized Pharmaceutical Tablets", Polymer Science USSR 2(1) · June 2016

"Novel Biomaterials Used in Medical 3D Printing Techniques", MDPI, February 2018

"A flexible-dose dispenser for immediate and extended-release 3D printed 5 tablets", European Journal of Pharmaceutics and Biopharmaceutics 2015

"3D Bioprinting Technology: Scientific Aspects and Ethical Issues", Science and Engineering Ethics 24(12) · June 2017

"3-dimensional bioprinting for tissue engineering applications", Biomaterials Research volume 20 (2016)
"Cardiothoracic Applications of 3-dimensional Printing", Journal of thoracic imaging 31(5) · May 2016
"Hydrogels for Liver Tissue Engineering", MDPI July 2019
aabme.asme.org
www.sc.edu
medcraveonline.com
sketchucation.com
3dmedicalconference.com
www.openpr.com
www.emjreviews.com
www.embodi3d.com
www.aranca.com
www.staph-infection-resources.com
cloudtweaks.com

CHAPTER THREE

3D BIOPRINTED HUMAN SKIN

S. ARUN KARTHICK, S. BAGYARAJ, B. DEVI

Abstract

Burns and diseases, like chronic ulcers, infections, cancer and other genetic and somatic diseases cause damage to the human skin. Approximately 265,000 out of the 11 million people with burn injuries die each year according to WHO estimates. Thus, effective medical attention and treatments are required to prevent a further rise in numbers, and to avoid future complications that may otherwise arise. To aid and restore the damaged skin, autologous grafts, acquired from the same patient, are generally used to avoid immune rejection. However, the availability of autografts for wound coverage is inadequate. To overcome this, a number of techniques have been explored to replace the usage of autografts. One such technique, cell-cultured epithelial autografts, showed promising results, but this technique is limited by the fragility, mechanical strength, and the difficulty of handling these autografts. In response to these limitations, engineers have developed and tested new approaches that have led to the growth and expansion of new, sophisticated, lab-grown artificial skin, with dermal and epidermal layers that interact after implantation, during *in vitro* maturation. In an autologous graft, fibrinogen has been utilised widely to construct human skin due to its availability, tissue tolerance, and low price. The limitations of this process, including the need for trained professionals, the high production cost, and the time required for production, have all shown that there is an increasing demand to develop a new technique that offers automation, thereby reducing production time, and which will also result in lesser production costs. To address all these needs, 3D bioprinting has emerged as a flexible tool by 3D bioprinting print cells, soluble factors, and biomaterials as tissues or organs, which are either autologous or allogeneic, with high precision. For bioprinting, a variety of biomaterials have been widely used by tissue engineers, and some examples

include hydrogels, polymers, and ceramics. This chapter will give a comprehensive review of the 3D bioprinting of human skin, skin printing methods, and the challenges, as well as of future perspectives in skin bioprinting.

Introduction: Skin importance

Skin acts as the first line of defence, an essential part of the immune system covering the whole-body surface, and it is the largest organ of the human body. Its other functions include body temperature regulation, control of evaporation, vitamin D synthesis, and maintenance of fluid balance. Skin represents 15% of the total adult body weight and is composed of three layers: the epidermis, the dermis, and the hypodermis (Vijayavenkataraman, Lu, and Fuh 2016, 032001; George 1997, 11). The outer layer of the skin is the epidermis, which contains mainly keratinocyte cells with melanocytes, Merkel cells, and Langerhans cells. The thickness of the epidermis varies from 0.05 to 1.5 mm in different areas of the human body. The epidermis is further divided into different sub-layers. From the outermost to the innermost they are: the stratum corneum, stratum lucidum, stratum granulosum, stratum spinosum, and the stratum basale (Gilaberte, Prieto-Torres, Pastushenko, and Juarranz 2016, 1–14).

Stratum corneum is a layer of dead cells and the basic component of the skin barrier, the outermost layer (Guerrero, Conti, Zapatero-Solana, Elisabeth, Fernando, Nechaevsky, and Marcela 2016, 107–120). The innermost sub-layer, stratum basale, is usually one cell thick, while the stratum spinosum is the thickest epidermal sub-layer of five to six cells (Gilaberte, Prieto-Torres, Pastushenko, and Juarranz 2016, 1–14).

The dermis is located under the epidermis, with a thickness of approximately 3mm. The dermis mostly consists of extracellular matrix (ECM) and fibroblast. Type 1 collagen is the main component of the ECM, while elastin and other types of collagen are also found in it. Together with the ECM, proteoglycans, polysaccharides and glycosaminoglycan are also incorporated in the dermis. The epidermis lacks nerves and is avascular in nature, whereas the dermis contains blood vessels, nervous tissue, hair follicles, sebaceous sweat glands, and the lymphatic system. The dermis acts as a support and aids in nutrient supply for the epidermis, while the epidermis and dermis are separated by a basement membrane which controls the exchange of molecules between them. Underneath the dermis is the hypodermis. The main function of the hypodermis is the storage of fat, as well as participating in adaptive immunity (Böttcher-Haberzeth, Thomas

and Ernst 2010, 450–460). The hypodermis consists of fibroblasts, macrophages, adipose cells, nerves, blood and the lymphatic system.

Need for artificial skin

Skin damage or wounds have commonly resulted from trauma, skin diseases, burn, or removal of the skin during surgery (Coyer, Anne, Anna, Rae Cole, et al. 2015, 199–201). Under such circumstances, the skin requires immediate therapeutic intervention to regain the structure and the normal function of the skin, and allow the usual mobility of the patient. If the wound at the superficial part is not treated immediately, it can lead to bacterial invasion and related complications (Horiuchi, Hiroyuki, Kouji, Satoru, et al. 2010, 501–503). Similarly, even minor deformities bring psychological distress to the affected individuals, especially to children. However, extensive skin burns and wide skin wounds are difficult to repair. The gold standard still used in the clinic is the autologous skin graft. Yet, the shortage of donor skin is a serious issue (Zöller, Eva, Manuel, Matthias, et al. 2014, 190–198).

Other types of skin grafts, such as allografts and xenografts, are associated with the risks of infection, immune reactions, and cultural issues (Nunery 2001, 389–394). Furthermore, polymer-based wound dressing materials, in combination with many substances, have been developed, but they are not living skin (Abrigo, Sally and Peter 2014, 772–792.). A possible solution to overcoming this problem is to fabricate a skin graft using biomaterials with incorporated living cells. Incidentally, tissue engineering provides some solutions with great promise as it uses biomaterials, living cells, and biological and chemical signals to prepare functional skin graft (Metcalfe and Mark 2007, 413–437). However, the tissue-engineering skin grafts available in the market have inherent problems of non-homogeneous distribution of cells, failure to integrate and vascularisation upon implantation with chances of rejection of the new formed skin (Verseijden, Sandra, Eric, Johan, et al. 2010, 1007–1020).

Among the number of available skin grafts on the market, most are made from collagen-based material with fibroblasts and keratinocytes (Shevchenko, Stuart, and S. Elizabeth 2010, 229–258). The recently developed injectable cell-laden gel, which employs cell-friendly processing techniques, is a highly promising approach to preparing skin grafts (Gao, Qingzhen, Xin, Guorui, et al. 2016, 746–756). Electrospinning may affect cell growth due to the applied high electric field. Cell-friendly spinnable polymers and solvents suitable for cells are the limiting factors (Yeo and GeunHyung

2014, 314–324; Augustine, Nandakumar, and Sabu 2016, 518–529). 3D printing was recently taken up as an important technique in the field of tissue engineering, with cell-laden constructs that have good control over cells and biomaterial organisation to replace the concept of scaffold-based tissue engineering. Here we summarise the types of 3D bioprinting, and review the challenges involved in 3D bioprinted skin grafts. But we still believe that 3D bioprinted skin grafts have the most potential to heal skin burns and wounds (Kang, Sang, In, Carlos et al. 2016, 312–319).

Skin printing methodologies

Conventional fabrication methods used for manufacturing skin scaffolds include electrospinning, fibre deposition, freeze-drying, gas foaming, and salt leaching. All these techniques lack precise control of internal skin structural features and topology. Therefore, techniques are needed for multifunctional scaffolds for the accurate fabrication of skin grafts (Zong, Harold, Chiung-Yin, Lihong et al. 2005, 5330–5338; Moroni, J. R. and C. A. 2006, 974–985). To overcome this limitation, additive manufacturing (AM) technology is progressively being recognised as a potential solution for constructing complex interfacial tissue engineering scaffolds. Complex 3D bioprinted structures can be formed by AM by automatic deposition of biomaterials and living cells on a substrate, using computer-aided design/computer-aided manufacturing (CAD/CAM) technology (Derby 2012, 921–926). In contrast to conventional methods, AM works around the principle of layer-by-layer material addition, thereby creating an object. Among various AM techniques, 3D bioprinting is an important method that focuses on printing living cells with biomaterials. The 3D bioprinting technique has control of the shape, size, porosity, and interconnectivity of the engineered printing scaffold (Figure 1). In the following topics, we will elaborate on the frequently utilised 3D bioprinting methods for preparing skin grafts.

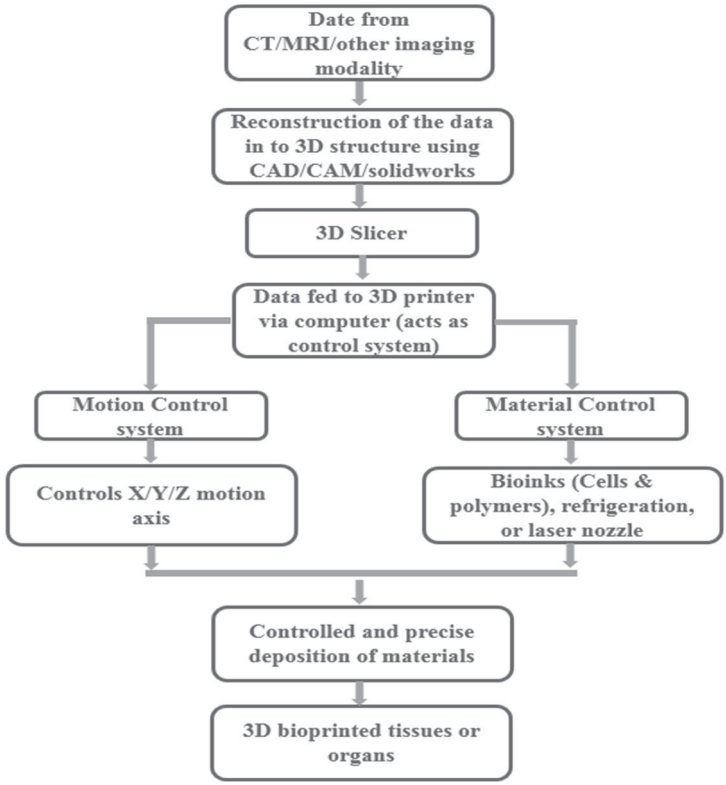

Figure 1. Technical Steps involved in 3D bioprinting

1. Inkjet based bioprinting

This bioprinting method is based on the conventional inkjet printing process with desktop inkjet printers. In this process, under computer control, precise droplets of "bio-ink" are deposited on the hydrogel substrate or the culture dish. The droplet actuation mechanism is further classified into the piezoelectric and thermal actuator method (Boland, Tao, Brook and Xiaofeng 2006, 910–917). In the thermal method, bio-ink droplets are generated by heating, which makes the ink drop out of the nozzle onto the substrate by inflated bubble force (Figure 2a). To generate the required pulse pressure, the nozzle temperature can reach hundreds of degrees in a few microseconds; this technology is broadly used as it is economically viable. But the

droplets are unequal in size and are mixed in an unordered manner when being prepared using the thermal actuator method (Cui, Delphine, Zaverio and Thomas 2010, 963–969; Murphy and Anthony 2014, 773–785; Cui, Thomas, Darryl and Martin 2012, 149–155). Frequent nozzle blockage affects the smooth printing when using the thermal method, which also affects the shear and thermal stress, along with the protein and cell viability in bio-inks. Using piezoelectric technology, the transient pressure is used to prepare bio-ink drops (Figure. 2b). In contrast to the thermal actuator method, the piezoelectric actuator method does not use heat and does not cause nozzle clogging, allowing droplets to remain directional with regular and equal size (Nakamura, Akiko, Fumio, Akihiko et al. 2005, 1658–1666; Saunders, Julie and Brian 2008, 193–203). On the other hand, the piezoelectric actuator method can cause damage to the cell membrane, and even cause cell lysis if used too frequently.

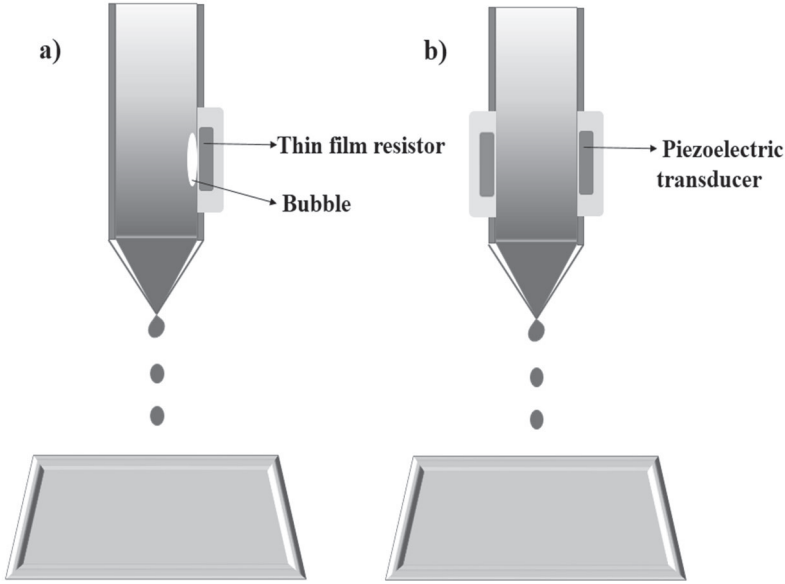

Figure 2. a) Thermal inkjet-based bioprinting and b) Piezoelectric transducer-based bioprinting.

2. Pressure assisted bioprinting (PAB)

PAB is based on the extrusion process to create and construct the desired 3D patterns. The biomaterials used for pressure-assisted bioprinting are usually solutions, pastes, or dispersions that are extruded via the nozzle, using the coordinated motion of pneumatic pressure or screw-based pressure in the form of a continuous filament onto a stationary substrate (Chia and Benjamin 2015, 4). The final 3D pattern is constructed by the layer-by-layer process. (Figure 3). Direct incorporation of cells, homogenous distribution of cells, and room temperature processing are the advantages of the PAB method.

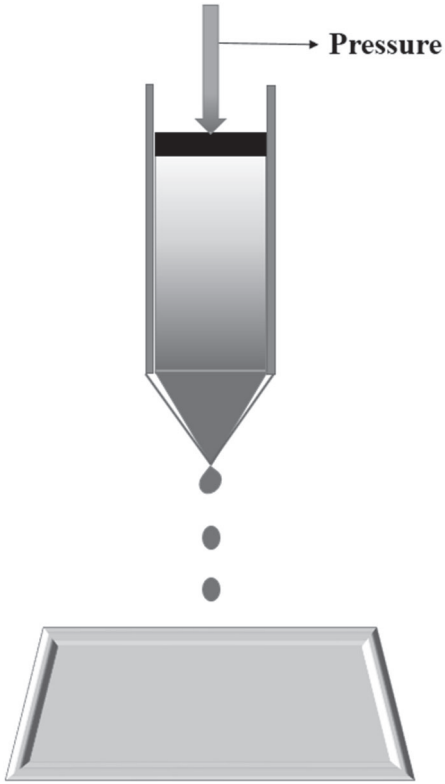

Figure 3. Pressure assisted bioprinting

3. Laser-assisted bioprinting (LAB)

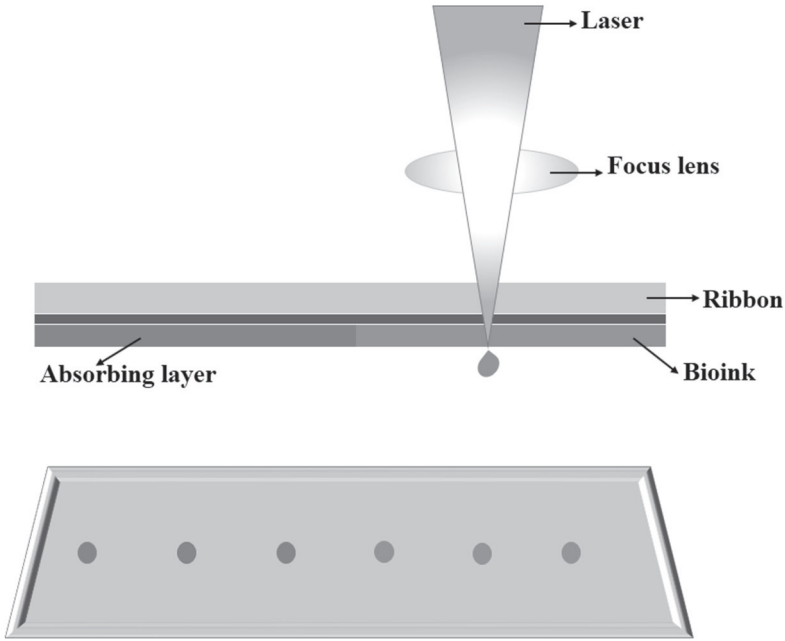

Figure 4. Laser-assisted bioprinting.

In LAB, the laser energy source is used to deposit biomaterials onto a substrate. This method typically consists of three parts: a pulsed laser source; a coated ribbon with biological materials that are deposited on the metal film; and a substrate (Jana and Amir 2015, 1503–1521). The laser's source is irradiated towards the ribbon, causing the biological materials to evaporate and reach the substrate in droplet form. The substrate contains a cell culture or biopolymer medium for cellular adhesion and sustained growth (Figure 4). Nanosecond lasers in the wavelength range of UV or near UV are mainly used as an energy source in the LAB to print cells, proteins, hydrogels, and ceramic materials (Catros, Jean-Christophe, Bertrand, Benjamin et al. 2011, 025001; Trombetta, Jason, Edward, Stephen, et al. 2017, 23–44). The resolution of LAB varies from pico to micrometer, and the same is affected by factors like the rheological property; the thickness of the biological materials;

energy of the laser source per pulse; wettability nature of the substrate; and the printing speed and 3D construction of the structure (Guillemot, Agnès, Sylvain and Bertrand 2010, 507–515; Guillemot, A., S., B., et al. 2010, 2494–2500). LAB offers unique advantages over other printing techniques, such as non-contact process, higher activity of the printing cells with higher resolution, and precise delivery of the bio-ink droplets (Patrascioiu, J. M., A., J. L., et al. 2014, 55–63; Ali, Emeline, Alexandre, Aurelien, et al. 2014, 045001).

4. Stereolithography (STL)

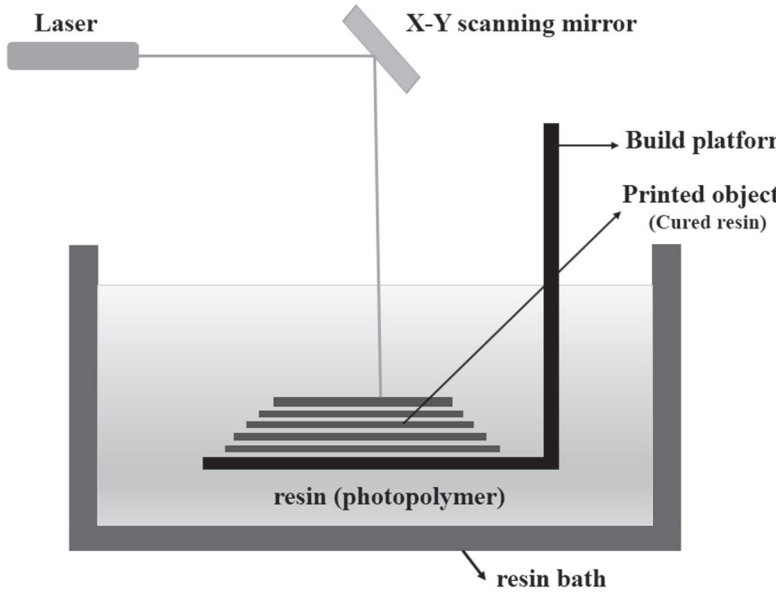

Figure 5. Stereolithographic bioprinting

The STL method was developed in the late 1980s. It utilises nozzle-free technology and solid freeform (Melchels, PW, Jan and Dirk 2010, 6121–6130). Upon illumination, the liquid photosensitive polymer gets solidified. A digital micromirror array is used to control and illuminate light on photosensitive polymer (Figure 5). STL is mainly used to prepare structures from epoxies and curable acrylics. Nowadays, the use of multiple resins and the number of photo cross-

linker usage has increased the preparation of a structure (Chia and Benjamin 2015, 4). STL offers a greater number of process materials and the highest fabrication accuracy compared to other solid freeform methods. Additionally, layer-by-layer printing of light-sensitive hydrogels can be prepared by STL and the printing time depends on the thickness of the 3D structure (Wang, Rafa, Benjamin, Roya, et al. 2015, 045009). Though STL offers more advantages, there are many restrictions, such as residual toxic effects of curing agent, need of proper biodegradable and biocompatible polymers, and the inability to remove the support structure completely from the constructed structure.

Fundamentals in 3D bioprinting

To fabricate tissues or organs, the tissue-engineered graft should consist of biomaterials, cells, and the environmental structure which mimics the nature of the human body. 3D bioprinting acts as an accurate and highly effective method to prepare artificial tissues or organs.

1. **Biomaterials**

   Generally, the biomaterial is classified into metals, ceramics, polymers, composite, and nanocomposite materials. Based on the physical property of the biomaterial, the optimum bioprinting technique will be chosen. For instance, low viscosity biomaterials are more often used in the 3D bioprinting process because of the low-pressure environment where the cells can grow well (Khatiwala, Richard, Benjamin, Scott, et al. 2012, 1–19). Properties, like pore size and interconnectivity inside the material, also influence the growth of incorporated cells (Tasoglu and Utkan 2013, 10–19).

2. **Biocompatibility**

   The first parameter to be taken into consideration when fabricating grafts is the biocompatibility of the material used; this considerably limits the number of suitable materials used. Thus, the materials used in 3D bioprinting should not cause immunogenic reaction and inflammation but should support cell growth, attachment, proliferation, and migration of cells for the host body (Wüst, Marie, Ralph, and Sandra 2014, 630–640).

3. **Pore size and porosity**

   Pore size, shape, and pore volume directly relates to the cell adhesion on and into the grafts. Development of extracellular matrix (ECM), cell organization, collagen assembly, and mineralisation depend on the pore size of the scaffold (Matsiko, John and Fergal 2015, 486–497). Cells in growth depend on the porosity and interconnectivity. Also, porosity and interconnectivity help in oxygen and nutrient transport and the removal of waste by cellular metabolism.

4. **Mechanical property**

   In addition to biocompatibility, pore size and porosity, the appropriate matching of the required mechanical property of the native human condition is very important. For example, when a high elastic moduli artificial bone is implanted in situ, it suffers from stress shielding and obstructs new bone-cell formation. 3D bioprinting technology offers grafts that exactly mimic the mechanical property of the native human body (Nadeem, Carol-Anne, Matthew, RM Dominic, et al. 2015, 015005).

5. **Bio-inks and bioprinting cells**

   Among the wide range of bio-inks are polymers, ceramics, and composites. Hydrogel bio-ink has received much attention due to the stringent bioprinting condition and the large range of the hydrogel-based bio-inks formulation being made and utilised (Jose, Maria, Thomas, Fiorenzo, et al. 2016, 1662–1678).

Cell printing and the behaviour of the cell depends on the surface morphology, stiffness, and functional group of the biomaterial used for printing. Usually, an environment that mimics a tissue-like property is made using hydrogels with encapsulated cells as bioprinting ink. The function of the hydrogel is to protect the cells from the shear force being generated during the printing process, as well as to maintain the bio function of the cells (Bertassoni, Juliana, Vijayan, Ana, et al. 2014, 024105).

Challenges in skin bioprinting

Printed skin grafts have shown promising potential with recent advancements, but several barriers remain that limit the clinical translation of the engineered 3D printed skin grafts. The most important task is to develop a large constructed skin with a highly-developed vasculature

(Hendrickx, Jan and Aernout 2011, 13–24). The anastomosis of blood vessels is the critical property needed for the 3D constructed skin grafts after implantation. Preparing a multi-layered 3D complex structure still remains a big challenge (Groeber, Monika, Martina, Svenja, et al. 2011, 352–366). For example, the 3D-constructed skin grafts should maintain the thickness and texture of hypodermis, dermis, and epidermis to match with the native skin.

3D printed skin grafts must also contain functional structures like sweat glands, hair follicles, and sebaceous glands. It is important to engineer fully functional 3D bioprinted skin grafts in the future which exactly mimic the native anatomy and physiology of the skin. For the successful regeneration of the skin, the controlled release of active molecules is also an important parameter. With a combination of solutions, recent advancements, and by the use of slow or partially degrading biomaterials, vascularised flaps, nanoparticle incorporation of angiogenic agents can be utilised to overcome the present challenges in 3D skin printing (Augustine, Pan, Alejandro, Nandakumar, et al. 2017, 3358–3376). To expedite 3D bioprinted skin grafts to clinical trials, the regulatory bodies must define the protocols and the manufacturing process. With a group of engineers from biomedical, mechanical, materials science, bioengineering, medical practitioners, and regulatory bodies, we believe that 3D bioprinted skin grafts will solve many, if not all, of the existing barriers.

Future perspectives of skin bioprinting

3D bioprinted skin grafts offer a great advantage to construct complex skin structures that are difficult to heal using the normal clinical process. The ultimate goal of skin bioprinting is to construct a fully functional skin graft with all vascular channels and with functional structures (sweat glands, hair follicles, and sebaceous glands) included in it, subsequent transplantation, and anastomosed with the circulation of native blood. A hand-held device (Biopen) for intraoperative bioprinting was developed to simplify clinical translation of 3D bioprinted grafts (D O'Connell, Claudia, Fletcher, Cheryl, et al. 2016, 015019). Such a device offers deposition of cells precisely in the wounded area. 4D printing, which depends on stimuli response, is an additional revolutionary advancement that will result in 4D bioprinting (Gao, Arndt, Tomo, Jiang et al. 2014, 1304–1311; An, Chee, and Vladimir 2016). Like a lab on a chip, integration of biosensors into the skin results in skin on a chip, and such a system can be used to stimulate inflammation, and to test drug-based treatments to study the pathophysiology of skin

defects and drug testing for skin problems (Wufuer, GeonHui, Woojune, Byoungjun, et al. 2016, 37471). 3D bioprinting using multipotent stem cells and induced pluripotent stem cells have countless opportunities and new possibilities for skin printing. Scientists may also attempt to develop skin grafts with certain growth factors, and anti-inflammatory drugs for diabetic wound healing. Finally, this field requires the joint effort of researchers and scientists from various fields and continuous funding support to see the successful translation of the printed skin grafts to the clinic.

Conclusion

3D printing, an additive manufacturing process, is the basis of bioprinting that allows for the printing of biomaterials with incorporated cells to function as tissues and organs. Bioprinting steps involve the design of the 3D architecture of the tissue or organ to be printed, the selection of suitable biomaterials with incorporated cells and lastly, the printing of the tissue or organ construct. Bioprinting of skin utilises biomaterials, cells, and other ingredients to make a multi-layered functional structure for preparing skin grafts for *in vitro* and *in vivo* application. For making skin grafts matrix, polymers made of natural and synthetic material are widely used. The sources of these cells include fibroblasts, keratinocytes, multipotent stem cells, and induced pluripotent stem cells. Still, such 3D bioprinted skin has limitations, like poor vascularisation, absence of hair follicles, other additional functions, and aesthetics. To overcome these limitations, clues, such as electrical/mechanical stimuli, growth factors, or nanoparticles are actually used to mimic the nature of native skin structure and organisation. The clinical translation of the 3D bioprinted skin graft has been delayed by regulatory bodies due to the structural complexity of the printed skin and the need to have extended *in vitro* and *in vivo* clinical studies. By the use of innumerable technologies and advances, like conductive polymers, bio-nano composites, 4D printing, and microfluidics, one can accomplish fully functional bioprinted skin grafts. Skin grafts prepared by this approach will be structurally, functionally, and aesthetically similar to that of native skin. Additionally, there is a need for more data on technical standardisation, random trails, and longstanding follow up to know the effectiveness and the risk of oncology on the developed 3D bioprinted skin grafts.

References

Abrigo, Martina, Sally L. McArthur, and Peter Kingshott. 2014. "Electrospun Nanofibers as Dressings for Chronic Wound Care: Advances, Challenges, and Future Prospects". *Macromolecular bioscience,* No. 14(6): 772–792.

Ali, Muhammad, Emeline Pages, Alexandre Ducom, Aurelien Fontaine, and Fabien Guillemot. 2014. "Controlling Laser-Induced Jet Formation for Bioprinting Mesenchymal Stem Cells with High Viability and High Resolution". *Biofabrication*, No. 6(4): 045001.

An Jia, Chee Kai Chua, and Vladimir Mironov. 2016. "A Perspective on 4D Bioprinting". *International Journal of Bioprinting*, No. 2(1).

Augustine, Robin, Nandakumar, Kalarikkal, and Sabu, Thomas. 2016. "Clogging-Free Electrospinning of Polycaprolactone Using Acetic Acid/Acetone Mixture". *Polymer-Plastics Technology and Engineering*, No. 55(5): 518–529.

Augustine, Robin, Pan Dan, Alejandro Sosnik, Nandakumar Kalarikkal, Nguyen Tran, Brice Vincent, Sabu Thomas, Patrick Menu, and Didier Rouxel. 2017. "Electrospun Poly (Vinylidene Fluoride-Trifluoroethylene)/Zinc Oxide Nanocomposite Tissue Engineering Scaffolds with Enhanced Cell Adhesion and Blood Vessel Formation". *Nano Research*, No. 10(10): 3358–3376.

Bertassoni, Luiz E, Juliana C. Cardoso, Vijayan Manoharan, Ana L. Cristino, Nupura S. Bhise, Wesleyan A. Araujo, Pinar Zorlutuna, NE Vrana, AM Ghaemmaghami, MR Dokmeci, and A. Khademhosseini. 2014. "Direct-Write Bioprinting of Cell-Laden Methacrylated Gelatin Hydrogels". *Biofabrication*, No. 6(2): 024105.

Boland, Thomas, Tao Xu, Brook Damon, and Xiaofeng Cui. 2006. "Application of Inkjet Printing to Tissue Engineering". *Biotechnology Journal: Healthcare Nutrition Technology*, No. 1(9): 910–917.

Böttcher-Haberzeth, Sophie, Thomas Biedermann, and Ernst Reichmann. 2010. "Tissue Engineering of Skin". *Burns*, No. 36(4): 450–460.

Catros, Sylvain, Jean-Christophe Fricain, Bertrand Guillotin, Benjamin Pippenger, Reine Bareille, Murielle Remy, Eric Lebraud, Bernard Desbat, Joëlle Amédée, and Fabien Guillemot. 2011. "Laser-Assisted Bioprinting for Creating on-Demand Patterns of Human Osteoprogenitor Cells and Nano-Hydroxyapatite". *Biofabrication*, No. 3(2): 025001.

Chia, Helena N, and Benjamin M. Wu. 2015. "Recent Advances in 3D Printing of Biomaterials". *Journal of biological engineering*, No. 9(1): 4.

Coyer, Fiona, Anne Gardner, Anna Doubrovsky, Rae Cole, Frances Mary Ryan, Craig Allen, and Greg McNamara. 2015. "Reducing Pressure Injuries in Critically Ill Patients by Using a Patient Skin Integrity Care Bundle (InSPiRE)". *American Journal of Critical Care,* No. 24(3): 199–209.

Cui, Xiaofeng, Delphine Dean, Zaverio M. Ruggeri, and Thomas Boland. 2010. "Cell Damage Evaluation of Thermal Inkjet-Printed Chinese Hamster Ovary Cells". *Biotechnology and bioengineering,* No. 106(6): 963–969.

Cui, Xiaofeng, Thomas Boland, Darryl DD'Lima, and Martin K Lotz. 2012. "Thermal Inkjet Printing in Tissue Engineering and Regenerative Medicine". *Recent patents on drug delivery & formulation*, No. 6(2): 149–155.

Derby, Brian. 2012. "Printing and Prototyping of Tissues and Scaffolds". *Science*, No. 338(6109): 921–926.

D O'Connell, Cathal, Claudia Di Bella, Fletcher Thompson, Cheryl Augustine, Stephen Beirne, Rhys Cornock, Christopher J. Richards, J Chung, S Gambhir, Z Yue, and J Bourke. 2016. "Development of the Biopen: A Handheld Device for Surgical Printing of Adipose Stem Cells at a Chondral Wound Site". *Biofabrication*, No. 8(1): 015019.

Gao, Bin, Qingzhen Yang, Xin Zhao, Guorui Jin, Yufei Ma, and Feng Xu. 2016. "4D Bioprinting for Biomedical Applications". *Trends in biotechnology*, No. 34(9): 746–756.

Gao, Guifang, Arndt F. Schilling, Tomo Yonezawa, Jiang Wang, Guohao Dai, and Xiaofeng Cui. 2014. "Bioactive Nanoparticles Stimulate Bone Tissue Formation In Bioprinted Three-Dimensional Scaffold and Human Mesenchymal Stem Cells". *Biotechnology journal*, No. 9(10): 1304–1311

George, F. Murphy. 1997. "Histology of the Skin". In *Lever's Histopathology of the Skin*, edited by R. Elenitsas, C. Jaworsky and B. Johnson, 5–50. Philadelphia: Lippincott-Raven.

Gilaberte, Y., L. Prieto-Torres, I. Pastushenko, and A. Juarranz. 2016. "Anatomy and Function of the Skin". *Nanoscience in Dermatology*, 1–14.

Groeber, Florian, Monika Holeiter, Martina Hampel, Svenja Hinderer, and Katja Schenke-Layland. 2011. "Skin Tissue Engineering—In Vivo and In Vitro Applications". *Advanced drug delivery reviews*, No. 63(4–5): 352–366.

Guerrero-Aspizua, Sara, Conti, Claudio, Zapatero-Solana, Elisabeth, Larcher, Fernando, Del Rio-Nechaevsky, Marcela. 2016. "Current Applications for Bioengineered Skin". In *Translating Regenerative*

Medicine to the Clinic, edited by Jeffrey Laurence, Pedro Baptista, and Anthony Atala, 107–120. Cambridge, MA, USA: Academic Press.

Guillemot, Fabien, Agnès Souquet, Sylvain Catros, and Bertrand Guillotin. 2010. "Laser-Assisted Cell Printing: Principle, Physical Parameters Versus Cell Fate and Perspectives in Tissue Engineering". *Nanomedicine*, No. 5(3): 507–515.

Guillemot, Fabien, A. Souquet, S. Catros, B. Guillotin, J. Lopez, M. Faucon, B. Pippenger, R. Bareille, M. Rémy, S. Bellance, and P. Chabassier. 2010. "High-Throughput Laser Printing of Cells and Biomaterials for Tissue Engineering". *Acta biomaterialia*, No. 6(7): 2494–2500.

Hendrickx, Benoit, Jan J. Vranckx, and Aernout Luttun. 2011. "Cell-Based Vascularization Strategies for Skin Tissue Engineering". *Tissue Engineering Part B: Reviews*, No. 17(1): 13–24.

Horiuchi Tetsuya, Hiroyuki Tanishima, Kouji Tamagawa, Satoru Sakaguchi, Yoshiharu Shono, Hideaki Tsubakihara, Katsuyoshi Tabuse, and Yukiyasu Kinoshita. 2010. "A Wound Protector Shields Incision Sites from Bacterial Invasion". *Surgical infections*, No. 11(6): 501–503.

Jana, Soumen, and Amir Lerman. 2015. "Bioprinting a Cardiac Valve". *Biotechnology advances*, No. 33(8): 1503–1521.

Jose, Rod R, Maria J. Rodriguez, Thomas A. Dixon, Fiorenzo Omenetto, and David L. Kaplan. 2016. "Evolution of Bio-inks and Additive Manufacturing Technologies for 3D Bioprinting". *ACS biomaterials science & engineering*, No. 2(10): 1662–1678.

Kang, Hyun-Wook, Sang Jin Lee, In Kap Ko, Carlos Kengla, James J. Yoo, and Anthony Atala. 2016. "A 3D Bioprinting System to Produce Human-Scale Tissue Constructs with Structural Integrity". *Nature biotechnology*, No. 34(3): 312–319.

Khatiwala, Chirag, Richard Law, Benjamin Shepherd, Scott Dorfman, and Marie Csete. 2012. "3D Cell Bioprinting for Regenerative Medicine Research and Therapies". *Gene Therapy and Regulation*, No. 7(1): 1–19.

Matsiko, Amos, John P. Gleeson, and Fergal J. O'Brien. 2015. "Scaffold Mean Pore Size Influences Mesenchymal Stem Cell Chondrogenic Differentiation and Matrix Deposition". *Tissue Engineering Part A*, No. 21(3–4): 486–497.

Melchels, Ferry PW, Jan Feijen, and Dirk W. Grijpma. 2010. "A Review on Stereolithography and Its Applications in Biomedical Engineering". *Biomaterials*, No. 31(24): 6121–6130.

Metcalfe, Anthony D, and Mark WJ Ferguson. 2007. "Tissue Engineering of Replacement Skin: the Crossroads of Biomaterials, Wound Healing,

Embryonic Development, Stem Cells, and Regeneration". *Journal of the Royal Society Interface*, No. 4(14): 413–437.

Moroni, Lorenzo, J. R. De Wijn, and C. A. Van Blitterswijk. 2006. "3D Fiber-Deposited Scaffolds for Tissue Engineering: Influence of Pores Geometry and Architecture on Dynamic Mechanical Properties". *Biomaterials*, No. 27(7): 974–985.

Murphy, Sean V, and Anthony Atala. 2014. "3D Bioprinting of Tissues and Organs". *Nature biotechnology*, No. 32(8): 773–785.

Nadeem, Danish, Carol-Anne Smith, Matthew J. Dalby, RM Dominic Meek, Lin Sien, Li Gang, and So Bo. 2015. "Three-Dimensional CaP/Gelatin Lattice Scaffolds With Integrated Osteoinductive Surface Topographies for Bone Tissue Engineering". *Biofabrication*, No. 7(1): 015005.

Nakamura, Makoto, Akiko Kobayashi, Fumio Takagi, Akihiko Watanabe, Yuko Hiruma, Katsuhiro Ohuchi, Yasuhiko Iwasaki, Mikio Horie, Ikuo Morita, and Setsuo Takatani. 2005 "Biocompatible Inkjet Printing Technique for Designed Seeding of Individual Living Cells". *Tissue engineering*, No. 11(11–12): 1658–1666.

Nunery, William R. 2001. "Risk of Prion Transmission with the Use of Xenografts and Allografts in Surgery". *Ophthalmic plastic and reconstructive surgery*, No. 17(6): 389–394.

Patrascioiu, A, J. M. Fernández-Pradas, A. Palla-Papavlu, J. L. Morenza, and P. Serra. 2014. "Laser-Generated Liquid Microjets: Correlation between Bubble Dynamics and Liquid Ejection". *Microfluidics and nanofluidics*, No. 16(1–2): 55–63.

Saunders, Rachel E, Julie E. Gough, and Brian Derby. 2008. "Delivery of Human Fibroblast Cells by Piezoelectric Drop-on-Demand Inkjet Printing". *Biomaterials*, No. 29(2): 193–203.

Shevchenko, Rostislav V, Stuart L. James, and S. Elizabeth James. 2010. "A Review of Tissue-Engineered Skin Bioconstructs Available for Skin Reconstruction". *Journal of the Royal Society Interface*, No. 7(43): 229–258.

Tasoglu, Savas, and Utkan Demirci. 2013. "Bioprinting for Stem Cell Research". *Trends in biotechnology*, No. 31(1): 10–19.

Trombetta, Ryan, Jason A. Inzana, Edward M. Schwarz, Stephen L. Kates, and Hani A. Awad. 2017. "3D printing of Calcium Phosphate Ceramics for Bone Tissue Engineering and Drug Delivery". *Annals of biomedical engineering*, No. 45(1): 23–44.

Verseijden, Femke, Sandra J. Posthumus-Van Sluijs, Eric Farrell, Johan W. Van Neck, Steven ER Hovius, Stefan OP Hofer, and Gerjo JVM Van Osch. 2010. "Prevascular Structures Promote Vascularization in

Engineered Human Adipose Tissue Constructs Upon Implantation". *Cell Transplantation*, No. 19(8): 1007–1020.

Vijayavenkataraman, Sanjairaj, W. F. Lu, and J. Y. H. Fuh. 2016. "3D Bioprinting of Skin: A State-of-the-Art Review on Modelling, Materials, and Processes". *Biofabrication*, No. 8(3): 032001.

Wang, Zongjie, Raafa Abdulla, Benjamin Parker, Roya Samanipour, Sanjoy Ghosh, and Keekyoung Kim. 2015. "A Simple and High-Resolution Stereolithography-Based 3D Bioprinting System using Visible Light Crosslinkable Bioinks". *Biofabrication*, No. 7(4): 045009.

Wufuer, Maierdanjiang, GeonHui Lee, Woojune Hur, Byoungjun Jeon, Byung Jun Kim, Tae Hyun Choi, and SangHoon Lee. 2016. "Skin-on-a-Chip Model Simulating Inflammation, Edema and Drug-Based Treatment". *Scientific reports*, No. 6: 37471.

Wüst, Silke, Marie E. Godla, Ralph Müller, and Sandra Hofmann. 2014. "Tunable Hydrogel Composite with Two-Step Processing in Combination with Innovative Hardware Upgrade for Cell-Based Three-Dimensional Bioprinting". *Acta biomaterialia*, No. 10(2): 630–640.

Yeo, MyungGu, and GeunHyung Kim. 2014. "Cell-Printed Hierarchical Scaffolds Consisting of Micro-Sized Polycaprolactone (PCL) and Electrospun PCL Nanofibers/Cell-Laden Alginate Struts for Tissue Regeneration". *Journal of Materials Chemistry B*, No. 2(3): 314–324.

Zöller, Nadja, Eva Valesky, Manuel Butting, Matthias Hofmann, Kippenberger S, Bereiter-Hahn J, Bernd A, and Kaufmann R. 2014. "Clinical Application of A Tissue-Cultured Skin Autograft: An Alternative for the Treatment of Non-Healing or Slowly Healing Wounds?" *Dermatology*, No. 229 (3): 190–198.

Zong, Xinhua, Harold Bien, Chiung-Yin Chung, Lihong Yin, Dufei Fang, Benjamin S. Hsiao, Benjamin Chu, and Emilia Entcheva. 2005. "Electrospun Fine-Textured Scaffolds for Heart Tissue Constructs". *Biomaterials*, No. 26(26): 5330–5338.

CHAPTER FOUR

HEART 3D PRINTING

HELEN MARY M C, DILBAG SINGH,
K. K. DEEPAK

Abstract

This chapter describes the methods used to display the electrical activity of the heart as a 3D image. The methods used to acquire a 3D image of the heart are mainly conventional vectorcardiography and Cardiogoniometry. In conventional vectorcardiography, the projected vector planes are aligned to the different body axes (Peters et al., 2003). The result of this is that the anterior and posterior walls of the heart are partially superimposed, and the observer requires good visual thinking to understand the orientation of the vector loops. Cardiogoniometry displays heart activity, and the major planes are rotated by 45 degrees. The anterior and posterior walls provide a vector loop, which lies in the diagonal sagittal plane. It helps the physician to analyse the recordings more accurately, without the influence of heart rotation.

Introduction

The electrocardiogram (ECG) is an electrical representation of the polarisation of the heart by placing electrodes on the skin's surface at specific locations on the body.[1,2] The heart is stimulated by the natural pacemaker of the heart, which applies an electric current to it, such that the heart (coronary) muscles contract and expand rhythmically. These contractions and expansions of the heart are obtained from the ECG, and it

[1] Leif Sörnmo and Pablo Laguna, "Electrocardiogram (ECG) signal processing," *Wiley Encyclopedia of biomedical engineering*, 2006,
[2] Yun-Hsuan Chen et al., "Soft, comfortable polymer dry electrodes for high-quality ECG and EEG recording," *Sensors* 14, no. 12 (2014): 23758–23780.

identifies the health of the heart. The ECG is recorded using the 12-lead system, which consists of 4 probes placed on the body's extremities (legs and hands) and 6 precordial leads on the chest (near the heart).[3] The electrodes convert the ionic current from our body to electrical signals (as potential differences). The ECG measures the regularity of heartbeats, the presence of heart abnormalities, and the effects of drugs or devices (like a pacemaker). In order to identify the status of the heart in more depth, we have to obtain the 3D image of the heart. The two main methods to acquire a 3D image of the heart, as noted above, are conventional vectorcardiography and Cardiogoniometry.

Vectorcardiography

Vectorcardiography (VCG) develops a three-dimensional (3D) image of the electrical activity of the heart by calculating the magnitude and direction of the electrical signals generated from the heart.[4,5] It displays the spatial locations of ECG waveforms as vector figures, using a cathode-ray oscillograph. Hence the name vectorcardiogram (VCG) was recommended as a suitable term for these records. The initial idea for the VCG was introduced in the 1920s.[6] The author manually graphed a series of vectors to represent the electric forces of depolarisation and repolarisation according to the recordings of the electric cardiac activity. The first technique for recording the VCG was explained in detail by Wilson et al., in 1937.[7] An improved system for vectorcardiography was developed by Frank's system,[8] and it made the VCG clinically useful.

---

[3] Anthony Dupre, Sarah Vincent, and Paul A Iaizzo, "Basic ECG theory, recordings, and interpretation," in *Handbook of cardiac anatomy, physiology, and devices* (Springer, 2005), 191–201.

[4] B Surawicz, "Comprehensive Electrocardiology: Theory and Practice in Health and disease," *Eds PW McFarlane, TDV Laurie. New York* 1 (1989): 512–63.

[5] Plonsey Malmivuo, Jaakko Malmivuo, and Robert Plonsey, *Bioelectromagnetism: principles and applications of bioelectric and biomagnetic fields* (Oxford University Press, USA, 1995).

[6] Hubert Mann, "A method of analyzing the electrocardiogram," *Archives of Internal Medicine* 25, no. 3 (1920): 283–294.

[7] FN Wilson, FD Johnston, and PS Barker, "The use of the cathode-ray oscillograph in the study of the monocardiogram," *J. din. Invest* 16 (1937): 664.

[8] Ernest Frank, "An accurate, clinically practical system for spatial vectorcardiography," *circulation* 13, no. 5 (1956): 737–749.

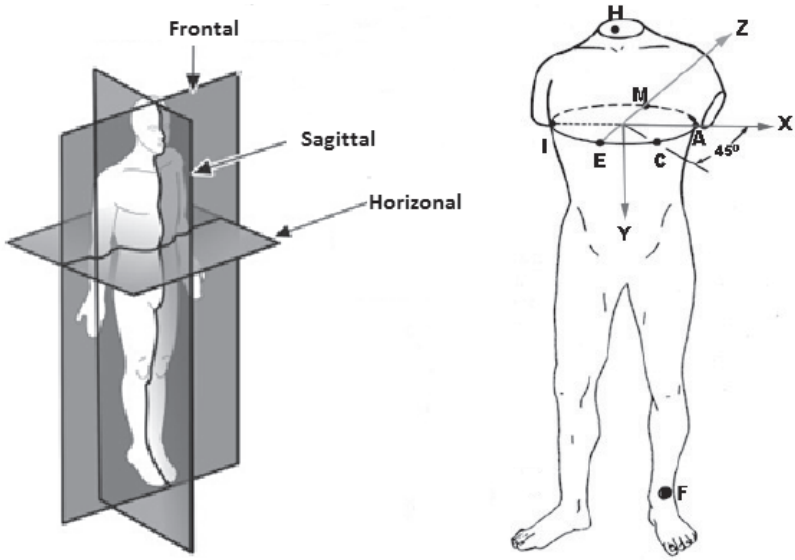

Figure 1: The orthogonal anatomical imaging planes of the human body and location on electrodes for VCG recording

VCG requires only three orthogonal leads: one in the right-to-left axis (X lead); one in the head-to-feet axis (Y lead); and one in the front-back (anteroposterior) axis (Z lead). This method generates orthogonal projections directly on frontal, horizontal and sagittal planes.[9] These three projection planes are given in Figure 1. Each orthogonal projection simultaneously records the same electric episodes on two perpendicular axes.[10,11] The frontal plane simultaneously records the episodes on the X and Y axes; the horizontal plane records simultaneous episodes on the X and Z axes; and the sagittal plane records simultaneous episodes on the Y and Z axes. Figure

[9] Paul H Langner JR et al., "Comparison of four orthogonal systems of vectorcardiography," *Circulation* 17, no. 1 (1958): 46–54.
[10] Franklin D Johnston, "The clinical value of vectorcardiography," *Circulation* 23, no. 2 (1961): 297–303.
[11] Malmivuo, Plonsey, Jaakko Malmivuo, and Robert Plonsey. Bioelectromagnetism: principles and applications of bioelectric and biomagnetic fields. Oxford University Press, USA, 1995.

2 shows a view of the VCG signal from three Cartesian planes (X-Y, Y-Z, and X-Z).

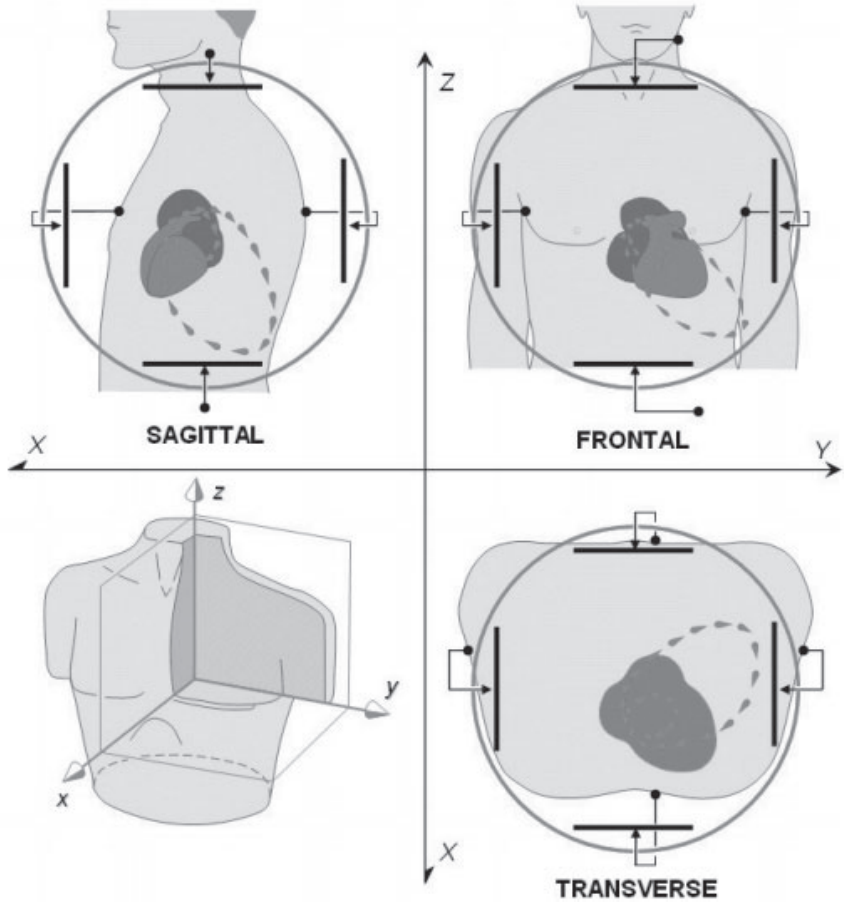

Figure 2: The basic principle of vectorcardiography from three Cartesian planes (X-Y, Y-Z, and X-Z) (Malmivuo et. al 1995)

There are several ways to mathematically derive VCGs from 12-lead ECGs and vice versa.[12,13] These derivations of VCG from a 12-lead ECG and a 12-lead ECG from VCG are only an approximation. The expression derived for X, Y, and Z from the standard leads system is given in Equation 1, 2, 3. V1 to V6 are chest leads and DI, DII are limb leads.

$$X = -(-0.172\,V1 - 0.074\,V2 + 0.122\,V3 + 0.231\,V4 + 0.239\,V5 + 0.194\,V6 + 0.156\,DI - 0.010\,DII) \quad \ldots\ldots(1)$$

$$Y = (0.057\,V1 - 0.019\,V2 - 0.106\,V3 - 0.022\,V4 + 0.041\,V5 + 0.048\,V6 - 0.227\,DI + 0.887\,DII) \quad \ldots\ldots(2)$$

$$Z = -(-0.229\,V1 - 0.310\,V2 - 0.246\,V3 - 0.063\,V4 + 0.055\,V5 + 0.108\,V6 + 0.022\,DI + 0.102\,DII) \quad \ldots\ldots(3)$$

From these scalar coordinates, the instantaneous cardiac vector can be obtained, beat after beat, along with the vectorcardiographic loops, and many others based on our area of interest.[14,15,16,17] The vectors, during one cardiac cycle, are displayed with their origin on a common baseline; their ends form a vector loop beginning at and returning to this point. Because the ECG components—the P wave, QRS complex and T wave—start from and return to the same baseline, three loops can be recorded, one for each

---

[12] George Eastman, "A new method of deriving the vectorcardiogram from routine clinical electrocardiographic leads," *Journal of the American Geriatrics Society* 8, no. 9 (1960): 708–723.

[13] Gordon E Dower, H Bastos Machado, and JA Osborne, "On deriving the electrocardiogram from vectorcardiographic leads," *Clinical Cardiology* 3, no. 2 (1980): 87–95.

[14] Drew Dawson et al., "Linear affine transformations between 3-lead (Frank XYZ leads) vectorcardiogram and 12-lead electrocardiogram signals," *Journal of Electrocardiology* 42, no. 6 (2009): 622–630.

[15] David M Schreck and Robert D Fishberg, "Derivation of the 12-lead electrocardiogram and 3-lead vectorcardiogram," *The American journal of emergency medicine* 31, no. 8 (2013): 1183–1190.

[16] Rik Vullings et al., "Bayesian approach to patient-tailored vectorcardiography," *IEEE transactions on biomedical engineering* 57, no. 3 (2009): 586–595.

[17] Thomas Schau et al., "Baseline vectorcardiography as a predictor of invasively determined acute hemodynamic response to cardiac resynchronization therapy," *Clinical Research in Cardiology* 102, no. 2 (2013): 129–138.

of these three deflections.[18] In some cases, the U wave is also present. The depolarisation of the ventricles is called systole and the relaxation of ventricles is called diastole. The schematic representation of the ECG is given in Figure 3.

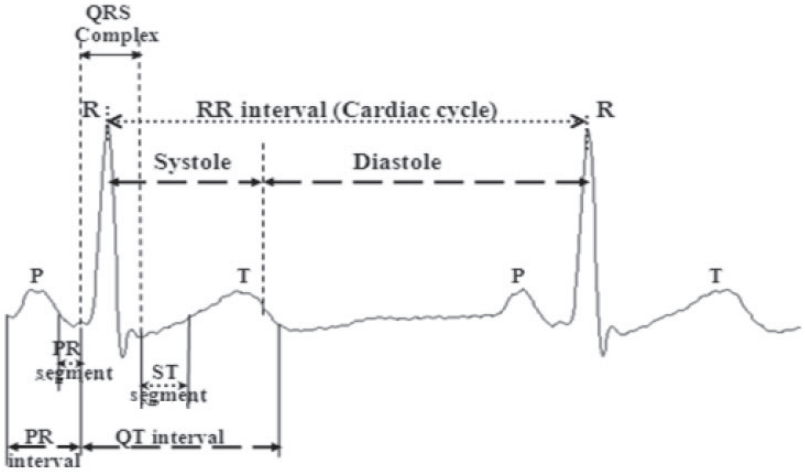

Figure 3: Components of the ECG waveform. Electrical and mechanical events

To obtain a vectorcardiogram (VCG), two leads must be recorded simultaneously. The modern VCG system employs a set of three orthogonal leads, and also includes a set of appropriate resistors to correct these leads for the varying distances of the electrodes from the heart.[19,20] To enhance the accuracy of the orthogonal lead system and the ability to measure the accurate direction of instantaneous vectors at frequent intervals, the VCG displays the rotation of the loop, which adds valuable information not available from an ECG.

Although the principle of an ECG and VCG have essentially the same content, under certain VCG displays a diagnostic pattern more distinctly than those displayed using the ECG. Thus, the VCG helps to clarify the

[18] MC Helen Mary, Dilbag Singh, and KK Deepak, "Detecting changes in cardiovascular interaction during postural stress using directed coherence," *Signal, Image, and Video Processing* 13, no. 8 (2019): 1521–1528.
[19] Johnston, Franklin D. "The clinical value of vectorcardiography." Circulation 23, no. 2 (1961): 297–303.
[20] Arthur Grishman, Leonard Scherlis, and Richard P Lasser, "Spatial vectorcardiography," *The American journal of medicine* 14, no. 2 (1953): 184–200.

diagnosis by providing more accurate information by measuring intervals and by displaying the rotations of the QRS loop and its components. These details pertain most often to the analysis of the early portion of the QRS complex.[21] The VCG has been most useful in clarifying the diagnosis of inferior, anteroseptal, and posterior myocardial infarction, and bundle branch infarction and other blocks.[22] The VCG is also more sensitive and is capable of detecting left atrial enlargement (the magnitude of the maximum posterior P vectors in the horizontal and sagittal planes), and right ventricular hypertrophy (demonstration of a clockwise QRS loop displaced anteriorly and to the right).

Notwithstanding the diagnostic dominance of the VCG compared with the ECG, the net yield of such advantages in clinical practice is not adequate to overcome the disadvantages of the more costly equipment.[23,24] A longer time is required to apply the electrodes and produce the records, and the occasional need to provide the loops made from the orthogonal leads by semidirect precordial leads. Such minor inconveniences, combined with the increasing availability of technology for the assessment of abnormality in cardiac wall motion and myocardial perfusion, have resulted in the reduced use of the VCG in clinical practice. The necessity for using the ECG for analysis of cardiac activity is another reason that has led to this trend.

The VCG is a useful teaching tool for explaining the ECG, but equally helpful is the vectorial display of the magnitude and direction of the ECG components. Plotting the vectors of the individual components of the QRS complex sequentially from the same point of origin allows us to trace a vectorcardiographic loop.[25,26] Conversely, scalar ECG components can be derived from the VCG. The healthy subject's three-dimensional plot of

---

[21] Johnston, Franklin D. "The clinical value of vectorcardiography." Circulation 23, no. 2 (1961): 297–303.
[22] Johnston, Franklin D. "The clinical value of vectorcardiography." Circulation 23, no. 2 (1961): 297–303.
[23] Langner JR, Paul H, Robert H Okada, Samuel R Moore, and Harry L Fies. "Comparison of four orthogonal systems of vectorcardiography." Circulation 17, no. 1 (1958): 46–54.
[24] Johnston, Franklin D. "The clinical value of vectorcardiography." Circulation 23, no. 2 (1961): 297–303.
[25] Hui Yang, Satish TS Bukkapatnam, and Ranga Komanduri, "Spatiotemporal representation of cardiac vectorcardiogram (VCG) signals," *Biomedical engineering online* 11, no. 1 (2012): 16.
[26] Grishman, Arthur, Leonard Scherlis, and Richard P Lasser. "Spatial vectorcardiography." The American journal of medicine 14, no. 2 (1953): 184–200.

VCG is shown in Figure 4, but for an unhealthy subject the VCG plot obtained will be different.

Cardiogoniometry

Cardiogoniometry (CGM) provides a three-dimensional display of the heart's activity which is more precise than a VCG.[27] In this method, the major planes are rotated by 45 degrees in the major axis of the heart. The heart is split into the anterior and posterior walls, which provides a better insight into the vector loop formation, which normally lies in the diagonal sagittal plane.[28,29]

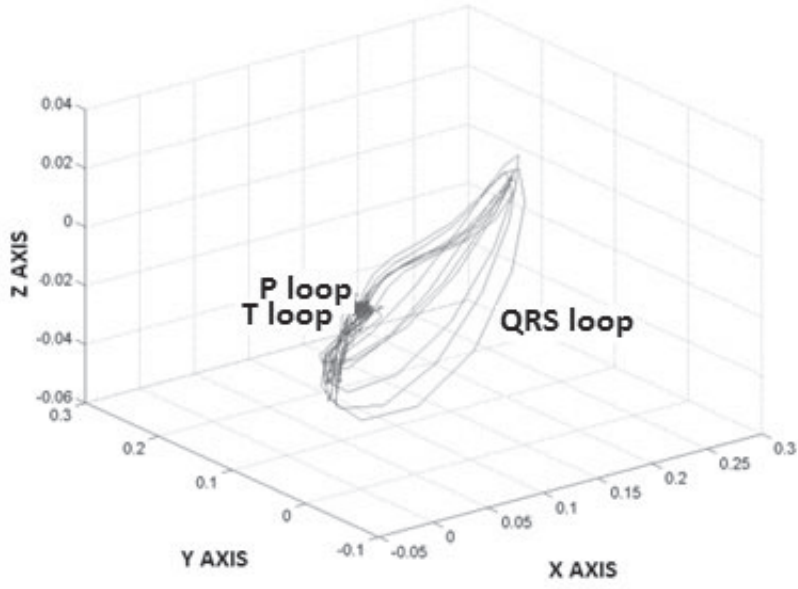

Figure 4: Three-dimensional VCG plot (Yang et. al 2012)

[27] E Sanz, JP Steger, and W Thie, "Cardiogoniometry," *Clinical Cardiology* 6, no. 5 (1983): 199–206.
[28] H Saner et al., "Cardiogoniometry: a new noninvasive method for detection of ischemic heart disease," *Clinical Cardiology* 6, no. 5 (1983): 207–210.
[29] Sotirios Spiliopoulos et al., "Diagnosis of obstructive coronary artery disease by cardiogoniometry: A field test in a real-life setting," *Journal of electrocardiology* 48, no. 3 (2015): 420–422.

The diagonal sagittal plane hence becomes the main plane. CGM is also a non-invasive method for the quantitative three-dimensional analysis of myocardial depolarisation and repolarisation. It helps the physician to analyse the recordings more accurately without the influence of heart rotation.[30]

Mechanical heart activities are based on the conduction of electrical impulses, resulting in measurable potentials. These potentials are captured and converted into digital values using bipolar leads (A, D, and Ve).[31] For data recording, in addition to the ground electrode, only four thoracic electrodes are required, positioned in a predefined geometric configuration. The location of the electrodes is shown in Figure 5. The particular electrode positioning provides a three-dimensional reading, and spatial display of the cardiac potentials over a specific period.[32]

The data obtained from A, D, and Ve is summarised vectorially and analysed as a three-dimensional time signal. Although the leads are in a fixed geometric configuration, for physiological reasons, it is not orthogonal. Transformation of the data, supplied by leads A, D, and Ve to X, Y and Z, convert the digitalised data into an orthogonal form, whereby X and Y represent the diagonal sagittal plane, and Z runs orthogonally to the sagittal plane.[33] With these three leads in an orthogonal configuration, it is now possible to build a three-dimensional vector display. This offers a considerably more realistic visualisation of cardiac conduction stimuli processes than the previously described vectorcardiography.[34] The three-dimensional CGM plot of the healthy subject is shown in Figure 6.

---

[30] Sanz, E, JP Steger, and W Thie. “Cardiogoniometry.” Clinical Cardiology 6, no. 5 (1983): 199–206.

[31] Sanz, E, JP Steger, and W Thie. “Cardiogoniometry.” Clinical Cardiology 6, no. 5 (1983): 199–206.

[32]Ralf Birkemeyer et al., “Comparison of cardiogoniometry and electrocardiography with perfusion cardiac magnetic resonance imaging and late gadolinium enhancement,” *Europace* 14, no. 12 (2012): 1793–1798.

[33] Spiliopoulos et al., “Diagnosis of obstructive coronary artery disease by cardiogoniometry: A field test in a real-life setting.”

[34] Oliver Brown et al., *95 A Systematic Review of The Clinical Applications of Cardiogoniometry in Cardiovascular Disease*, 2016.

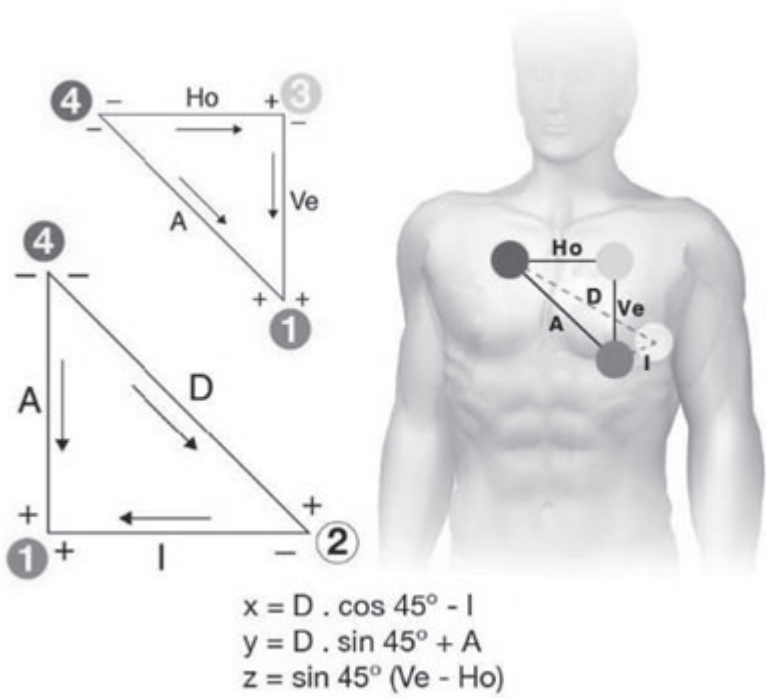

Figure 5: Location of placement of electrodes during Cardiogoniometry

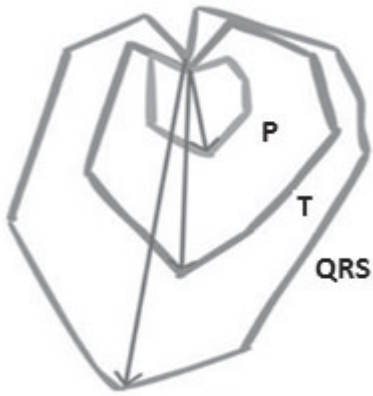

Figure 6: Three-dimensional plot of cardiogoniometry

Difference between Cardiogoniometry and Conventional Vectorcardiography

The body axis could be aligned in horizontal, vertical and saggital plane as required during vectorcardiography.[35] The result of this is that the anterior and posterior walls of the heart are partially superimposed, and the observer requires good visual thinking to understand the orientation of the vector loops, and is given in Figure 7.

In cardiogoniometry, the major planes can be rotated by 45 degrees to align with the major axis of the heart.[36] It allows the heart to be split into different planes thereby providing a better insight of internal parts and the vector loop which lies in the diagonal sagittal plane. The diagonal sagittal plane thus becomes the main plane, and is given in Figure 7.

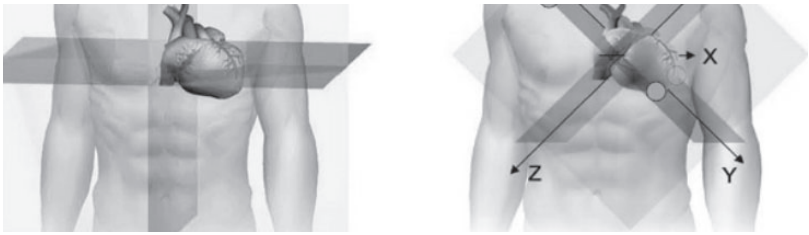

Figure 7: The projected vector plane for Vectorcardiography (left side) and Cardiogoniometry (right side)

References

Birkemeyer, Ralf, Ralph Toelg, Uwe Zeymer, Rainer Wessely, Sebastian Jäckle, Bajram Hairedini, Mike Lübke, Manfred Aßfalg, and Werner Jung. "Comparison of cardiogoniometry and electrocardiography with perfusion cardiac magnetic resonance imaging and late gadolinium enhancement." Europace 14, no. 12 (2012): 1793–1798.

Brown, Oliver, Jennifer Rossington, Andrew Clark, and Angela Hoye. 95 A Systematic Review of The Clinical Applications of Cardiogoniometry in Cardiovascular Disease, 2016.

[35] Eastman, George. "A new method of deriving the vectorcardiogram from routine clinical electrocardiographic leads." Journal of the American Geriatrics Society 8, no. 9 (1960): 708–723.

[36] Sanz, E, JP Steger, and W Thie. "Cardiogoniometry." Clinical Cardiology 6, no. 5 (1983): 199–206.

Chen, Yun-Hsuan, Maaike Op De Beeck, Luc Vanderheyden, Evelien Carrette, Vojkan Mihajlovic, Kris Vanstreels, Bernard Grundlehner, Stefanie Gadeyne, Paul Boon, and Chris Van Hoof. "Soft, comfortable polymer dry electrodes for high quality ECG and EEG recording." Sensors 14, no. 12 (2014): 23758–23780.

Dawson, Drew, Hui Yang, Milind Malshe, Satish TS Bukkapatnam, Bruce Benjamin, and Ranga Komanduri. "Linear affine transformations between 3-lead (Frank XYZ leads) vectorcardiogram and 12-lead electrocardiogram signals." Journal of electrocardiology 42, no. 6 (2009): 622–630.

Dower, Gordon E, H Bastos Machado, and JA Osborne. "On deriving the electrocardiogram from vectorcardiographic leads." Clinical Cardiology 3, no. 2 (1980): 87–95.

Dupre, Anthony, Sarah Vincent, and Paul A Iaizzo. "Basic ECG theory, recordings, and interpretation." In Handbook of cardiac anatomy, physiology, and devices, 191–201. Springer, 2005.

Eastman, George. "A new method of deriving the vectorcardiogram from routine clinical electrocardiographic leads." Journal of the American Geriatrics Society 8, no. 9 (1960): 708–723.

Frank, Ernest. "An accurate, clinically practical system for spatial vectorcardiography." circulation 13, no. 5 (1956): 737–749.

Grishman, Arthur, Leonard Scherlis, and Richard P Lasser. "Spatial vectorcardiography." The American journal of medicine 14, no. 2 (1953): 184–200.

Johnston, Franklin D. "The clinical value of vectorcardiography." Circulation 23, no. 2 (1961): 297–303.

Langner JR, Paul H, Robert H Okada, Samuel R Moore, and Harry L Fies. "Comparison of four orthogonal systems of vectorcardiography." Circulation 17, no. 1 (1958): 46–54.

Malmivuo, Plonsey, Jaakko Malmivuo, and Robert Plonsey. Bioelectromagnetism: principles and applications of bioelectric and biomagnetic fields. Oxford University Press, USA, 1995.

Mann, Hubert. "A method of analyzing the electrocardiogram." Archives of Internal Medicine 25, no. 3 (1920): 283–294.

Mary, MC Helen, Dilbag Singh, and KK Deepak. "Detecting changes in cardiovascular interaction during postural stress using directed coherence." Signal, Image, and Video Processing 13, no. 8 (2019): 1521–1528.

Peters S, Brattström A, Meyners W, Trümmel M, "Lokalisierte rechtspräkordiale QRS-Prolongation als diagnostisches Kriterium der arrhythmogenen rechtsventrikulären Dysplasie-Kardiomyopathie:

Tatsache oder Messfehler", Journal für Kardiologie, 2003, 10(5), 200-203

Saner, H, HR Baur, E Sanz, and HP Gurtner. "Cardiogoniometry: a new noninvasive method for the detection of ischemic heart disease." Clinical Cardiology 6, no. 5 (1983): 207–210.

Sanz, E, JP Steger, and W Thie. "Cardiogoniometry." Clinical Cardiology 6, no. 5 (1983): 199–206.

Schau, Thomas, Walter Koglek, Josef Brandl, Martin Seifert, Jürgen Meyhöfer, Michael Neuss, Georg Grimm, Robert Bitschnau, and Christian Butter. "Baseline vectorcardiography as a predictor of invasively determined acute hemodynamic response to cardiac resynchronization therapy." Clinical Research in Cardiology 102, no. 2 (2013): 129–138.

Schreck, David M, and Robert D Fishberg. "Derivation of the 12-lead electrocardiogram and 3-lead vectorcardiogram." The American journal of emergency medicine 31, no. 8 (2013): 1183–1190.

Sörnmo, Leif, and Pablo Laguna. "Electrocardiogram (ECG) signal processing." Wiley encyclopedia of biomedical engineering, 2006.

Spiliopoulos, Sotirios, Christof Wald, Vera Hergesell, Alexandros Merkourios Dimitriou, Reiner Koerfer, and Gero Tenderich. "Diagnosis of obstructive coronary artery disease by cardiogoniometry: A field test in a real-life setting." Journal of electrocardiology 48, no. 3 (2015): 420–422.

Surawicz, B. "Comprehensive Electrocardiology: Theory and Practice in Health and disease." Eds PW McFarlane, TDV Laerie. New York 1 (1989): 512–63.

Vullings, Rik, Chris HL Peters, Iman Mossavat, S Guide Oei, and Jan WM Bergmans. "Bayesian approach to patient-tailored vectorcardiography." IEEE transactions on biomedical engineering 57, no. 3 (2009): 586–595.

Wilson, FN, FD Johnston, and PS Barker. "The use of the cathode-ray oscillograph in the study of the monocardiogram." J. din. Invest 16 (1937): 664.

Yang, Hui, Satish TS Bukkapatnam, and Ranga Komanduri. "Spatiotemporal representation of cardiac vectorcardiogram (VCG) signals." Biomedical engineering online 11, no. 1 (2012): 16.

CHAPTER FIVE

BIOPRINTED TISSUES AND ORGANS

KIRTI AGRAWAL, S. VARADHARAJAN,
S.V. KIRTHANASHRI

Abstract

Three-dimensional (3D) bioprinting has wide spread applications in tissue regeneration for creating functional artificial organs. Additionally, it helps to create 3D models for drug testing. With primary focus on tissue regeneration, there are various research groups around the world trying to recreate functional human organs. With advancement of this technology, the different printing strategies would create a benchmark in regenerative medicine, and improve the efficiency and delivery speed to the intended target. They can be employed in 3D disease modelling as well. The use of stem cells in bio-inks could marked impact and lead to an enhancement of cell survival. This chapter focusses on role of 3D printing for tissue regeneration and the development of artificial organs.

1.0 Introduction

Three-dimensional (3D) bioprinting finds application in tissue regeneration for creating functional artificial organs. Additionally, it helps to create 3D models for drug testing. While the primary focus lies in tissue regeneration, there are various research groups around the world trying to recreate functional human organs. The requisites for developing these organs are the cells, the extracellular matrix (ECM), and the growth factors, which are the primary elements of tissue engineering. Using the bioprinting process, these primary elements are taken in optimised ratios to make 3D organs *in vitro* that can then be implanted into the human body. Three-dimensional bioprinting occurs in different phases that can be categorised into pre-printing, printing, and post-printing phases. The selection and preparation

of bio-ink, that is, the choosing of the required dimensions of the printed structures, forms the pre-printing phase. The process of printing on the bioprinter with the bio-inks contributes to the printing phase, while the process after printing, like cross-linking or biological evaluation, forms the post-printing phase. The use of 3D printing has led to reduced processing time and has offered ground-breaking solutions for the commercialisation of microfluidic systems. Each phase of printing has to be optimised to get the desired product. The tissue/organ post-printing can be referred to as a construct, matrix, scaffold, or bioprinted structure. Once the bioprinted structures are printed, they are provided with biologically sterile conditions (maintained in the carbon-dioxide incubator at 37°C with the appropriate medium).

Additionally, 3D printing minimises the processing time, and increases the production/sample number, which, in turn, speeds up the overall process. In other words, the printing phase is faster and more successful if the pre-printing phase parameters are accurate.

The pre-printing and printing phases require enormous data collection and involve a great deal of literature review, which will eventually help in selecting the appropriate bio-ink. The type of bio-ink and selection of the bioprinter are critical parameters for a successful three-dimensional printed organ/model. The success of 3D printing lies in its ability to achieve the resolution size of printing nanodroplets of cells on the predetermined position.

2.0 Bio-Inks

The main element of bioprinting is the bio-ink, which help in cell adhesion, and further exhibits cellular function. Bio-inks are classified into different types from the origin of their source, which are as follows:

- Natural bio-ink: Alginate, Gelatine, Collagen, fibrin, hyaluronic acid, dECM (decellularized ECM), silk, chitosan, agarose, etc.
- Synthetic bio-ink: PEG, Pluronic acid, Polyhydroxy butyrate-co-valerate (PHBV), polycaprolactone (PCL), polyvinyl alcohol (PVA), polylactic acid (PLA), poly lactide-co-glycolide (PLGA), etc.
- Self-assembled: cd-Ad HA, DNA peptide, peptide nanofibres, etc.

The natural bio-inks of animal origin have been widely researched because of the cell attachment motifs they contain. This type of bio-ink helps in faster and prolonged cell attachment and also increases the cell-material

interaction. The rapid degradation rate and biocompatibility issue are the main concerns, however. Due to their origin, they are prone to raise some immunological complications when implanted. Gelatine, collagen, and silk are high protein-containing materials from animal sources that have been modified to make them compatible and able to be used in various products for regeneration. To neutralise the effect of natural origin products, synthetic polymers are mixed with the natural polymers to gain a dual effect. Such mixed/combined bio-inks overcome the shortcomings of each one and helps to establish stable cell-cell & cell-ECM interactions. The synthetic polymers lack recognition motifs but are biocompatible and show a slower rate of degradation upon implantation. In the self-assembled bio-inks, the cells and ECM interact with each other before the printing process, and hence have been found to establish a stronger interaction that is favourable for cell culture. Table 1 details a few of the bio-inks employed for bioprinting tissues.

Table 1: Widely used bio-inks for bioprinting organs/tissues

Biomaterial	Cells	Organ/Tissue	Reference
Alginate and PEG-Fibrinogen	HUVECs, iPSC-CMs	Cardiac Tissue	[1]
Gelatine	HCASMCs, HUVECs, hMSCs	Vascular constructs	[2]
Silk fibroin	Schwann cells	Neural tissue repair	[3]
HA-SH hydrogel (HA-hydroxyapatite)	Human dermal fibroblast (HDF cells)	Skin tissue regeneration	[4]

[1] Fabio Maiullari et al., "A Multi-Cellular 3D Bioprinting Approach for Vascularized Heart Tissue Engineering Based on HUVECs and IPSC-Derived Cardiomyocytes," *Scientific Reports*, 2018, https://doi.org/10.1038/s41598-018-31848-x.

[2] Haitao Cui et al., "In Vitro and in Vivo Evaluation of 3D Bioprinted Small-Diameter Vasculature with Smooth Muscle and Endothelium," *Biofabrication*, 2019, https://doi.org/10.1088/1758-5090/ab402c.

[3] Ya Hong Zhao et al., "Novel Conductive Polypyrrole/Silk Fibroin Scaffold for Neural Tissue Repair," *Neural Regeneration Research*, 2018, https://doi.org/10.4103/1673-5374.235303.

[4] Haopeng Si et al., "3D Bioprinting of the Sustained Drug Release Wound Dressing with Double-Crosslinked Hyaluronic-Acid-Based Hydrogels," *Polymers*, 2019, https://doi.org/10.3390/polym11101584.

PCL-HA	TGFβ3	Articular cartilage regeneration	5
nano-ink (nHA+ TGF-β1- PLGA nanosphere+ PEG-Da:PEG hydrogel)	hMSCs	osteochondral scaffold	6
dECM and Pluronic F127	(HDF-n), (HA-VSMCs), (HUVECs)	Arteriovenous structures	7
Collagen, Alginate, And Fibrin	C2C12 myoblasts and MC3T3 fibroblasts	The femur, branched coronary arteries, trabeculated embryonic heart, and human brain	8

HUVEC: Human Umbilical Cord Vascular Endothelial Cells;
iPSC: Induced Pluripotent Stem Cells;
HCASMC: Human Coronary Artery Smooth Muscle Cells;
hMSC: Human Mesenchymal Stem Cell;
TGFβ: Transforming Growth Factor β;
C2C12: Mouse Myoblast Cell Line,
MC3T3: Mouse Osteoblast Cell;
HA-VSMC: Human Aortic Vascular Smooth Muscle Cells

3.0 Bioprinters

The bioprinter is the instrument used for bioprinting. This instrument is commercially available or can be made in-house, and is suitable for ejecting

[5] Chang H. Lee et al., "Regeneration of the Articular Surface of the Rabbit Synovial Joint by Cell Homing: A Proof of Concept Study," *The Lancet*, 2010, https://doi.org/10.1016/S0140-6736(10)60668-X.
[6] Nathan J. Castro, Joseph O'Brien, and Lijie Grace Zhang, "Integrating Biologically Inspired Nanomaterials and Table-Top Stereolithography for 3D Printed Biomimetic Osteochondral Scaffolds," *Nanoscale*, 2015, https://doi.org/10.1039/c5nr03425f.
[7] Yuanyuan Xu et al., "A Novel Strategy for Creating Tissue-Engineered Biomimetic Blood Vessels Using 3D Bioprinting Technology," *Materials*, 2018, https://doi.org/10.3390/ma11091581.
[8] Thomas J. Hinton et al., "Three-Dimensional Printing of Complex Biological Structures by Freeform Reversible Embedding of Suspended Hydrogels," *Science Advances*, 2015, https://doi.org/10.1126/sciadv.1500758.

the bio-ink onto the support platform to yield the bioprinted structures. The instrument is maintained in a sterile cleanroom environment in order to make the environment of bioprinting free from contamination. A few of the commercially available bioprinters are: EnvisionTEC, Organovo, RegenHU, Cellink, etc. Each of the commercial or in-house bioprinters has a particular style of ejecting the bio-inks. The different classifications of bioprinters are listed as follows:

3.1 Droplet-based printing technology

This printing technology is one of the fastest, and has the capacity for high-resolution printing. The cells along with bio-ink or only cells are made into droplets and deposited at high speed to form the desired structure.[9] The droplets are formed by employing acoustic ejectors. This type of bioprinting was reported by Fang et al., with dextran and polyethylene glycol (PEG) along with different types of cells.[10] The 3D-printed ovine mesenchymal stem cells (MSCs), and human embryonic kidney cells from acoustic ejectors, showed chondrogenic lineages.[11]

Application: Droplet-based bioprinting has been used for printing cells and 3D tissue constructs of vascularised tissue, hollow vessels, etc.

3.2 Jet-based bioprinting technology

This technology is very popular and is a high throughput-based printing technology. It can be sub-divided into various classifications: electrostatic, thermal and piezoelectric printers, based on the forces that are generated for printing.[12]

---

[9] Mohamed Ali et al., "Three-Dimensional Bioprinting for Organ Bioengineering: Promise and Pitfalls," *Current Opinion in Organ Transplantation*, 2018, https://doi.org/10.1097/MOT.0000000000000581.

[10] Yu Fang et al., "Rapid Generation of Multiplexed Cell Cocultures Using Acoustic Droplet Ejection Followed by Aqueous Two-Phase Exclusion Patterning," *Tissue Engineering - Part C: Methods*, 2012, https://doi.org/10.1089/ten.tec.2011.0709.

[11] Yu Zhao et al., "The Influence of Printing Parameters on Cell Survival Rate and Printability in Microextrusion-Based 3D Cell Printing Technology," *Biofabrication*, 2015, https://doi.org/10.1088/1758-5090/7/4/045002.

[12] Dezhi Zhou et al., "Bio-inks for Jet-Based Bioprinting," *Bioprinting*, 2019, https://doi.org/10.1016/j.bprint.2019.e00060.

a) Electrostatic inkjet printing:

According to the volume of the fluid chamber, the droplets are generated. The voltage between the electrode and pressure plate gets increased, which raises the volume in the ink chamber, causing the ink to flow. When the voltage goes down, the plates go back to the original position. The small nozzle diameter often leads to the clogging of pores in the printer. They are an inexpensive and preferred choice of printers.

b) Thermal inkjet printing:

The operation of thermal inkjet printers is supported by a current pulse for a few microseconds to increase the temperature to 300°C in the thermal actuators. This process of heating causes the ink to create small air bubbles that eject the ink from the nozzle. The pulse current and viscosity of ink decide the formation of a droplet. This, however, cannot be applied to a wide range of bio-inks that are heat sensitive.[13]

c) Piezoelectric inkjet printing:

The piezoelectric actuators are employed to eject the droplets. A strong pulse voltage changes the shape of the actuator that deforms the fluid chamber. Due to this pressure change, the droplet is pushed out. About four variants of print heads are available in piezoelectric printers that include squeeze mode, bend mode, push mode and shear mode.[13,14] The inkjet printers demand high viscosity solutions than can interfere with the cell encapsulation, but low cost and high speed make them the most demanding bioprinters.

Application: Inkjet bioprinting has been used for printing cells and tissue constructs of bone, cartilage, skin, cardiac, and nervous tissue.

3.3 Extrusion-based printing

This type of bioprinter is an excellent choice for the dual printing of cells with bioactive compounds. In this technique, pneumatic pressure is used to deliver the bio-inks to the nozzle, which then falls on the platform.

---

[13] Xinda Li et al., "Inkjet Printing for Biofabrication," in *3D Printing and Biofabrication*, 2018, https://doi.org/10.1007/978-3-319-45444-3_26.

[14] Satyajit Patra and Vanesa Young, "A Review of 3D Printing Techniques and the Future in Biofabrication of Bioprinted Tissue," *Cell Biochemistry and Biophysics*, 2016, https://doi.org/10.1007/s12013-016-0730-0.

Continuous strips of biomaterials are ejected out of the nozzle which get arranged in x, y, and z-axis, as per the computer-aided design (CAD) programme to form the required 3D structure. Hence, high viscosity and cell encapsulated materials can be used. Factors, such as shear stress, affecting the cell viability because of high viscosity are often reported.[15]

Application: Extrusion-based bioprinters have been massively used in R&D for printing cells and tissue constructs of bone, blood vessel, neuron, cartilage, skin, muscle, and cardiac tissue.

3.4 Laser Bioprinting

Laser bioprinting is a nozzle-less printing technique that applies the principle of laser-induced forward transfer technique (LIFT) for bioprinting. A high-pulsed laser hits the biomaterials that are covered with a laser-absorbing layer (titanium, gold, platinum), and the bubble created from the biomaterials falls on the substrate. A range of viscous materials can be used, which maintains the cellular viability. Since the nozzle is not part of the bioprinter, the problems related to shear stress are not exhibited, thus making them highly preferable for cell printing. This can also be referred to in optical-based bioprinting technology, where laser lights can be used for polymerisation. Arrays of cells are printed using this technique to prepare the 3D structure by maintaining the viability of the cell. The size of the droplets and the resolution can be affected by different factors, such as the laser energy, the substrate thickness, the ink properties, and the diameter of the focal point on the upper layer.

Application: Laser bioprinting has been utilised for printing cells and 3D tissue constructs of bone, blood vessel, cartilage, skin, muscle, adipose tissue, etc., on a large scale.

3.5 Acoustic printing/Ultrasound bioprinting

The acoustic waves or the ultrasound waves and the conducting interface (e.g. gold) are employed to 3D print the tissues via this nozzle-free technique. The ink is placed on the fluidic chamber and in the interface between the ink and the substrate; the circular acoustic waves are created, which then overcomes the surface tension and pulls the droplet downwards. Interestingly, voltage and heat are not used in the process. The size of the

[15] by S. Kamisuki et al., "Low Power, Small, Electrostatically-Driven Commercial Inkjet Head," in *Proceedings of the IEEE Micro Electro Mechanical Systems (MEMS)*, 1998, https://doi.org/10.1109/memsys.1998.659730.

droplets printed on the surface depends on the time of the pulse created.[16] It has been reported that the proliferation and differentiation of stem cells are enhanced using low-intensity ultrasound (ultrasound BP approaches).

Application: Ultrasound bioprinting has been employed for printing cell-laden biological matrices.

3.6 Optical-Based Bioprinting Techniques

The interaction of light with the ink to deposit on the donor plate is the characteristic feature of optical-based techniques also referred to as light-assisted printing.

The light interaction with the bio-ink to polymerise a photo-curable ink or helps the deposition of the ink from a donor plane onto a substrate. We classify the optical-based bioprinting technique into three main technologies: laser-induced forward transfer (LIFT), stereolithography (SLA), and two-photon lithography (TPL).

a) SLA (stereolithography)

Light is used as the source for polymerisation. The different layers are allowed to get polymerised with the light source. They can incur some resolution problems with the light source and hence digital light processing (DLP) utilises arrays of micromirrors to concentrate light on the resins that get polymerised. The thickness of the curing required for printing is addressed by increasing the pulse duration, wavelength and energy, repetition rate, and beam focus diameter of the light, and improvising the resin properties (viscosity and surface tension).

b) TPL (two-photon lithography)

The light source in this technique is near-infrared. The penetration of light occurs which hastens the printing process.

Application: Optical-based bioprinters have been extensively used in R&D for printing 3D tissue constructs of a blood vessel, osteochondral scaffold, neuron, cartilage, etc.

Table 2 lists the various types of 3D bioprinter with their application.

[16] by Yufeng Zhou, "The Application of Ultrasound in 3D Bio-Printing," *Molecules*, 2016, https://doi.org/10.3390/molecules21050590.

Table 2: Types of a 3D bioprinter for bioprinting organs/tissues

	Droplet-Based	**Jet-Based**	**Micro-Valve Printing**	**Extrusion Printing**	**Laser Bio-Printing**	**Ultrasound Bioprinting**	**Optical Bioprinting**
Pros	Low cost Moderate print speed Good resolution	Low cost Fast printing speed Moderate cell viability	Low cost High resolution High droplet deposition	Compatible with cross linkers Control thermally sensitive hydrogel Multi-head dispensing system	High cell viability No issues of biomaterial clogging and viscosity High resolution	Nozzle-free technique High cell viability	Low cost High cell viability Good resolution Fast printing speed
Cons	Larger droplets (50–300 µm) lead to a lower resolution Uneven deposition and nozzle clogging	Generation of small deformation Clogging of the printer head	Need for constant external pressure supply Nozzle blockage common	High mechanical stress to cells slow printing speed	High equipment cost Potential genetic damage to cells The requirement for laser-absorbing material	Poor depositional control	Scarcity of biocompatible resins Use of photo-initiators and radicals (cytotoxic)

Bio-inks	Gelatine, Alginate, dECM, fibrin, PEG, GelMA	Collagen, Alginate, PEGDMA, HA-PEG, n-HA, PCL	Alginate, Collagen, PEG, Gelatine	Collagen, dECM, Gelatine, Alginate, Fibrin, Agarose, PCL, GelMA	Collagen, Matrigel, Nano-hydroxyapatite (n-HA), Alginate	PEG, Collagen, GelMA	GelMA, silk fibroin, n-HA, GelMA-PEGDA hybrid hydrogel
Organ/tissue	Vascularized structures, Blood vessels	Blood vessel, Neuron	Liver, Lung	Bone, Cartilage	Bone, Skin	Cell-laden biological matrices	Blood Vessel
Reference	17	18	19	20	21	22	23

[17] Pranabesh Sasmal et al., "3D Bioprinting for Modelling Vasculature," *Microphysiological Systems*, 2018, https://doi.org/10.21037/mps.2018.10.02.
[18] Yi Zhang et al., "Biomaterials Based on Marine Resources for 3D Bioprinting Applications," *Marine Drugs*, 2019, https://doi.org/10.3390/md17100555.
[19] Sanjairaj Vijayavenkataraman et al., "3D Bioprinting of Tissues and Organs for Regenerative Medicine," *Advanced Drug Delivery Reviews*, 2018, https://doi.org/10.1016/j.addr.2018.07.004.
[20] Željka P. Kačarević et al., "An Introduction to 3D Bioprinting: Possibilities, Challenges, and Future Aspects," *Materials*, 2018, https://doi.org/10.3390/ma11112199.
[21] Gregor Skeldon, Baltasar Lucendo-Villarin, and Wenmiao Shu, "Three-Dimensional Bioprinting of Stem-Cell Derived Tissues for Human Regenerative Medicine," *Philosophical Transactions of the Royal Society B: Biological Sciences*, 2018, https://doi.org/10.1098/rstb.2017.0224.
[22] Daniele Foresti et al., "Acoustophoretic Printing," *Science Advances*, 2018, https://doi.org/10.1126/sciadv.aat1659.
[23] Christian Mandrycky et al., "3D Bioprinting for Engineering Complex Tissues," *Biotechnology Advances*, 2016, https://doi.org/10.1016/j.biotechadv.2015.12.011.

4.0 The Applications of 3D Bioprinting for Tissue Regeneration

4.1 Vascular Tissues

Vascular tissues are bioprinted, being in great demand as they help in the transport of nutrients and gases. The preferred choice of cells for evaluating the *in vitro* vascular behaviour is by using HUVEC (Human Umbilical Cord Vascular Endothelial Cells). Various natural and synthetic polymers are employed for regenerating vascular tissue-like structures. Commonly bioprinted polymers include Alginate, GelMA, agarose, pluronic F127, etc.[24]

4.2 Bone-Like Structures

Restoring the function of bones requires designing and selecting highly compressible polymer that can withstand the load-bearing capabilities of the bone. Bone marrow stromal cells are the most preferred choice of cells. Natural polymer-based hydrogels, such as collagen, alginate, and gelatine, combined with bone-related stem cells for printing 3D bone tissue constructs, have shown promising developments in bioprinting technology.[25]

4.3 Cartilage

Avascular tissue is subjected to high levels of research to explore its regenerative potential. Thermoresponsive injectable hydrogels from various polymeric sources are also very well researched. The human chondrocytes are the most widely used choice of cells. Cartilage tissue engineering includes biocompatible materials with good mechanical strength, such as cellulose, dECM, hydroxyapatite, chondroitin sulfate, nano-silicates, etc.[26]

---

[24] David Angelats Lobo and Paola Ginestra, "Cell Bioprinting: The 3D-Bioplotter™ Case," *Materials*, 2019, https://doi.org/10.3390/ma12234005.

[25] Mitchell A. Kuss et al., "Short-Term Hypoxic Preconditioning Promotes Prevascularization in 3D Bioprinted Bone Constructs with Stromal Vascular Fraction Derived Cells," *RSC Advances*, 2017, https://doi.org/10.1039/c7ra04372d.

[26] Joydip Kundu et al., "An Additive Manufacturing-Based PCL-Alginate-Chondrocyte Bioprinted Scaffold for Cartilage Tissue Engineering," *Journal of Tissue Engineering and Regenerative Medicine*, 2015, https://doi.org/10.1002/term.1682.

The HUVEC is the best cell choice for evaluating the parameters for cardiac tissue regeneration. The 3D bioprinted cardiac tissue, using human cardiac progenitor cells (hCPCs) and human coronary artery endothelial tissues, along with gelatine methacrylate and alginate hydrogels, are reported.[27]

4.5 Liver Tissues

The liver is a highly complex organ for regeneration because of the involvement of numerous cells carrying out various biochemical metabolisms. Bioprinting a liver is still a challenge due to the selection of bio-ink. Organovo Inc., a California based company, claims to have bioprinted a liver for *in vitro* drug testing, but the stability of the liver lasted for only up to 28 days. The cells and the bio-inks employed were not disclosed and the cost factor also limits its viability as a suitable treatment. Primary human cells, endothelial cells, macrophages are used in co-culture for developing liver tissue *in vitro*. Gelatine, silk, pluronic, and decellularised extracellular matrix are some of the more highly researched polymers for liver regeneration.[28]

4.6 Schwann Cells

The purpose of nerve tissue regeneration is to address the small and long gaps caused by injury, for which the Schwann cells are the ideal ones for nerve tissue regeneration. Apart from the selection of appropriate biomaterials for providing micro-environmental cues, the alignment of the scaffolds is to be considered. Bio-inks, comprising alginate, agarose, carboxymethyl-chitosan, PEG, etc., to fabricate 3D scaffolds encapsulating Schwann cells, show potential for nerve tissue engineering.[29]

---

[27] Mohammad Izadifar et al., "UV-Assisted 3D Bioprinting of Nanoreinforced Hybrid Cardiac Patch for Myocardial Tissue Engineering," *Tissue Engineering - Part C: Methods*, 2018, https://doi.org/10.1089/ten.tec.2017.0346.

[28] Phillip L. Lewis et al., "Directing the Growth and Alignment of Biliary Epithelium within Extracellular Matrix Hydrogels," *Acta Biomaterialia*, 2019, https://doi.org/10.1016/j.actbio.2018.12.039.

[29] M. D. Sarker et al., "Bio-Fabrication of Peptide-Modified Alginate Scaffolds: Printability, Mechanical Stability and Neurite Outgrowth Assessments," *Bioprinting*, 2019, https://doi.org/10.1016/j.bprint.2019.e00045.

4.7 Skin Tissues

Skin is one of the largest organs, and is a highly sought-after tissue that requires regeneration or artificial implants. Fibroblast and keratinocytes are the cells that are widely used for *in vitro* skin tissue regeneration. Alginate, gelatine, fibrinogen, human dermal fibroblasts bio-ink and encapsulated hMSCs bio-ink-based skin tissue scaffold have been reported.[30]

4.8 Muscle Cells

Artificial skeletal muscle tissue gains a lot of attention in the field of tissue engineering to restore severe tissue loss. Myoblasts are the muscle cells that are incorporated with the biomaterials for muscle tissue formation. PEG, fibrinogen, pluronic/alginate blends, and PEGDA blended into GelMA are used to print fibre scaffolds.[31]

4.9 3D organoid models - Cancer Cells

Various cancer cells are introduced to biomaterials to form *in vitro* 3D models/disease models. The cancer cells from numerous sources have been researched to obtain similar 3D models mimicking *in vivo* conditions. The breast cancer cell line, 21PT, was cultured by cross-linking hyaluronic acid and gelatine to form a bioprinted 3D organoid model. Human liver cell lines are bioprinted with pluronic to obtain the 3D organoid models. The role of creating these models is for *in vitro* drug screening or for understanding the molecular mechanism of the diseases. The cell viability is found to be higher in 3D bioprinted models as the cells are provided with the topographical and biochemical cues to sustain it in the micro-environment. In a recent study, 3D bioprinting using alginate or chitosan biomaterials has been reported to create *in vitro* glioma tumour model. A neuroblastoma tumour model, based on chitosan and gelatine hydrogel, has also been reported. Alginate,

[30] Farshad Oveissi et al., "Tough and Processable Hydrogels Based on Lignin and Hydrophilic Polyurethane," *ACS Applied Bio Materials*, 2018, https://doi.org/10.1021/acsabm.8b00546.

[31] A. R. Pereira, D. Trivanović, and M. Herrmann, "Approaches to Mimic the Complexity of the Skeletal Mesenchymal Stem/Stromal Cell Niche in Vitro," *European Cells & Materials*, 2019, https://doi.org/10.22203/eCM.v037a07.

gelatine, and fibrinogen containing glioma stem cells were also used to create a brain glioma model.[32]

5.0 New Directions: 4D Bioprinting

Both microfluidics and tissue engineering have contributed to engineering *in vitro* models and devices for addressing various physiological conditions. 3D printing has opened up a new horizon in regenerative medicine that has helped in the development of artificial organs. Though 3D printing has offered numerous advantages, it failed to develop hollow constructs. Recently, the emergence of 4D printing overcame this challenge by integrating the time factor to 3D printing.[33]

The 4D bioprinting is based on:

(i) The shape deformation of the material (either conventional materials or smart materials).

(ii) The maturation of the engineered construct (time-dependent process).

This technology can indeed help to address the challenges of 3D printing by helping to develop native tissue/organs, and can change the prospects of regenerative medicine.

6.0 Future Direction and Conclusion

Printing strategies are set to create a benchmark in regenerative medicine, and improve the efficiency and speed of delivering to the intended target. They can be used for various approaches, including 3D disease modelling. The use of stem cells in bio-inks can have a marked impact and lead to an enhancement of cell survival.

---

[32] Ying Wang et al., "3D Bioprinting of Breast Cancer Models for Drug Resistance Study," *ACS Biomaterials Science and Engineering*, 2018, https://doi.org/10.1021/acsbiomaterials.8b01277.

[33] Bin Gao et al., "4D Bioprinting for Biomedical Applications," *Trends in Biotechnology*, 2016, https://doi.org/10.1016/j.tibtech.2016.03.004.

References

1. Maiullari, Fabio, Marco Costantini, Marika Milan, Valentina Pace, Maila Chirivì, Silvia Maiullari, Alberto Rainer, et al. "A multi-cellular 3D bioprinting approach for vascularized heart tissue engineering based on HUVECs and iPSC-derived cardiomyocytes." *Scientific Reports* 8, no. 1 (2018): 13532.
2. Cui, Haitao, Wei Zhu, Yimin Huang, Chengyu Liu, Zu-Xi Yu, Margaret Nowicki, Shida Miao et al. "In vitro and in vivo evaluation of 3D bioprinted small-diameter vasculature with smooth muscle and endothelium." *Biofabrication* 12, no. 1 (2019): 015004.
3. Zhao, Ya-Hong, Chang-Mei Niu, Jia-Qi Shi, Ying-Yu Wang, Yu-Min Yang, and Hong-Bo Wang. "Novel conductive polypyrrole/silk fibroin scaffold for neural tissue repair." *Neural regeneration research* 13, no. 8 (2018): 1455.
4. Si, Haopeng, Tianlong Xing, Yulong Ding, Hongbo Zhang, Ruixue Yin, and Wenjun Zhang. "3D Bioprinting of the Sustained Drug Release Wound Dressing with Double-Crosslinked Hyaluronic-Acid-Based Hydrogels." *Polymers* 11, no. 10 (2019): 1584.
5. Lee, Chang H., James L. Cook, Avital Mendelson, Eduardo K. Moioli, Hai Yao, and Jeremy J. Mao. "Regeneration of the articular surface of the rabbit synovial joint by cell homing: a proof of concept study." *The Lancet* 376, no. 9739 (2010): 440–448.
6. Castro, Nathan J., Joseph O'Brien, and Lijie Grace Zhang. "Integrating biologically inspired nanomaterials and table-top stereolithography for 3D printed biomimetic osteochondral scaffolds." *Nanoscale* 7, no. 33 (2015): 14010–14022.
7. Xu, Yuanyuan, Yingying Hu, Changyong Liu, Hongyi Yao, Boxun Liu, and Shengli Mi. "A Novel Strategy for Creating Tissue-Engineered Biomimetic Blood Vessels Using 3D Bioprinting Technology." *Materials* 11, no. 9 (2018): 1581.
8. Hinton, Thomas J., Quentin Jallerat, Rachelle N. Palchesko, Joon Hyung Park, Martin S. Grodzicki, Hao-Jan Shue, Mohamed H. Ramadan, Andrew R. Hudson, and Adam W. Feinberg. "Three-dimensional printing of complex biological structures by the freeform reversible embedding of suspended hydrogels." *Science advances* 1, no. 9 (2015): e1500758.
9. Ali, Mohamed, Sang Jin Lee, and John D. Jackson. "Three-dimensional bioprinting for organ bioengineering: promise and pitfalls." *Current opinion in organ transplantation* 23, no. 6 (2018): 649–656.
10. Fang, Yu, John P. Frampton, Shreya Raghavan, Rahman Sabahi-

Kaviani, Gary Luker, Cheri X. Deng, and Shuichi Takayama. "Rapid generation of multiplexed cell cocultures using acoustic droplet ejection followed by aqueous two-phase exclusion patterning." *Tissue Engineering Part C: Methods* 18, no. 9 (2012): 647–657.

11. Zhao, Yu, Yang Li, Shuangshuang Mao, Wei Sun, and Rui Yao. "The influence of printing parameters on cell survival rate and printability in microextrusion-based 3D cell printing technology." *Biofabrication* 7, no. 4 (2015): 045002.
12. Zhou, Dezhi, Jianwei Chen, Boxun Liu, Xinzhi Zhang, Xinda Li, and Tao Xu. "Bio-inks for jet-based bioprinting." *Bioprinting* (2019): e00060.
13. Li, Xinda, Jianwei Chen, Boxun Liu, Xiong Wang, Dongni Ren, and Tao Xu. "Inkjet Printing for Biofabrication." *3D Printing and Biofabrication* (2018): 1–19.
14. Patra, Satyajit, and Vanesa Young. "A review of 3D printing techniques and the future in bio-fabrication of bioprinted tissue." *Cell biochemistry and biophysics* 74, no. 2 (2016): 93–98.
15. Kamisuki, S., T. Hagata, C. Tezuka, Y. Nose, M. Fujii, and M. Atobe. "A low power, small, electrostatically-driven commercial inkjet head." In *Proceedings MEMS 98. IEEE. Eleventh Annual International Workshop on Micro Electro Mechanical Systems. An Investigation of Micro Structures, Sensors, Actuators, Machines, and Systems (Cat. No. 98CH36176*, pp. 63–68. IEEE, 1998.
16. Miri, Amir, Ebrahim Mostafavi, Danial Khorsandi, Shu-Kai Hu, Matthew Malpica, and Ali Khademhosseini. "Bioprinters for organs-on-chips." *Biofabrication* (2019).
17. Zhou, Yufeng. "The application of ultrasound in 3D bioprinting." *Molecules* 21, no. 5 (2016): 590.
18. Sasmal, Pranabesh, Pallab Datta, Yang Wu, and Ibrahim T. Ozbolat. "3D bioprinting for modeling vasculature." *Microphysiological systems* 2 (2018).
19. Zhang, Yi, Dezhi Zhou, Jianwei Chen, Xiuxiu Zhang, Xinda Li, Wenxiang Zhao, and Tao Xu. "Biomaterials Based on Marine Resources for 3D Bioprinting Applications." *Marine drugs* 17, no. 10 (2019): 555.
20. Vijayavenkataraman, Sanjairaj, Wei-Cheng Yan, Wen Feng Lu, Chi-Hwa Wang, and Jerry Ying Hsi Fuh. "3D bioprinting of tissues and organs for regenerative medicine." *Advanced drug delivery reviews* (2018).
21. Kačarević, Željka, Patrick Rider, Said Alkildani, Sujith Retnasingh, Ralf Smeets, Ole Jung, Zrinka Ivanišević, and Mike Barbeck. "An introduction to 3D bioprinting: possibilities, challenges and future

aspects." *Materials* 11, no. 11 (2018): 2199.
22. Skeldon, Gregor, Baltasar Lucendo-Villarin, and Wenmiao Shu. "Three-dimensional bioprinting of stem-cell-derived tissues for human regenerative medicine." *Philosophical Transactions of the Royal Society B: Biological Sciences* 373, no. 1750 (2018): 20170224.
23. Foresti, Daniele, Katharina T. Kroll, Robert Amissah, Francesco Sillani, Kimberly A. Homan, Dimos Poulikakos, and Jennifer A. Lewis. "Acoustophoretic printing." *Science advances* 4, no. 8 (2018): eaat1659.
24. Mandrycky, Christian, Zongjie Wang, Keekyoung Kim, and Deok-Ho Kim. "3D bioprinting for engineering complex tissues." *Biotechnology advances* 34, no. 4 (2016): 422–434.
25. Angelats Lobo, David, and Paola Ginestra. "Cell Bioprinting: The 3D-Bioplotter™ Case." *Materials* 12, no. 23 (2019): 4005.
26. Kuss, Mitchell A., Robert Harms, Shaohua Wu, Ying Wang, Jason B. Untrauer, Mark A. Carlson, and Bin Duan. "Short-term hypoxic preconditioning promotes pre-vascularization in 3D bioprinted bone constructs with a stromal vascular fraction derived cells." *RSC advances* 7, no. 47 (2017): 29312–29320.
27. Kundu, Joydip, Jin-Hyung Shim, Jinah Jang, Sung-Won Kim, and Dong-Woo Cho. "An additive manufacturing-based PCL–alginate–chondrocyte bioprinted scaffold for cartilage tissue engineering." *Journal of tissue engineering and regenerative medicine* 9, no. 11 (2015): 1286–1297.
28. Izadifar, Mohammad, Dean Chapman, Paul Babyn, Xiongbiao Chen, and Michael E. Kelly. "UV-assisted 3D bioprinting of nano-reinforced hybrid cardiac patch for myocardial tissue engineering." *Tissue Engineering Part C: Methods* 24, no. 2 (2018): 74–88.
29. Lewis, Phillip L., Ming Yan, Jimmy Su, and Ramille N. Shah. "Directing the growth and alignment of biliary epithelium within extracellular matrix hydrogels." *Acta biomaterialia* 85 (2019): 84–93.
30. Sarker, M. D., Saman Naghieh, Adam D. McInnes, Liqun Ning, David J. Schreyer, and Xiongbiao Chen. "Bio-fabrication of peptide-modified alginate scaffolds: Printability, mechanical stability, and neurite outgrowth assessments." *Bioprinting* 14 (2019): e00045.
31. Oveissi, Farshad, Sina Naficy, Thi Yen Loan Le, David F. Fletcher, and Fariba Dehghani. "Tough and Processable Hydrogels Based on Lignin and Hydrophilic Polyurethane." *ACS Applied Bio Materials* 1, no. 6 (2018): 2073–2081.
32. Pereira, A. R., D. Trivanović, and M. Herrmann. "Approaches to mimic the complexity of the skeletal mesenchymal stem/stromal cell niche in vitro." *European cells & materials* 37 (2019): 88–112.

33. Wang, Ying, Wen Shi, Mitchell Kuss, Sameer Mirza, Dianjun Qi, Alexey Krasnoslobodtsev, Jiping Zeng, Hamid Band, Vimla Band, and Bin Duan. "3D bioprinting of breast cancer models for drug resistance study." *ACS Biomaterials Science & Engineering* 4, no. 12 (2018): 4401–4411.
34. Gao, Bin, Qingzhen Yang, Xin Zhao, Guorui Jin, Yufei Ma, and Feng Xu. "4D bioprinting for biomedical applications." *Trends in biotechnology* 34, no. 9 (2016): 746–756.

CHAPTER SIX

ASSISTIVE DEVICE FOR OSTEOCHONDROMA AT TALUS

DIKSHA ZUTSHI,
DR. REEMA SHYAMSUNDER SHUKLA

Abstract

The non-cancerous protuberance of the bone or the cartilage present at the distal end of the bone near the growth plate causes the overall shifting of the load to one particular end, which may cause severe pain and damage to the spine and the patella. These are called secondary damages; the objective of the study is to develop an assistive device to eliminate secondary damages. Obliterating these damages is the prime objective of this study. Titanium would be used in its alloy form because of its durability and also for its lightweight characteristics. We aim to bring the shifted weight back to its normal level by counterbalancing it, using the magnetising effect of electromagnets. With this, we add a pressure strap to enhance stability and assist the talus in effective weight transfer. The circuit is only completed when the foot is in contact with the force-sensitive resistors. Two of them are placed in parallel, and their resistance values compared, which depends upon the amount of pressure applied to each of them. The pressure isn't equal because of the unequal body weight on either side. A current passes to the corresponding electromagnet to bring the pressure on both sides to equilibrium by bringing about a physical alleviation or depression on both sides.

Aim: To develop an assistive device for osteochondroma at the talus to eliminate secondary damages.

Introduction

The excessive and benign outgrowth of cartilage or bone at the distal end, near the epiphyseal plate, could lead to the misalignment of body mass, which may result in damage to the spine and patella. Osteochondroma is the most common primary bone tumour. It is the abnormal cartilaginous growth at the surface of the bone, and is not a true neoplasm. It constitutes the fully developed bone component, along with a cap that is cartilaginous in origin, and the damaged tissue which is referred to as a lesion, along with the medullar cavity of the long bones. It increases in size depending on the patient's age, and this lesion usually turns into a dormant state after completion of the growth plate. These tumours gradually grow, creating subtle but progressive symptoms. These mostly occur at the extreme sides of the long bones. The most uncommon presence has been reported at the patella or tarsal region. Osteochondroma at the talus is extremely unwonted: very few cases have ever been seen or reported.[1]

Primary tumours, or osteochondroma, can be in different forms: single or numerous, and with or without a stalk; they are outgrowths from the bone's surface, comprising the bone part, having a hyaline cartilage cap. Excision is the only solution.[2] Solitary osteochondromas are usually asymptomatic, but can also result in hemarthrosis at the ankle. An osteochondroma at the talus may be present with various symptoms, including pain, ankle swelling, painless mass, and a limited range of ankle motion.[3]

Pain is the result of friction between nerves and bones, which, in turn, causes irritation and constricted joint motion. Osteochondroma is found as a scattered mass between the articulations in the talus region. This asymptotic outgrowth may severely interfere with the normal functioning of adjacent structures, such as tendons and blood vessels. Osteochondroma, in such cases, may even go through unconstrained regression. It has a characteristic radiographic appearance, and it grows in the opposite direction to the

[1] Boya, Hakan, Ozal Ozcan, and Cigdem Tokyol. "Osteochondroma of the talus: an unusual location." *Acta Orthop Traumatol Turc* 48, no. 2 (2014): 236–9.
[2]Atik, O. Sahap, Baran Sarikaya, Cemalettin Kunat, Ramin Muradi, Bahadır Ocaktan, and Hüseyin Topçu. "Osteochondroma of the talus." *Eklem Hastalik Cerrahisi* 21, no. 2 (2010): 116–117.
[3] Kim, Sung-Hun, Whan-Yong Chung, Seung-Hwan Kim, and Woo-Suk Lee. "Osteochondroma of the talus-a report of two cases." *Journal of the Korean Orthopaedic Association* 43, no. 1 (2008): 135–138.

adjacent joint, which is located at the metaphysis of the long bones.[4] Osteochondromas which aren't causing any serious effects, such as extreme motion restriction and swellings, can be treated without excision, otherwise surgery is the best solution for cases which are symptomatic and repetitive in nature.[5]

At least 4 per cent of tumours are located around the foot and ankle. At the foot, the metatarsal is the most common site of occurrence. Swelling usually characterises the condition, where even a small outgrowth (caused by a significant biochemical imbalance) leads to pain and diminishes the normal foot-ankle functioning, thereby becoming a significant indicator of early abnormality.[6]

- Osteochondroma at the talus is an extremely rare condition of tarsal tunnel syndrome where the only cure is excision.
- Osteochondroma at the talus leads to the eventual shifting of body weight to an extreme side. This results in secondary damage at the patella and spine.
- With the help of an assistive device, and not a curative device, the only cure available is to surgically remove the outgrowth.

Previous Works

1) Ankle motion is improvised by using llizarov external fixation, and pain is alleviated.[7]
2) A 54-year-old male suddenly experienced solitary osteochondroma growing proximal to the left first metatarsal, and multiple osteochondromas around the talus. Multiple non-surgical therapies were

[4] Ibrahim, M., and A. Hakeem. "Osteochondroma of Talus–An Unusual Site." *Unique Journal of Medical and Dental Sciences* 1, no. 2 (2013): 61–62.
[5] Suranigi, Shishir, Kanagasabai Rengasamy, Syed Najimudeen, and James Gnanadoss. "Extensive osteochondroma of talus presenting as tarsal tunnel syndrome: report of a case and literature review." *Archives of Bone and Joint Surgery* 4, no. 3 (2016): 269.
[6] Al Mutani, Mohammed, Aatif Mahmood, and C. R. Chandrasekar. "Giant osteochondroma of the talar neck." *The Foot* 23, no. 1 (2013): 45–49.
[7] Shawen, CPT Scott B., COL Kathleen A. McHale, and H. Thomas Temple. "Correction of ankle valgus deformity secondary to multiple hereditary osteochondral exostoses with llizarov." *Foot & ankle international* 21, no. 12 (2000): 1019–1022.

tried, such as shoe equipment and arch support. Total surgical excision was the only solution.[8]

3) A minor swelling was noticed on the dorsum of the right ankle of a six-year-old, and pain was experienced while running. The swelling gradually progressed and the presence of a solid mass of slight tenderness came to light after a detailed physical examination.[9]
4) A radiogram revealed a hard, bony structure at the talus of a 21-year-old who was experiencing pain and swelling in the right foot.[10]
5) A patient was seen with a kidney-shaped, small swelling at the dorsum of the right ankle. Sports activities intensified the pain. After a physical examination, a stagnant, firm lump was found.[11]
6) A solid painless mass was found to be slowly progressing on the left ankle of a 35-year-old male. Limitation in motion was experienced. An oval-shaped hard structure was seen at the dorsum of the talonavicular joint, after a full physical examination.[12]
7) Numbness, pain, swelling, and a burning sensation were felt by a 60-year-old woman in the right foot. CT scan with 3D-reconstruction made visible the presence of multiple exor growths on the different sides of the talus, sticking out in all directions and protruding into the soft tissues.[13]
8) Solitary osteochondroma of the sinus tarsi was seen in an 8-year-old boy who had a history of repeated ankle sprains and limited motion range

[8] Shtofmakher, Garry, Michelle A. Kaufman, Prashant H. Bhoola, Ankur A. Patel, Susan M. Rice, and Randy E. Cohen. "Multiple osteocartilaginous exostoses of the lower extremity: A case report." *The Foot* 25, no. 1 (2015): 62–65.

[9] Boya, Hakan, Ozal Ozcan, and Cigdem Tokyol. "Osteochondroma of the talus: an unusual location." *Acta Orthop Traumatol Turc* 48, no. 2 (2014): 236–9.

[10] Atik, O. Sahap, Baran Sarikaya, Cemalettin Kunat, Ramin Muradi, Bahadır Ocaktan, and Hüseyin Topçu. "Osteochondroma of the talus." *Eklem Hastalik Cerrahisi* 21, no. 2 (2010): 116–117.

[11] Kim, Sung-Hun, Whan-Yong Chung, Seung-Hwan Kim, and Woo-Suk Lee. "Osteochondroma of the talus-a report of two cases." *Journal of the Korean Orthopaedic Association* 43, no. 1 (2008): 135–138.

[12] Kim, Sung-Hun, Whan-Yong Chung, Seung-Hwan Kim, and Woo-Suk Lee. "Osteochondroma of the talus-a report of two cases." *Journal of the Korean Orthopaedic Association* 43, no. 1 (2008): 135–138.

[13] Suranigi, Shishir, Kanagasabai Rengasamy, Syed Najimudeen, and James Gnanadoss. "Extensive osteochondroma of talus presenting as tarsal tunnel syndrome: report of a case and literature review." *Archives of Bone and Joint Surgery* 4, no. 3 (2016): 269.

around the subtalar joint. Surgical excision of the tumour was the only way forward.[14]

9) A 12-year-old boy reported difficulty walking, pain in the left ankle, and swelling for a year, which gradually grew. After examination, it was revealed that there was a huge, hard chunk of mass around the talus, fixed to the underlying bone. MRI showed continuity between cortical and medullary sides of the lesion.[15]

From past research, it has been noticed that an engineering approach had not been extensively applied concerning correction of osteochondroma. Only the support structures were developed to hold the affected area, and to alleviate pain. Hence, it is hypothesised that the electronic footwear for the correction of the above said deformity would be of great assistance to suffering patients.

Materials and Methods

Titanium alloy would be used because of its cost efficiency, durability and lightweight characteristics. Two force-sensitive resistor plates are used as the topmost layer. The circuit completes and a current is allowed to pass when the foot comes in contact with the force sensitive resistor. The two are placed in parallel, and their resistance values compared, which depends upon the amount of pressure applied, as shown in Fig. 3.

If, because of body weight, the pressure isn't equal on both sides, then the current passes to the respective electromagnet, causing a change in the angle of alleviation of the plane and hence counterbalancing the shifted weight. The change is quite insignificant in millimetres, but it successfully neutralises the unequal pressure. This process is repeated until the resistance on both sides is equal.

[14] Andreacchio, Antonio, Lorenza Marengo, and Federico Canavese. "Solitary osteochondroma of the sinus tarsi." *Journal of Pediatric Orthopaedics B* 27, no. 1 (2018): 88–91.

[15] Krishna, Ch V. Murali, P. Ashok Kumar, P. Rambabu, K. Srinivasa Rao, and M. Satish. "Osteochondroma talus: a case report." *Journal of Evolution of Medical and Dental Sciences* 4, no. 3 (2015): 496–500.

Alongside this, a strap of pressure cuff is used around the talus and calcaneus to provide support, to assess the weight transfer, and to alleviate pain.

- Relays: to switch between electromagnets to be magnetised
- Springs
- Electromagnets
- Arduino (Microcontroller)
- PLA (Polylactic acid)

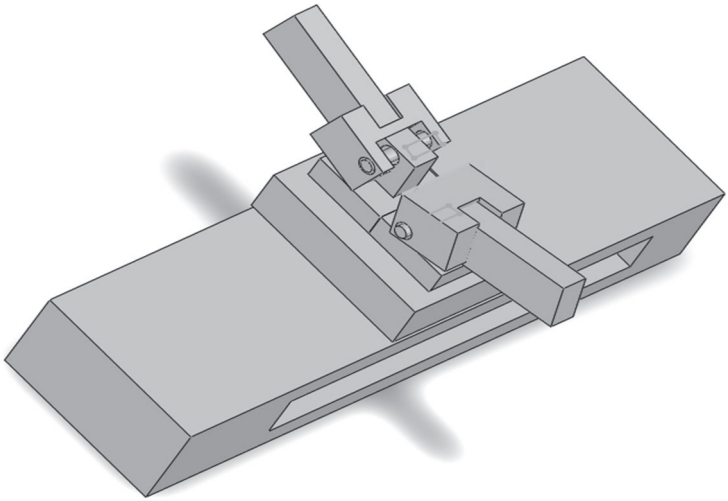

Fig.1: DESIGN 1. Used motors and windings to change the angle, which was not all that feasible.

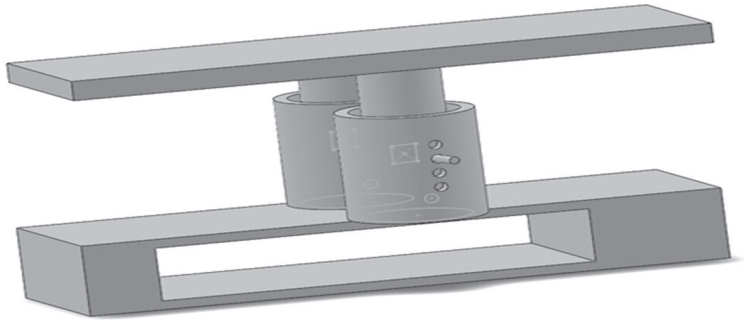

Fig. 2: DESIGN 2. Improvised design.

Electromagnetic actuators magnetise and demagnetise to change the angle of alleviation. The designs have been mentioned in Fig.1 and Fig. 2.

Resistive Touch Sensing

Force Sensing Resistors (FSR) take pressure as input, and output the resistance as shown in Fig. 3. After appropriate calibration, the accurate value of pressure applied can be estimated by knowing its resistance output. It was invented and patented by Franklin Eventoff, in 1977. It has a conductive polymer in its structure, which changes its resistance, along with the varying pressure.

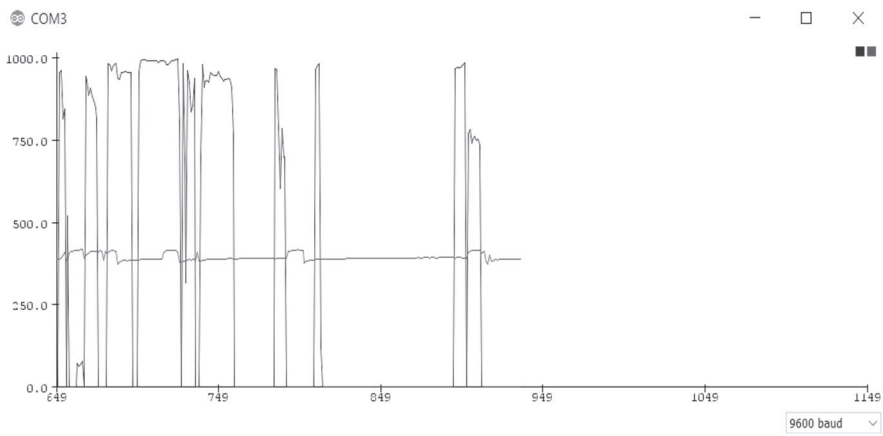

Fig. 3: Force-Sensing resistor output.

If the resistance—pressure readings—of plate “a” is more than “b”, this means the pressure at “a” is more, which implies that “b” needs to be brought down by electro- magnetising the respective actuator. The 3D printed models are shown in Fig. 4 and Fig. 5. Having the base and electromagnetic actuators controlled by relays, according to pressure-resistance readings of FSR, is shown in Fig. 6.

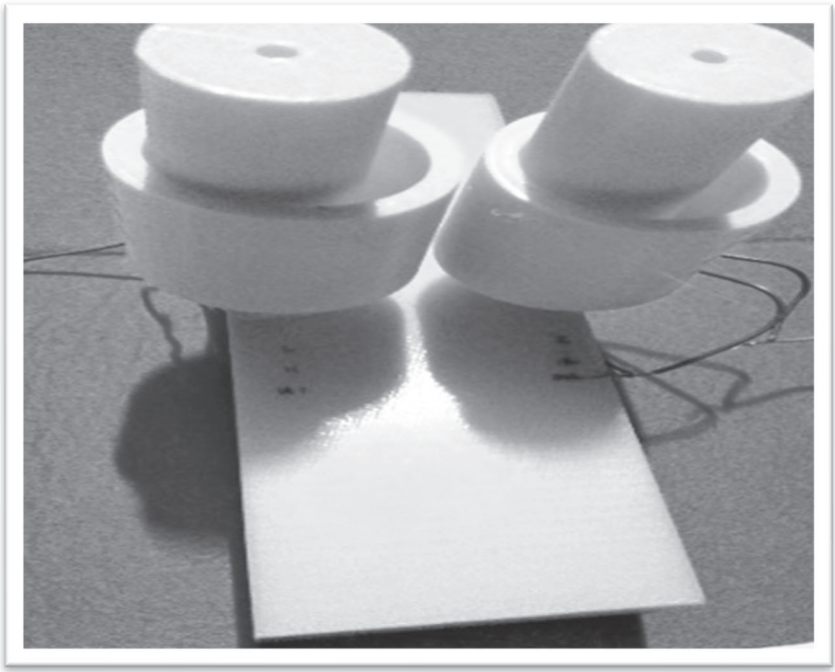

Fig. 4: Printed model.

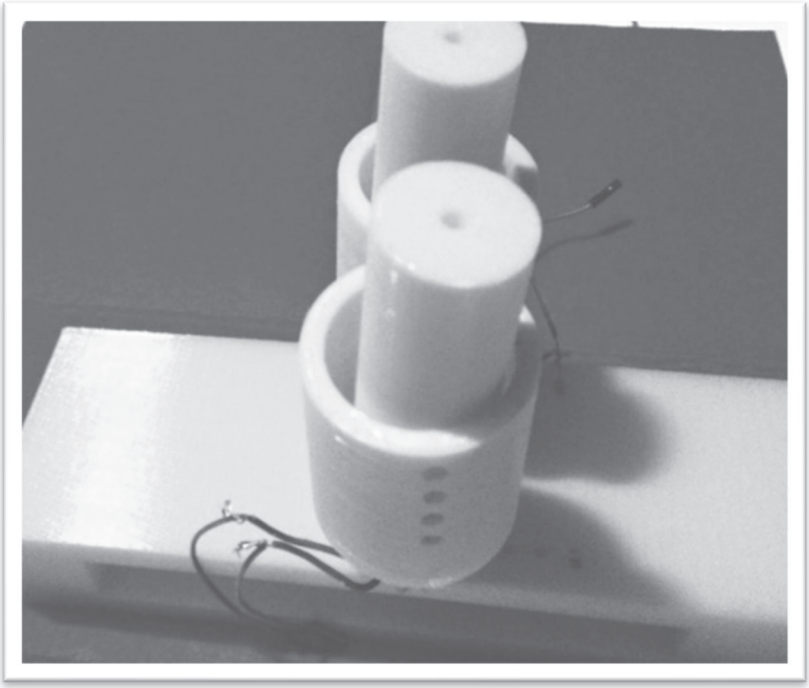

Fig. 5: Interconnections of the model with the rod.

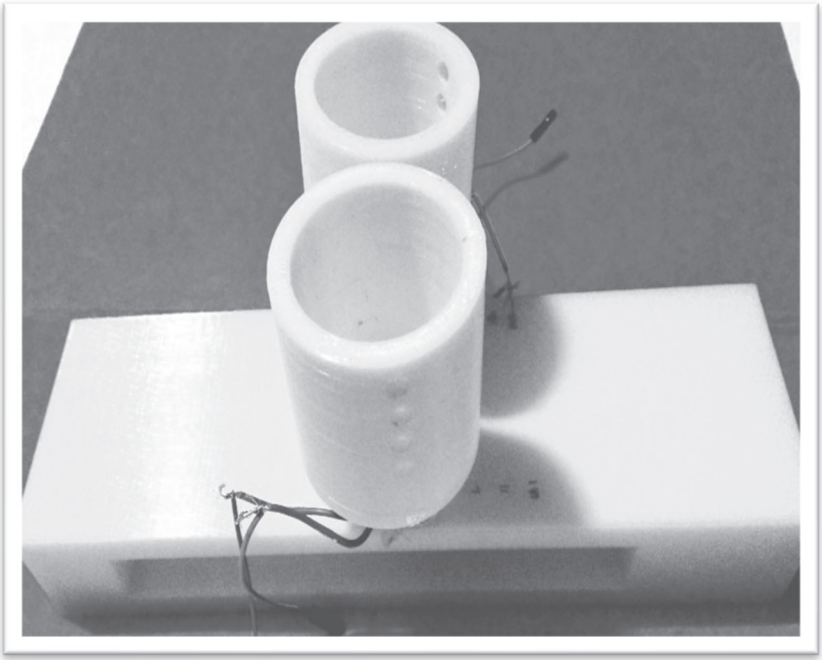

Fig. 6: Interconnections of the model without rod.

"Raise3D N2" 3D Printer

- Build Volume (W*D*H): (12*12*12) inch/ (305*305*305) mm
- Layer Resolution: 0.01 to 0.25 mm
- Filament type: PLA, ABS, PETG, Nylon, Carbon Fibres, TPU/TPE (Flexibles) Metal Composites, etc.
- Filament size:1.75mm
- Printing surface: Buildtak

Printing Material

PLA (Polylactic acid)

Pressure Activation

This would activate the entire mechanism, i.e. the entire device would be activated by the body weight. It works as a switch, where pressure leads to joining of the two terminals, hence the circuit is completed and it starts working as shown in Fig.7.

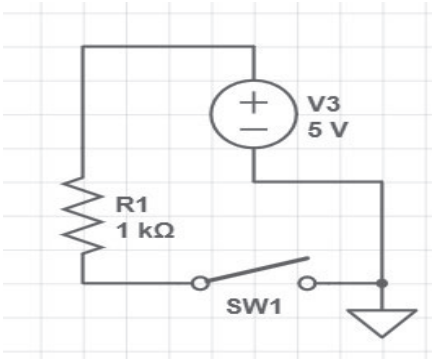

Fig.7: Current flow circuit.

Electromagnetic Locking

When resistance in both force-sensitive resistors equalises, the signal is passed and the electromagnet magnetises and locks at the closest slot. The linear actuator will work on the spring effect as shown in Fig. 8.

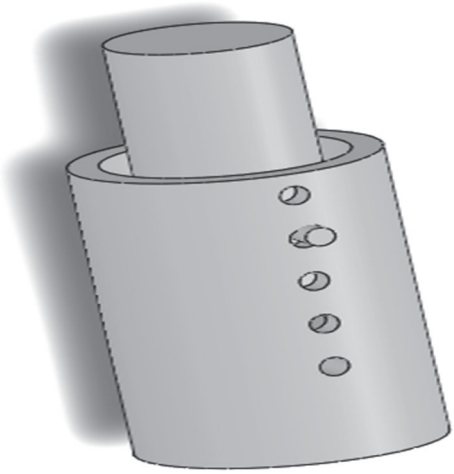

Fig. 8: Design of electromagnetic actuator.

Result and Conclusion

To neutralise the pressure on the foot, the reading of two adjacently placed force-sensitive resistances (FSRs) is compared and counterbalanced until FSR readings become equal, that is, pressure on both sides of the platform becomes equal. By displacing the angle of orientation of the platform below the foot, that is, by counterbalancing the bodyweight that has been shifted in an extreme direction because of the outgrowth at the talus, this results in neutralising the pressure at the foot and, in turn, alleviating the foot from pain and correcting the posture, thereby preventing secondary damages. The pressure cuff around the talus will hold the area to assist efficient bodyweight transfer, providing support to the affected area, and will apply slight pressure.

Thus, the devised assistive device would prove very helpful to patients suffering from this rare condition of osteochondroma at the talus. The only known cure for symptomatic osteochondroma is surgical excision. It should be pointed out that the device is an assistive, not a corrective, one. It will assist patients by helping them manoeuvre a correct gait, post or pre-excision, along with prescribed medications, and hence assist in rehabilitation.

Future Scope

In the future, the design may be further improved with additional modes, using sensors for the measurement of the collapse of patients, and respiratory and heart rates, which could be transmitted using the Internet of Things to the mobile phones of the patient's closest associates, which would issue safety warnings.

Applications

- Prescribed by doctors for rehabilitation purposes post-surgical excision of osteochondroma at the talus.
- Prescribed by doctors in minor conditions, along with medications to manoeuvre normal gait.

Limitations

- Cannot be used for patients with implants because of the interference of electromagnetic fields.
- Low-weight patients generally targeted; for paediatrics, not adults.

CHAPTER SEVEN

3D PRINTING IN HEALTHCARE: A PRAGMATIC APPROACH TRANSFORMING THE FIELD OF MEDICAL SCIENCE

SWATI SIKDAR

Abstract

In the world of advanced healthcare technology, 3D printing is playing a pragmatic role in both saving and improving lives. It has paved the way for the fabrication of prototypes for pre-surgical planning, pharmaceutical research in terms of drug discovery-dosage-delivery, customised limbs or prosthetics, and implants as well as providing anatomical replicas of *in vivo* organs. The software and the printer used for 3D printing can convert the scanned data from imaging into 3D models, leading to the recreation of a patient's injured areas to enable an in-depth interpretation of their present status. A better understanding of the ailment, the development of a superior therapeutic plan, the enhanced outcomes of the treatment with a reduction of patients' dissatisfaction, accusations of malpractice and complaints made regarding the medical support being provided are all made possible with the incorporation of patient-specific, customised 3D-printed models and effective doctor-patient conversations. 3D printing incorporates a rapid prototyping method that ensures quick manufacturing of the specimen's prototypes by high-speed and cost-effective tooling, and also allows an easy demonstration of products, to check the viability of design choices and fast redesigning of faulty prototypes. The selection of materials, the lack of an effective way of integration in hospitals and actual end-users, along with a lack of regulation on 3D printing production process are a few areas that require special attention to overcome some of the challenges in the evolution of this smart technology. This chapter focuses on the relevant information regarding the basics of 3D printing, fabrication methods, the

global scenario, an analysis of the costs, the challenges, and the recent developments of 3D bio-printing in medical applications.

Introduction

Developments in technology have widely affected people's lives in the twentieth century. Computer and Internet technologies are changing the fundamental ways in which things are done on a massive scale. Our lives are thus getting better and better with many new possibilities opening up for the future. 3D printing technology comprises different processes that are widely used to produce parts or products using different materials suited for specific applications.[1] The traditional methods of producing the parts or products were subtractive in nature, or processes that were dependent upon moulding or casting. The most popular and standard traditional methods (subtractive in nature) that were used before 3D printing were injection moulding and CNC machining. These methods, however, have a few limitations.

Traditional methods used a process involving the removal of material until the desired replica of the part of the product was created. Owing to the excess waste created due to this method, and the requirement of multiple machining to reach the end product of the desired specifications, a more efficient production method was required. Apart from this, costs were also higher due to the need to create prototypes as well as the realisation of proof of concept that required several reiterations during the designing process, which also involved the use of expensive tooling machines. Here, if a reiteration were needed for a single product, the entire process had to be repeated from the beginning. Therefore, these methods were also time-consuming.[2] The advantages were only seen in cases when production had reached the mass-production stage, but limitations were found in the product development stage. 3D-printing technology has revolutionised the traditional manufacturing process. It is also known as additive manufacturing, and this is indeed the key because 3D-printing is a radically different manufacturing method that is based on the use of advanced technology. It builds up parts in an additive manner, in layers which may be at a millimetre-scale, i.e. millimetre-thick layers of different materials used one above another. A variety of materials like nylon, carbon, metal or plastics,

[1] Rengier F, Mehndiratta A, von Tengg-Kobligk H, et al. 3D printing based on imaging data: a review of medical applications. Int J Comput Assist Radiol Surg 2010;5:335–41.
[2] Malik HH, Darwood AR, Shaunak S, et al. Three-dimensional printing in surgery: a review of current surgical applications. J Surg Res 2015;199:512–22.

etc., are used per the need for a particular product. This is different from any other existing traditional manufacturing process by changing the fundamental technique of production. The 3D-printing process has become easier due to advances in the associated software. Several steps need to be taken care of before the creation of a prototype or end-use product.[3] The advances in 3D-printing technologies are enhanced by CAD software. It is a crucial tool that contributes to creating 3D objects that can help product development personnel to test and adjust all the key elements in a product before the final shipping of the end-use products. It is used in most product development processes in one form or another. The software and the additive manufacturing techniques are the primary driving forces for the wide adoption of 3D-printing mechanisms in many fields.

The application of 3D printing in every domain of the industry is quite noticeable. Rapid development is witnessed in the fields of automotive, mechanical engineering, civil engineering, aerospace and biomedical engineering, etc. This can be used across the myriad fields of medicine as well. Its emerging trend is most prominent in the medical field where a 3D printed part of the specific organ system can be used in making the patient understand the critical levels of their disease or injury.[4] This in turn leads to the betterment of the doctor-patient relationship, which also leads to better treatment planning, and better healthcare services to the society as a whole. It is evident that we have begun to see the true and massive potential of 3D printing.[5,6] Some key points that should be taken care of during the designing process of the part that is to be 3D printed are as follows:

i) The portion must have some real volume, i.e. a solid one.
ii) Printing of very small and delicate features depends on the type of printer to be used. In many cases, those features may not be printed properly.
iii) For those parts having some overhanging features, support is to be provided for proper printing. It is an area of concern as the model

[3] Friedman T, Michalski M, Goodman TR, et al. 3D printing from diagnostic images: a radiologist's primer with an emphasis on musculoskeletal imaging-putting the 3D printing of pathology into the hands of every physician. Skeletal Radiol 2016;45:307–21
[4] Jabbar Qasim, Alaa Jabbar Qasim, The process and technologies of 3D printing, International Journal of Advances in Computer Science and Technology, Oct 2015.
[5] C.K. Chua, K.F. Leong, C.S. Lim. Rapid prototyping – principles and applications. World Scientific Publishing, Singapore, 2003
[6] Gu, Q., Hao, J., Lu, Y., Wang, L., Wallace, G.G. and Zhou, Q., 2015. Three-dimensional bio-printing. Science China. Life Sciences, 58(5), p.411.

so developed will be cleaned after removing those supports. If the part is delicate, then it might break.

iv) Before using the printer, calibration must be done each time. The orientation of the part should also be considered as some printers have precise X and Y axes rather than the Z-axis.

v) It must be ensured that the part must stick properly for the building plate. Without this, at some point the portion may loosen and ruin the entire task.[7,8]

The conventional 3D printing that uses living cells and growth factors together to produce tissue-like structure that mimic natural tissue is known as Bio-printing. This technique has huge potential for application in the fields of bioengineering & medicine.[9,10,11]

General Requirements for Fabrication with 3D Printing Technology

Technology is the key driving force for most of the instruments that are designed. Most of the 3D printers available use several available technologies.[12,13] The technology bridges the process between printing the

[7] Shafiee, A. and Atala, A., 2016. Printing technologies for medical applications. Trends in molecular medicine, 22(3), pp.254–265.

[8] Ludmila Novakova-Marcincinova, Ivan Kuric, Basic and Advanced Materials for Fused Deposition Modeling Rapid Prototyping Technology, Manuf. and Ind. Eng., 11(1), 2012, ISSN 1338-6549

[9] Sabina Luisa Campanelli, Nicola Contuzzi, Andrea Angelastro, and Antonio Domenico Ludovico (2010). Capabilities and Performances of the Selective Laser Melting Process, New Trends in Technologies: Devices, Computer, Communication, and Industrial Systems, Meng Joo Er (Ed.), ISBN: 978-953-307-212-8, InTech

[10] Fazil O. Sonmez, H. Thomas Hahn- Thermomechanical analysis of the laminated object manufacturing, Rapid Prototyping Journal Volume 4 · Number 1 · 1998 · 26–36

[11] Paulo Jorge Ba´rtolo and Ian Gibson, History of Stereolithographic Processes, Centre for Rapid and Sustainable Product, Polytechnic Institute of Leiria, Leiria, Portugal

[12] Beaumont Newhall (May 1958) “Photosculpture,” Image, **7** (5) : 100–105 François Willème, “Photo-sculpture,” U.S. Patent no. 43,822 (August 9, 1864). Available on-line at: U.S. Patent 43,822 François Willème (May 15, 1861) “La sculpture photographique”, Le Moniteur de la photographie, p. 34

[13] Huotilainen E, Jaanimets R, Valášek J, et al. Inaccuracies in additive manufactured medical skull models caused by the DICOM to STL conversion process. J Craniomaxillofac Surg 2014;42:e259–e265.

material and the finished product. All of the technologies are different from one another in terms of the method of usage of materials and depositing the same for printing the end-product. Some techniques liquefy the printing material to form the different layers whereas other techniques simply soften the materials to make the different layers. Other techniques may use a high-powered UV laser to cure the photo-reactive resin and then print the desired object. Some broadly used 3D printing technologies are discussed below:

1) Fused deposition modelling (FDM)

One of the technologies used in 3D printing is FDM. This is used for prototyping. In this method, from the data that has been inputted in the printer, layer-by-layer is developed in a bottom-up approach by heating and extrusion of the thermoplastic filament from the chosen printed material.[14] Each layer bonds with the previous layer and settles down. The control mechanism is there for the horizontal and vertical movements of the extrusion head. This method is cheaper, but less accurate. Post-processing is required for printed parts. For certain kinds of geometry, the process can be time-consuming. Printing material is limited to thermoplastic.

2) Selective Laser Sintering (SLS)

In this method, heat from a high-power driven laser beam is used to join small plastic, ceramic, glass particles to form solid structures.[15] As per the source or data file, tracing of the laser is done across the powder bed containing compressed powder. After the sintering of a layer, the direction of the laser is changed. This process continues until printing of the whole object is finished. The object needs to be cooled down. Finally, the powder bed is removed & excess powder is taken out from the end product. The advantage of this technique is that it does not require any supporting structure for complex parts or additional tooling. It saves material and reduces the time and cost of production; post-processing time is shorter.[16] This technology is quite useful in printing particular geometries that other

[14] Ploch CC, Mansi C, Jayamohan J, et al. Using 3D Printing to create personalized brain models for neurosurgical training and preoperative planning. World Neurosurg 2016;90:668–74.

[15] Kondo K, Harada N, Masuda H, et al. A neurosurgical simulation of skull base tumors using a 3D printed rapid prototyping model containing mesh structures. Acta Neurochir 2016;158:1213–9.

[16] Chan HH, Siewerdsen JH, Vescan A, et al. 3D rapid prototyping for otolaryngology head and neck surgery: applications in image-guidance, surgical simulation, and patient-specific modeling. PLoS One 2015;10:e0136370.

methods cannot. The printed objects are durable, robust and easy to use.[17] A variety of printing materials can be used, ranging from plastic to glass to ceramics to metals.

3) Laminated object manufacturing (LOM)

In this method, high temperature and pressure are used for laminating or fusing the sheets of plastic or plastic materials. They are then shaped to the required form with a computer-controlled laser or blade. This type of printer uses a continuous sheet of plastic or paper-like material coated with adhesive, and a roller feed spreads these materials along the build platform. By using a computer-controlled laser or blade, the material is cut into the desired pattern and the laser removes the excess material. Once the printing is complete, the object is detached from the build platform and the surplus materials are then removed.[18] Some slight post-processing like sanding, painting or varnish is required to keep out moisture. The cost of this technique is quite low as the material is readily available, and it does not involve chemical reactions or the usage of an enclosed chamber. Developing a larger model is easier using this method, but it is not ideal for developing objects with complex geometries or developing functional prototypes. That's why LOM is only used to make scale models and conceptual prototypes that can be tested for design or form.

4) Stereo-lithography (SLA)

This method is described as the original 3D-printing process. It is mostly used to create models, prototypes, and patterns. It is a laser-based process and uses an ultraviolet laser and a vat of resin for making parts. The laser beam projects the design onto the surface of the liquid polymer. Chains of atoms in the polymer gum connect once they have been exposed to the UV laser.[19] The laser beam is focused as per the data input fed to the printer. After one layer is formed, the platform in the vat is dropped and the direction is changed for tracing the next layer on to the top of the previous layer. After completion of the entire product, the platform is removed from the vat. This process is less time-consuming and is relatively cheaper. It requires support structures for parts with overhanging structures. Support structures are

[17] Levi D, Rampa F, Barbieri C, et al. True 3D reconstruction for the planning of surgery on malformed skulls. Childs Nerv Syst 2002;18:705–6.
[18] Wong KV, Hernandez A. A review of additive manufacturing. ISRN Mech Eng2012; 2012:1–10.
[19] Guo N, Leu MC. Additive manufacturing: technology, applications, and research needs. Front Mech Eng 2013; 8:215–43.

manually detached during the post-processing stage which includes a chemical bath for object cleaning and subjecting the object in an oven-like machine for the purpose to fully harden the resin. SLA serves as one of the most accurate 3D printing processes because it enables a superb surface finish, ensuring smoothness when compared to most other rapid prototyping methods.[20]

Materials

Together with 3D printing technologies, the materials to be used are of great importance while one attempts to recreate a model of the subject of interest. The selection of materials plays a key role in the quality of the fabricated product. Plastics, alloys, ceramics and metals are the most common materials used. Along with these materials, living cells have also become popular fabrication materials as they have major applications in medical education or research, and in the biomedical and healthcare domains.

Prototype manufacturing uses the fusion and deposition of the printing material on the build plate.[21] The type of printer and the corresponding technologies also contribute to the material selection criteria for a 3D printing job. Some commonly used examples of printing materials are: plaster, paper, metal foil, plastic film, metal alloys, thermoplastics, liquid resin and photopolymer, etc. 3D printing in the medical field is revolutionising the industry as it uses a special type of material rather than the alloy or plaster or plastic, etc. This specific material used in bio-printers is bio-ink which uses a computer-guided pipette to layer living cells one above the other. Thus, artificial living tissues are created. Here the biomaterials such as the living cells are combined with different growth factors and are called bio-ink. Instead of thermoplastic or resin, the living cell suspension is utilised.

Printers

It is the device that helps the healthcare professionals to develop the customised anatomical model, implants, prosthetics, a replica of the specific

[20] Kim GD, Oh YT. A benchmark study on rapid prototyping processes and machines: quantitative comparisons of mechanical properties, accuracy, roughness, speed, and material cost. Proc Inst Mech Eng B J Eng Manuf 2008; 222:201–15.
[21] C. L. Ventola, "Medical applications for 3D printing: current and projected uses," *Pharmacy and Therapeutics*, vol. 39, no. 10, pp. 704–711, 2014.

region of interest, and tissue fabrication, or organ fabrication.[22] The three-dimensional models of the *in vivo* body parts that have been fabricated help the health care service providers provide better services. The commonly used file format for the 3D printers is STL: the 3D models are converted into a readable format for the printer to print.

Generally, the available 3D printers are of the following types, using different technologies and materials for the development of the prototypes:

a) Extrusion Type Printer → This uses fused deposition modelling. The materials used by them are thermoplastics and eutectic metal.
b) Granular Type Printer → This type of printer can use any one of the mentioned technologies like direct metal laser sintering, electron beam melting, selective heat sintering, selective laser sintering, powder bed and inkjet head 3D printing or plaster-based 3D printing, and can use materials like metal alloys, titanium alloys, thermoplastic powder, thermoplastics, metal powders, ceramic powders or plasters, respectively, for the selected purposes.
c) Laminated Type Printer → Laminated object manufacturing is the technology used by this printer; paper, plastic film, and metal foil are the common materials used by this printer.
d) Light Polymerised Type Printer → In this type of printer, two techniques are used, namely stereo-lithography and digital light processing. The materials used by this printer differ, depending on the technology used. A photopolymer is used for stereo-lithography and liquid resin is the common material while using the Digital Light Projector (DLP) technique.
e) Bio-printer → This printer works in a similar way to conventional printers, and can use any of the technologies like laser, extrusion, inkjet, or acoustic. The material used by this printer is commonly known as a bio-ink which is a combination of the base materials like gelatine, collagen, silk, alginate, nano-cellulose, and biomaterials like living cells. The delivery of bio-ink depends on the type of tissue to be printed, along with the number of nozzles. It comes as a fluid with high viscosity from the nozzle.[23]

[22] D. B. Jones, R. Sung, C. Weinberg, T. Korelitz, and R. Andrews, "three-dimensional modeling may improve surgical education and clinical practice," *Surgical Innovation*, vol. 23, no. 2, pp. 189–195, 2016.
[23] O'Neill, B., Wang, D. D., Pantelic, M., Song, T., Guerrero, M., Greenbaum, A., & O'Neill, W. W. (2015). Transcatheter naval valve implantation using multimodality

Process Involved in Medical 3D Printing

3D printing is a new technological advance, and it is a process that is constantly evolving. Its rapid expansion in terms of its application is visible in the medical field.

Five technical stages are mandated for a complete printed model of patient-specific anatomical structures whose datasets are acquired from imaging modalities: time, expertise, and money are required for these stages.

- Selection of the area of interest or object of interest.
- Creation of the three-dimensional geometry from appropriate datasets.
- File optimisation for printing
- Proper selection of the materials
- Selection of the Printer

Since medical 3D printing also plays a vital role in medical education and research, a few essential characteristics of the model are therefore addressed for educational purposes. They are:

- The exact size needed for the specific organ or anatomical area is necessary;
- Whether the surrounding structures are required or not to describe the area of interest;
- Accuracy and resolution of the model that is appropriate for medical education and research purposes;
- If surgical manipulation needs to be performed, then the emphasis is given to the precise detail of the anatomy and material characteristics of the model for resects and sutures.

To describe a complete bio-printing method based on either laser technology, extrusion or inkjet, etc., these chronological sequences are maintained:

Imaging → To get the exact dimension of *in vivo* parts, medical imaging is performed. Pseudo 3D images are constructed thereafter.

Modelling → With the help of CAD software, a blueprint is generated. A fine adjustment is made in order to avoid the transfer of defects.

---

imaging: roles of TEE, CT, and 3D printing. JACC. Cardiovascular Imaging, 8(2), 221–5. http://doi.org/10.1016/j.jcmg.2014.12.006.

Inking → Bio-ink is prepared. Living cells and a compatible base is used. The base is the platform for the growth of scaffolds which also provides them with nutrients. Bio-ink is patient- and function-specific.

Printing → Bio-ink is deposited layer by layer where the thickness of each layer is 0.5 mm or maybe less.

Solidification → After deposition, the viscous liquid settles and solidifies to stabilise into shape. This process is called solidification. It can be aided by heat or some specific chemicals or UV light.

The entire bio-printing or medical 3D printing process with the above-mentioned chronological sequences revolves around three phases:

1. Pre-Bio-printing → In this phase, the three-dimensional digital models are created from the medical image data.
2. Bio-printing → Here, layer-by-layer deposition of bio-ink from the cartridge takes place based on the graphical model.
3. Post-Bio-printing → The final phase where the printed parts undergo mechanical and chemical stimulation for the creation of stable structures out of the biological materials.[24]

Recent Development in Medical Applications

Automobile manufacturers, aerospace industries, construction, architecture, etc., are the industries that widely use 3D printing technology. But the recent growth of 3D printing and its applications has led to its expansion in the field of medical science and healthcare engineering. The main importance of medical 3D printing or bio-printing is with the creation of the structures that mimic the native micro- and macro-environment of human physiology. It helps healthcare professionals to develop a customised anatomical model, implants, prosthetics, and replicas of the specific region of interest, tissue fabrication, or organ fabrication. Treatment procedure and their efficiency can be tested for some diseases by using the models of artificially affected tissues.

The three-dimensional models of the *in vivo* body parts that have been fabricated help the healthcare service providers to a better understanding of the pathology of the disease. They also help the physician to provide the

[24] Klein GT, Lu Y, Wang MY. 3D printing and neurosurgery—ready for prime time? World Neurosurg. 2013;80(3–4):233–235

patients with a superior description of the actual internal condition and this also makes it quite easy for them to develop a realistic and improved treatment plan whereby they may choose whether to approach the treatment surgically or therapeutically. The clarity that is brought about in the doctor-patient relationship is a step towards a better healthcare management system. Because of the expediency, flexibility, and customisation capability, 3D printing has a marked influence on multiple facets of the medical science or biomedical field.[25] The applications of 3D printing in medicine are now so numerous that an exhaustive and comprehensive study of all of them is practically impossible. Recent developments that show its remarkable impact in medical applications are described below:

a) Structural Assistance to Surgery: In the case of pre-procedural assessment for transcatheter naval valve implantation, 3D printing is used for prototyping the model of a targeted valve whose image is acquired by contrast-enhanced CT [23]. This anatomical replica enables the testing of the different implantable devices. Repeated procedures can thus be prevented which may potentially lead to morbidity, and the mortality rate of patients will be reduced as the usage of the replica will enable the possibility of attempting an optimal placement, to have a leakage evaluation, and afford an assessment of the possibility of the device to be a proper fit or not.

Before performing complicated surgery on the patients, surgeons can use this structural model of the parts that are patient-specific for trial.[26] This technique proved to be a way to speed-up the procedures and minimise surgery-related trauma.

b) Pharmacology: The field of personalised pharmacology also finds many possibilities through the application of three-dimensional printing. It is currently an integral part of pharmaceuticals. Here, the targeted dose of a generic drug is printed for the patient at a certain point of care.[27] Again, it can also help in the modification of the dosage profile with multiple

[25] Rosset, A., Spadola, L., & Ratib, O. (2004). OsiriX: an open-source software for navigating in multidimensional DICOM images. Journal of Digital Imaging, 17(3), 205–16. http://doi.org/10.1007/s10278-004-1014-6.

[26] Schmauss D, Schmitz C, Bigdeli AK, et al. Three-dimensional printing of models for preoperative planning and simulation of transcatheter valve replacement. Ann Thorac Surg 2012;93:e31–e33.

[27] Schmauss D, Haeberle S, Hagl C, et al. Three-dimensional printing in cardiac surgery and interventional cardiology: a single-center experience. Eur J Cardiothorac Surg 2015;47:1044–52.

sustained releases and immediate release layers. Since there is a difference in the patient's metabolism, the drug is dosed to the exact need of the patients.[28] This technique may prove to be beneficial to those patients with a narrow therapeutic window.[29] With personalised treatments, it is also capable of reducing the number of pills a person has to take. This proves to be very cost-effective as well as being an ethical option as the side effects of the drugs can be identified which in-turn will help in administering safe dosages. The potential in this field lies in the process of drug testing, clinical trials and the reduction of animal trials.[30,31]

c) Tissue Engineering: Some of the most complex care is associated with organ transplants. A serious phase of evaluation is associated both before and after a transplantation process. The complexity of the process is mainly due to the scarcity of compatible organs. In this application area, "bio-ink" is used to generate a scaffold and sometimes entire organs. Given the ability to "print" organs based on an individual's DNA, the concept of rejection could become a thing of the past.[32] Researchers are working on three-dimensional printed dermal grafts that can be directly applied to burn victims.

d) Custom-made Prosthetics: Many people suffer from the loss of a limb due to an injury during war or from an infection or accidents or due to some genetic condition. Prosthetics help them to have a better and improved quality of life in a way by restoring the function of the lost limb(s).

In earlier cases, prosthetics were generally designed whereby patients used some padding to fit into the prosthetic. Due to the existence of intricate

---

[28] Vukicevic M, Puperi DS, Grande-Allen KJ, et al. Erratum to 3D printed modeling of the mitral valve for catheter-based structural interventions. Ann Biomed Eng 2016;44:3432–12.

[29] Rengier, F., Mehndiratta, A., von Tengg-Kobligk, H., Zechmann, C.M., Unterhinninghofen, R., Kauczor, H.U. and Giesel, F.L., 2010. 3D printing based on imaging data: a review of medical applications. International journal of computer-assisted radiology and surgery, 5(4), pp.335–341.

[30] Martelli, N., Serrano, C., van den Brink, H., Pineau, J., Prognon, P., Borget, I. And El Batti, S., 2016. Advantages and disadvantages of 3-dimensional printing in surgery: a systematic review. Surgery, 159(6), pp.1485–1500

[31] Pati, F., Gantelius, J. and Svahn, H.A., 2016. 3D bioprinting of tissue/organ models. Angewandte Chemie International Edition, 55(15), pp.4650–4665.

[32] Arslan-Yildiz, A., El Assal, R., Chen, P., Guven, S., Inci, F. and Demirci, U., 2016. Towards artificial tissue models: past, present, and future of 3D bioprinting. Biofabrication, 8(1), p.014103.

details that are unique to different patients, this design feature is not sufficient as it does not suit every condition. But now, with the advent of 3D printing, a better solution can be provided for these patients where there is a possibility of a customised interface between the patient and their prosthetics. The friction in the interface and skin stress is reduced by this customisation, which gives an enriching experience in terms of advanced healthcare. It is also possible to design the prosthetics per the specific need of a single patient at a time.[33] The cheaper products are especially economically viable for children as they quickly outgrow their prosthetic limbs.

e) Medical Education: Human Anatomy is a complex subject that is not easily understood by the patient, patient party, and even doctors, who may be going through it for the first time. The traditional method was to use cadaveric materials for training purposes. Due to ethical issues and the costs involved in the process, controversies abound. Here, 3D printing serves as a novel, realistic, effective substitute by recreating the intricate anatomical structures accurately from the image data acquired from the high-resolution medical imaging modalities like CT Scanners and MRI machines, etc.[34,35] The physical three-dimensional models of *in vivo* body parts provide a detailed aid in understanding the structure-function relationship in reality. Besides, the ability of 3D printing to recreate several copies of any anatomical subject in different sizes gives a great advantage in training facilities, especially in situations where using a cadaver is not an option. For the patient who needs some treatment or surgical procedure, doctors use a 3D printed model of the section of interest to offer better treatment planning, which also enables the doctor to better explain the procedure to the patient. Instead of speaking in abstract terms, the physician would have a concrete object that the patient could directly interact with.[36] For medical students, this could create an additional tool in anatomy. While the use of medical

[33] Fahmy, M.D., Jazayeri, H.E., Razavi, M., Masri, R. and Tayebi, L., 2016. Three Dimensional Bioprinting Materials with Potential Application in Pre-prosthetic Surgery. Journal of Prosthodontics.

[34] Kong X, Nie L, Zhang H, et al. Do 3D Printing models improve anatomical teaching about hepatic segments to medical students? A randomized controlled study. World J Surg 2016;40:1969–76.

[35] Noecker AM, Chen JF, Zhou Q, et al. Development of patient-specific three-dimensional pediatric cardiac models. ASAIO J 2006;52:349–53.

[36] Walenga RL, Longest PW, Sundaresan G. Creation of an in vitro biomechanical model of the trachea using rapid prototyping. J Biomech 2014;47:1861–8.

cadavers will likely never be replaced, 3D models can demonstrate both normal and variant anatomy in a physical form to aid in the learning process.

f) Medical Research: Several tests need to be performed in medical research-oriented tasks. Automated structures of the cells are produced by the 3D printers.[37] Various toxicity tests for the development of new treatments are performed on these cells. This eliminates the chances of the drugs becoming toxic to humans when used in a practical scenario. The research process gets accelerated with reproducible tissue printing that matches the actual arrangement of the cells found in natural organs/tissues. The tissue constructs and organoids are helpful in medical research as they mimic the biological organs on a miniature scale, thus serving as alternatives in medical research.[38]

g) Organ Printing: The production of human organs or tissue structures can be integrated with biocompatible microfluidics for the creation of intense and complex structures that can easily mimic the native natural organs in their functioning. Advancements in this technology with its crucial application in the medical field holds the next step in printing organs that can be transplanted into human donors, or even printing *in vivo* organs in the body *in situ* in the operating room itself. This technology is yet to mature as compared to others, but has the potential to revolutionise medicine and healthcare by providing a system which would make the native organ transplants and current synthetic artificial organs obsolete. It will help in solving organ-related problems more quickly, and more easily.[39]

h) Cosmetic Surgery: For skin-grafting purposes or plastic surgery, this technology can be used.[40] Bioprinted skin tissue can be commercialised.

i) Surgical Instrument Printing: Forceps, clamps, scalpels, hemostats, etc., can be printed by medical 3D printers. Moreover, this technology can

[37] Singhal AJ, Shetty V, Bhagavan KR, et al. Improved surgery planning using 3-D printing: a case study. Indian J Surg 2016;78:100–4.
[38] Spottiswoode BS, van den Heever DJ, Chang Y, et al. Preoperative three-dimensional model creation of magnetic resonance brain images as a tool to assist neurosurgical planning. Stereotact Funct Neurosurg 2013;91:162–9
[39] Ho D, Squelch A, Sun Z. Modeling of aortic aneurysm and aortic dissection through 3D printing. J Med Radiat Sci 2017;64:10–17.
[40] Chae MP, Rozen WM, McMenamin PG, et al. Emerging applications of bedside 3D printing in plastic surgery. Front Surg 2015;2:25.

make surgical instruments very tiny in size, and with better precision.[41] The surgeons can operate on minuscule areas, reducing the amount of cutting that is done, and thus unnecessary damage to patients is avoided.

j) Miscellaneous: A few more applications are bone tissue regeneration, foodstuff production, hearing aid production, etc. Recently, advancements are seen in the case of cartilage tissue production that can be used in reconstruction or regeneration.[42] Dentistry has also witnessed the benefit of three-dimensional printing in producing dental retainers, orthodontics, etc.

Global Scenario & Costing

The global scenario of 3D printing technology can be well understood by looking at its utilisation in a wide range of modalities. Automobile manufacturers, aerospace industries, construction, architecture, biomedical engineering are some of the domains that have contributed to the wide-scale application of three-dimensional printing. High-technology-driven industries are capable of developing functional parts for testing purposes with the help of advanced 3D printing as a prototyping tool. With the reduction in the design cycle, design teams can create the specific part exactly as it needs to be, and considerably faster. It also enables the creation of complex geometrical structures more easily. The products so developed are lighter in weight but stronger than their machined counterparts. Civil engineers and architects find layer-by-layer manufacturing quite effective. They create the model of the layout or model of the building's shape for their project. The prototype printer system which is widely used can create a structure similar to a small house by using concrete or some other specialised materials.

The field of healthcare technology is also using this technology for the recreation of body parts or any specific organ that is required. The material used for developing limbs as a replacement for a part is different from the usual raw materials as they are biological cells that are synthesised in the laboratory. The chances of rejection by the body are minimised here as the cells that are grown are specific for a particular patient. Generally, the available prosthetic limbs are not customised to the needs of the patient and

[41] Ho, C.M.B., Ng, S.H. and Yoon, Y.J., 2015. A review of 3D printed bioimplants. International Journal of Precision Engineering and Manufacturing, 16(5), pp.1035–1046.

[42] Abduo, J., Lyons, K. and Bennamoun, M., 2014. Trends in computer-aided manufacturing in prosthodontics: a review of the available streams. International journal of dentistry, 2014.

are used to replace the parts that are lost due to disease or injury. 3D printing ensures the possibility of designing and developing prosthetic limbs based on the exact requirement of the patient and the scanned data from the existing structure of the patient. In all of these industries, the goal of 3D printing is based on the concept of creating entire parts that work like the original parts to be replaced that may be used as the final product that may be produced directly from the printer. In the near future, cars, aircraft, or an entire building can be developed by using 3D design modelling framed on CAD software, generating a layer-by-layer pattern just as a typical 3D printer works at the moment.[43] Most of the transformation in this area will be feasible from the origination of the appropriate materials. It will eliminate the testing phase as well as replace many cranes or construction workers. For bio-printing, a sterile printing condition is essential for optimising cell viability and achieving printing solutions appropriate for correct cell-matrix structure. Thus, accuracy in complex tissue is required and cell to cell distance is also to be ensured so that the exact output required is produced. Manufacturing a new product always involves reiterations of the same design with few modifications. This technology paves the way for the designers to visualise and assess their prototype without waiting for months. Thus, a version of every feasible idea is developed so that it can be reviewed by comparing and contrasting every feature for betterment of the actual end product.

With advanced 3D printing, one can create a part with the desired quality which will have the same look and feel of the finished product. Some segments can also be explored just like the real one as they have a direct impact on human lives. US-based medical laboratories and research companies are working with the liver and intestinal tissue models to study the organs for drug development purposes. Similarly, 3D brain organoids have also been developed for disease modelling. In Dubai, doctors have used a 3D printed model of arteries to allow for the safe navigation of the blood vessel before operating on a person suffering from a cerebral aneurysm. In 2018, in Belfast, a 3D printed model of a donor's kidney was used for kidney transplantation for a young woman. During the product development stage, the cost-to-benefit ratio is limited. For small- or

[43] Sheth, R., Balesh, E.R., Zhang, Y.S., Hirsch, J.A., Khademhosseini, A. and Oklu, R., 2016. Three-dimensional printing: an enabling technology for IR. Journal of Vascular and Interventional Radiology, 27(6), pp.859–865.

medium-scale production or requirements, industry cannot justify the cost concerning their benefit during the development of a product.[44,45]

But it is obvious that with a change in the requirements, the costs of this this technology are reduced, and the benefits are quite noticeable. The benefits of 3D printing in healthcare can be measured from the following: enabling better communication between a patient and the physician, better healthcare outcomes, proper treatment planning, less time during operation procedures, and reduced costs. By 2025, 3D printing in the medical technology field may be worth 3.5 billion dollars, and the industry's annual growth rate may reach 17.7%. Production costs using 3D printing are significantly lower than traditional manufacturing methods.

Challenges in Medical 3D Printing

Despite all the advantages and promises offered by any advanced technology, challenges are inevitable. Medical 3D printing is a very crucial area where the end-product has a major role in the provision of healthcare services. The recreation of the 3D model of the human anatomical part is solely dependent on the real-time DICOM data or perhaps data from any other modality. It is thus a must that the original dataset collected from the subject must be clean, accurate, and sufficient. Any kind of discontinuities or distortions observed in the model, or insufficient post-processing, can stop the entire process before completion.[46] The designing and prototyping process is speeded up by using the additive technology of modern printers. Fabricating one part at a time, or improvising the blueprint each time it is produced, is easier. Regeneration of the model can be done in a few hours. The design cycle for the prototype becomes a matter that can be addressed in a few days or weeks in contrast to the traditional methods that previously took months.

---

[44] Wu, X.B., Wang, J.Q., Zhao, C.P., Sun, X., Shi, Y., Zhang, Z.A., Li, Y.N., and Wang, M.Y., 2015. Printed three-dimensional anatomic templates for virtual preoperative planning before reconstruction of old pelvic injuries: initial results. Chinese medical journal, 128(4), p.477.

[45] Vodiskar, J., Kutting, M., Steinseifer, U., Vazquez-Jimenez, J.F. and Sonntag, S.J., 2017. Using 3D physical modeling to plan surgical corrections of complex congenital heart defects. The Thoracic and cardiovascular surgeon, 65(01), pp.031–035.

[46] Truscott, M., Booysen, G. and De Beer, D., 2010. Rapid prototyping and manufacturing in medical product development. Annals of DAAAM & Proceedings, pp.1573–1575

Though considerable improvements have been made in printer timing, the time required is still dependent on the type of the job to be done.[47] Post-processing time is determined by the method of printing used. Sometimes, smoothing is required to give a finished, fine-tuned look to the end product. The presence of irregularities in the three-dimensional model that may have occurred from the original scan which has been fed into the printer would require rectification. Some complications may also arise in the context of ownership. Till now, no FDA-approved 3D printer has been present, and there has been no standardization with regard to the grade of the printed models. The next issue that arises is the safety factor of the printer, which must be thoroughly checked before being purchased. In the case of rapid prototyping, the chances of mishandling are a matter of concern as some machines use heat, lasers, and a lot of different materials. Thus, these key parameters are also contributing factors to be considered while purchasing the three-dimensional printers. To ensure the safe use of the printers, a development team should be there to assess the location of the printer, its usage frequency, the efficiency of the user, etc., along with other allied safety features. From the results of different surveys, some more challenges in medical 3D printing include the regulatory environment, reimbursement policy, technology, and qualified workforce, because regulations for medical 3D printing and validation processes are still being formulated. Additionally, health insurance does not reimburse the cost of 3D printing, and technology-bio-ink-software is continuously evolving. There is also a demand for skilled technical personnel with the blending of biology and engineering for working in the healthcare sector.

Summary

Not every type of production may be possible or fulfilled by the use of three-dimensional printers. But the advancement of 3D printing is accelerating the production in some industrial areas more than ever before. The major areas of application are prototyping requirement-specific specialised parts in the fields of defence, aerospace, medical science, healthcare technology or home decor, etc. The advent of this may contribute to its future application in research, developing organoids, buildings, cars, etc. Through this chapter, we have a glimpse of the most utilised 3D printing techniques and the qualities that each technique is filled with. We also learned about the

[47] Marro, A., Bandukwala, T. and Mak, W., 2016. Three-dimensional printing and medical imaging: a review of the methods and applications. Current problems in diagnostic radiology, 45(1), pp.2–9.

material selections or choices depending upon the end-product to be developed. The strength and weaknesses of each printing technique in the context of rapid prototyping have also been highlighted. Another area of discussion is its field of application. The evolution of this technology is widely visible from its initial roots in manufacturing industries into the spectrum of medical science and healthcare technology. This has led to the overcoming of many experimental challenges before reaching the stage of implementation. All the earlier methods were subtractive in nature, quite expensive and time-consuming, and they also used some computer-aided designing models with a precise machining plan with clear instructions of the way to cut, mill, or drill the raw material into the desired object. Modern methods are promising.

The additive method based on the layer-by-layer production mechanism makes it more flexible and creative for users. It also eliminated the requirement of CAD designers for the creation of the object for the manufacturer. Now, the person can create the required object which is stronger in the specified areas that are desired and have a light overall weight by using better designing methods.

The printer and the software used nowadays are far more efficient, fast, and effective than traditional ones. Medical and healthcare technology is witnessing this phenomenon. The cardinal point of development and the driving force of 3D printing in clinical applications is in radiology. It is possible to convert the medical images captured from high-resolution MRI or CT devices into some 3D models which are then used to print 3D models of the concerned organs or parts. The doctors can thus use this recreated anatomical model to explain to the patient or their family members about their medical status and prospective treatment. This can also be used across diverse fields of medicine. Healthy doctor-patient communication is always desirable for effective healthcare services. Its impact is also remarkable in other industries. Many articles reviewed the industrial revolution created by this technology. With its use, designers are capable of creating the kind of required object, intricate building, medical implants or organoids or surgical tools, etc. It is even possible to make parts while working in space. Over the years, the cost of the 3D printers has decreased, thereby overcoming the limitations caused by earlier printers that used expensive hardware, materials, and which were much more complex in nature and required a CAD designer for making a trace of the object as per the customer's need. Now, these devices are within the financial reach of the majority of companies or healthcare organisations and customisation is also possible for

most of the jobs requiring precision. Skilled staff, temperature and atmospheric conditions can also affect the 3D printing process.

CHAPTER EIGHT

ASSISTIVE FOOT DEVICE FOR HEMIPLEGIC PATIENTS

SHREYAS PATIL, MIHIR KULKARNI,
SHUBHAM HAJARE, TANVI PARKHE,
RUTWIK PALASKAR, DIKSHA ZUTSHI,
DR. REEMA SHYAMSUNDER SHUKLA,
DR. SAMEER DESAI

Abstract

To develop a cost-effective assistive foot device for hemiplegic patients and also help them with the foot drop to avoid circumduction, strings and laces made of neoprene were incorporated as suspension straps for the foot. The materials used were neoprene fabric, industry-grade polyurethane, strings made up of polyester, and steel springs. A Think3D Printer model Raise3D N2 dual based on fused deposition modelling (FDM) technique was used to make a prototype, and the components were made of polylactic acid. The result was a foot brace with adjustable strings, with a tiny hinge near the heel which had two small strings to produce adequate force to push the patient's foot. This comprised a universally-sized plastic shoe inserted for providing support to the bottom of the foot, with gel pads near the arc of the foot extending to the top near the shin, with a semicircle brace to hold to the shin in line with the soft neoprene sweat-proof fabric. Laces were also tested with the same design, and a simple neoprene wrap around was also tested but did not provide adequate push forward. The foot drop brace comes with a dial, spectra cords, and eyelets. The dial is placed around the ankle with the eyelets fixed on the shoes in a cross pattern. The cord is then looped over the dial at least once. The string is subsequently attached to the eyelets so that its tension can be adjusted depending on how high up the patient

wants his/her foot to be. The second design of the foot drop brace consists of two straps attached to straps which further holds on to the two foot-cups. An additional strap attaches from below the cups to ensure the balance of the foot. The brace also has a heel rest to provide comfort. Lastly, the brace has a hollow cylindrical structure to hold the brace which will ultimately attach to the leg. Improved foot drop braces have thus been designed for hemiplegic patients.

Introduction

Patients suffering from hemiplegia suffer from unilateral weakness on the affected side, with flexed arms that are adducted, and internally rotated. The patient would clutch their arm to one side, and the leg would be dragged in a semi-circle to make up for the weakness in the distal muscles (foot drop) and the extensor hypertonia in the lower limb.[1] The inefficient gait cycle due to the hemiplegic cerebral palsy condition can be improved by using different types of orthoses.[2] The types of orthosis studied involved one that was made of plastic, and another metallic. Both showed improvement in addressing the hemiplegic gait. However, the metallic ankle foot orthosis (AFO) provided better stabilization of the ankle, allowing for an improved heel strike and push-off according to the previous findings.[3] Previous studies showed that a hinged AFO is better than a dynamic AFO since it provides better control of the plantarflexion by using a longer lever arm. The h-AFO to the proximal calf included a heel-toe gait pattern, which ensured reduced plantarflexion, thereby helping to increase step and stride length, and also reduced power absorption.[4] Further, a plastic AFO improves gait stability during walking. It also helps achieve better shock absorption thereby making the ankle foot orthosis a bit more comfortable

[1] Lehmann JF, Condon SM, Price R, deLateur BJ. Gait abnormalities in hemiplegia: their correction by ankle-foot orthoses. Archives of Physical Medicine and Rehabilitation. 1987 Nov; 68(11):763–71.

[2] Wang, Ray-Yau, Lu-Lu Yen, Chao-Chung Lee, Pei-Yi Lin, Mei-Fang Wang, and [3] Yea-Ru Yang. "Effects of an ankle-foot orthosis on balance performance in patients with hemiparesis of different durations." *Clinical rehabilitation* 19, no. 1 (2005): 37–44.

[3] Gök, Haydar, Ayse Küçükdeveci, Haydar Altinkaynak, Günes Yavuzer, and Süreyya Ergin. "Effects of ankle-foot orthoses on hemiparetic gait." *Clinical rehabilitation* 17, no. 2 (2003): 137–139.

[4] Romkes, Jacqueline, and Reinald Brunner. "Comparison of a dynamic and a hinged ankle-foot orthosis by gait analysis in patients with hemiplegic cerebral palsy." *Gait & posture* 15, no. 1 (2002): 18–24.

for the user.[5] Ankle foot orthosis is also known as a foot drop brace, which usually provides support intended to control the position and motion of the ankle, compensate for weakness, or correct deformities.[6] The goal was to stabilize the foot and ankle, and provide toe clearance during the swing phase of the gait, and provide optimal walking for people with hemiplegia.[7]

Methodology and Materials

A foot was devised where a foot brace with adjustable strings with a tiny hinge near the heel had two small strings to produce adequate force to push the patient's feet. Along with this, a universally sized plastic brace for the bottom of the feet with silicone gel pads near the arc of the foot was devised, which extended to the top near the shin, which had a semi-circular brace to hold on to the shin which was lined with soft neoprene sweat-proof fabric. Laces were also tested with the same design; a simple neoprene wrap-around was also tested, but this did not quite provide the push forward. The materials used were neoprene fabric, industry-grade polyurethane, polyethylene, and strings made of stainless steel.

The foot drop brace comes with a dial, spectra cords, and eyelets. The dial is placed around the ankle with the eyelets fixed on the shoes in a cross pattern. The cord is then looped over the dial at least once. Subsequently, the string is attached to the eyelets so that its tension can be adjusted depending on how far up the candidate wants his/her foot to be.

The second design of the foot drop brace consists of two straps attached to straps which further hold on to the two foot-cups. An additional strap

---

[5] Abe, Hiroaki, Akira Michimata, Kazuyoshi Sugawara, Naoki Sugaya, and Shin-Ichi Izumi. "Improving gait stability in stroke hemiplegic patients with a plastic ankle-foot orthosis." *The Tohoku journal of experimental medicine* 218, no. 3 (2009): 193–199.

[6] Park, Jin Hong, Min Ho Chun, Jun Su Ahn, Jong Yun Yu, and Si Hyun Kang. "Comparison of gait analysis between anterior and posterior ankle-foot orthosis in hemiplegic patients." *American journal of physical medicine & rehabilitation* 88, no. 8 (2009): 630–634

[7] Yamamoto, Sumiko, Masahiko Ebina, Shinji Miyazaki, Hideo Kawai, and Toshio Kubota. "Development of a new ankle-foot orthosis with dorsiflexion assist, part 1: desirable characteristics of ankle-foot orthoses for hemiplegic patients." *JPO: Journal of Prosthetics and Orthotics* 9, no. 4 (1997): 174–179
The paper hypothesises the development of an economical assistive foot device that would help in correcting the gait anomaly of a hemiplegic patient as well as the foot drop.

attaches from below the cups to ensure the balance of the foot. The brace also has a heel rest to provide comfort. The sole will be made out of foam to ensure the utmost comfort for the user. Lastly, the brace has a hollow cylindrical structure to hold the brace which will ultimately attach to the leg. See Fig. 1, design of the assistive foot for hemiplegic patients.

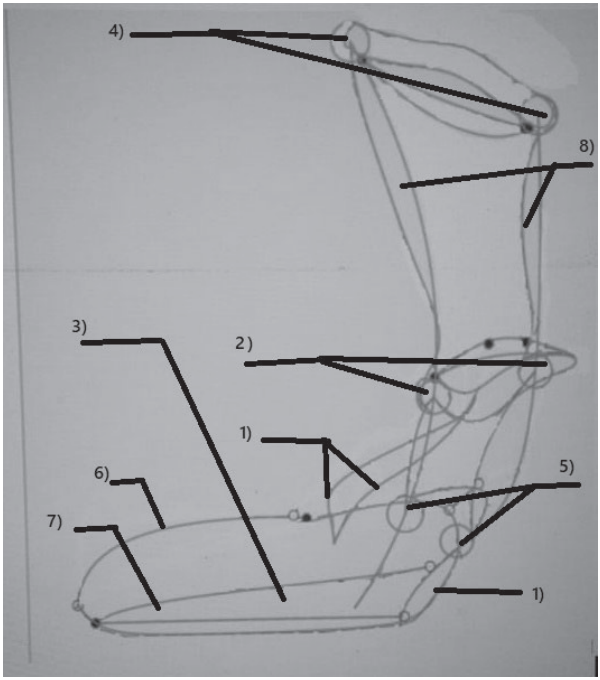

Fig. 1 Design of the Brace for correction of the gait anomaly in hemiplegic patients.

Note:
1) Two sets of strings are attached from the cuff on the bridge of the feet to the cuff above the ankle, transferring the movement of the foot lift as well as the heel cup to provide the necessary tension. 2) The neoprene and plastic brace coated with a sweatproof fabric on the inside of the brace to hold the strings in place provide a support structure. 3) Heel with the silicone gel pad is embedded on the footbed to give support to the foot arc and provide extra stability and reduce pain on the anterior and posterior tibialis, owing to pronation. 4) The neoprene brace above the ankle adds to the support. 5) The heel cup helps in pivoting and giving the foot an extra touch due to the strings moving through them. 6) The footbed structure frame that holds two braces before the toes and on the bridge before the heel cup. 7) The footbed made of a cushioning material like foam on various regions that helps in stabilizing the gait by maintaining the motion 8) The plastic and neoprene-covered

structure of the brace through which the strings move and complete an entire circular course maintaining the tension in the strings.

The sketches have been designed for shoes of the designed orthoses. Fig. 2 shows the lateral cross-section of the shoes. Fig. 3 indicates the top and rear view of the shoes, while Fig. 4 shows the top and rear view of the insole of the shoes. Fig. 5 illustrates the design of the shoes.

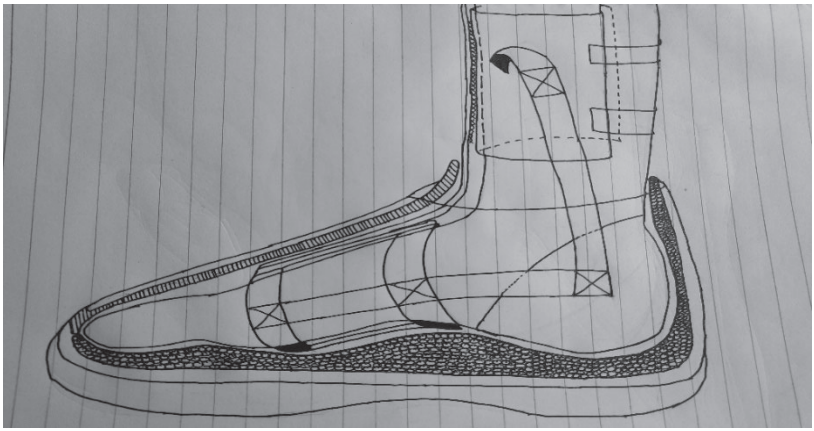

Fig. 2 lateral cross-section of the shoes.

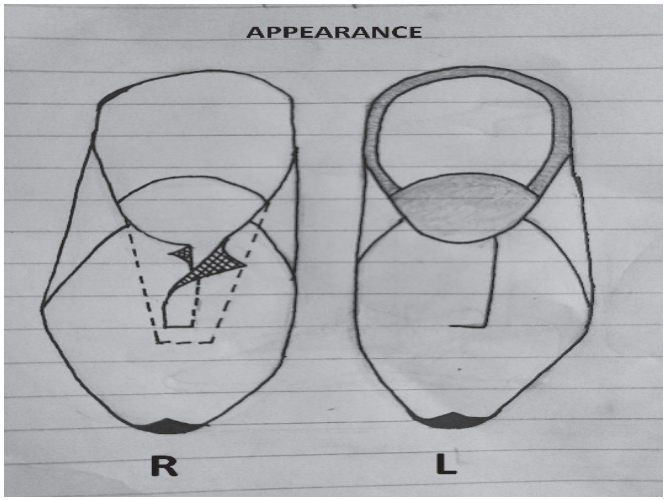

Fig. 3 Top and rear view of the shoes

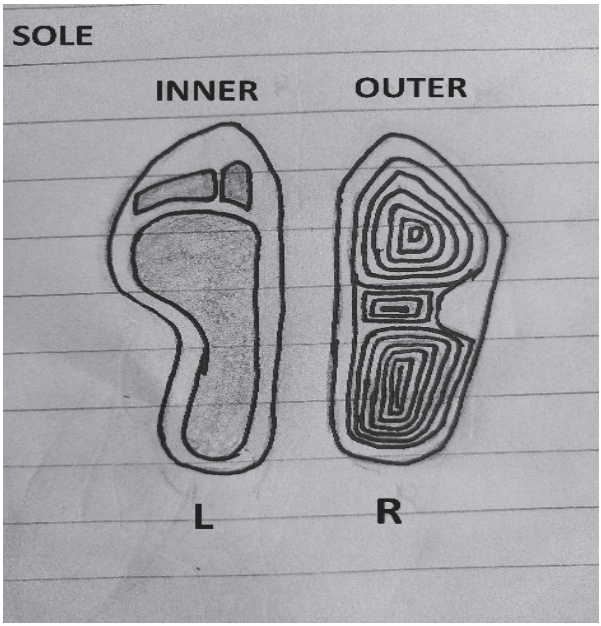

Fig. 4 Top and rear view of the insole of the shoes.

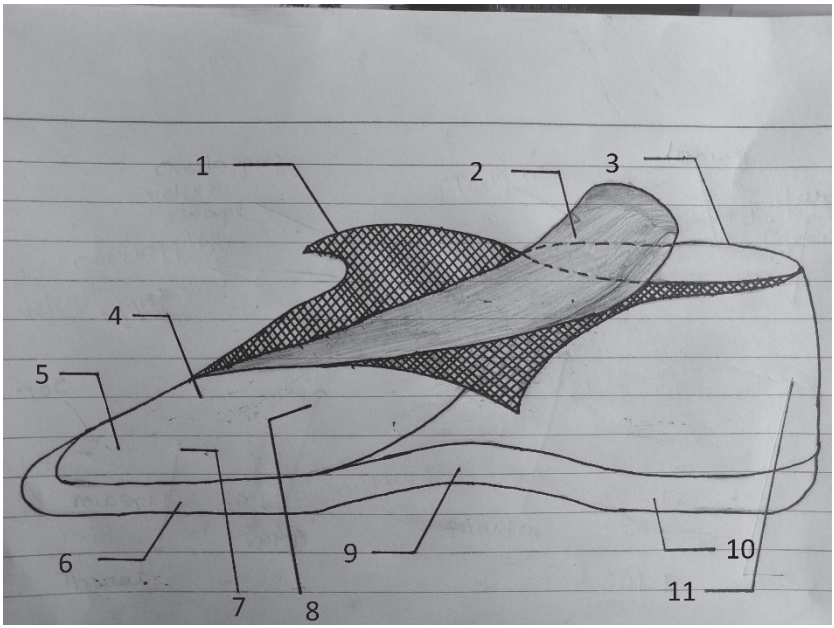

Fig. 5 Shoe design

Note:
1) Overlapping Velcro Straps; 2) Tongue; 3) Lining; 4) Upper; 5) Toe; 6) Mid-Sole; 7) Throat; 8) Vamp; 9) Out-Sole; 10) Heel; 11) Heel-Cap.

Related work

The gait velocity and lop-sidedness of stroke-victims were affected by different physical declensions. Essentially, the gait velocity was affected due to the feebleness of the hip flexors along with the knee extensors, and the imbalance of the gait was mainly influenced by the degree of erratic movement of the affected ankle plantar-flexors.[8] For people affected with hemiplegia, walking without wearing an ankle foot orthosis affected their gait. They had a shorter step, longer duration stance, and shorter duration swing than normal. There was an increased normal flexion of the affected

[8] Hsu, An-Lun, Pei-Fang Tang, and Mei-Hwa Jan. "Analysis of impairments influencing gait velocity and asymmetry of hemiplegic patients after mild to moderate stroke." *Archives of physical medicine and rehabilitation* 84, no. 8 (2003): 1185–1193.

hip during the midstance. The increased knee extension moment due to vertical force might be occurring owing to the centre of mass being placed farther outside the knee. The affected hip adduction during single support was found to be less in the hemiplegic patient than in a fit person, indicating a decreased lateral shift towards the affected side.[9,10] Stainless steel was used in our project because of its high tensile strength, and load-bearing strength, as compared to previous studies where nitinol was used, which was more expensive.[11]

Ankle foot orthoses built with a carbon fibre base offer great stiffness and tensile strength, and is lighter, which makes it an ideal material. They are manufactured by laying up thin layers of carbon fibre on a corrected positive cast, with a predefined bonding agent (generally epoxy resin), and alternating their placement until the desired thickness and stiffness are attained.[12] According to research done by Bartonek et al., carbon fibre-based ankle foot orthoses enhance the gait by improving the ankle plantarflexion moment, the ankle movement, and the stride length, but it should also be noted that the same orthosis does not suit every patient. This must be noted while designing the same, as the patients' individual requirements should be taken into consideration.[13]

Research done by Danielsson et al. indicates that using carbon fibre further reduces the energy cost and oxygen consumption, thereby making it easier

---

[9] Lehmann, Justus F., Sandra M. Condon, Robert Price, and Barbara J. deLateur. "Gait abnormalities in hemiplegia: their correction by ankle-foot orthoses." *Archives of Physical Medicine and Rehabilitation* 68, no. 11 (1987): 763–771.

[10] Whittle, Michael W. *Gait analysis: an introduction*. Butterworth-Heinemann, 2014.

[11] Amerinatanzi, Amirhesam, Hashem Zamanian, Narges Shayesteh Moghaddam, Ahmadreza Jahadakbar, and Mohammad Elahinia. "Application of the superelastic NiTi spring in ankle-foot orthosis (AFO) to create normal ankle joint behavior." *Bioengineering* 4, no. 4 (2017): 95.

[12] Munguia, J., and K. W. Dalgarno. "Ankle foot orthotics optimization using composite reinforcement of free-form structures." In *4th International Solid Freeform Fabrication Symposium.* [online] [accessed 19 Oct. 2016]. Available from Internet: http://sffsymposium.engr.utexas.edu/Manuscripts/2013/2013-61-Munguia.pdf. 2013.

[13] Bartonek, Åsa, Marie Eriksson, and Elena M. Gutierrez-Farewik. "Effects of carbon fiber spring orthoses on gait in ambulatory children with motor disorders and plantar flexor weakness." *Developmental Medicine & Child Neurology* 49, no. 8 (2007): 615–620.

for the patient to walk.[14,15] However, carbon fibre also comes with a huge cost and is not affordable to most, rendering it out of use for this ankle foot orthosis. Neoprene is used to line the semi-circular brace near the shin as it will provide a compact fit while providing the user with more comfort. Neoprene is a very pliable rubber-like material, and its open cell form is breathable which will make the ankle foot orthosis sweat-proof.[16,17]

The main structure of the brace was constructed using polyethylene. It would be utilised in a way that ensures it would provide resistance to plantar flexion. Polyethylene offers low strength, and hardness as well as rigidity, along with having high ductility and impact strength with low friction: the fact that it is also very affordable makes it easily bendable material[18,19] It would allow a high degree of flexibility to the orthosis. If used for an erratic foot, care should be taken to file down the leading edges of the brace in order to avoid marginal pressure on the foot.[20] Straps are band-like devices that wrap around the bottom or top of ankles and knees, etc. They help in relieving stress and pain, and also help in correcting deformities. It is stated in an article (the Development and Use of a Floating T-Strap on a Double Upright Metal AFO to Correct Coronal–Plane Pathologies and Reduce Skin Shear) that a floating T-strap (T-shaped strap) is as efficient in correcting frontal plane deformities as a conventional T-strap. It reduces friction between the strap and the skin which prevents the formation of blisters or

[14] Danielsson, Anna, and Katharina Stibrant Sunnerhagen. "Energy expenditure in stroke subjects walking with a carbon composite ankle-foot orthosis." *Journal of rehabilitation medicine* 36, no. 4 (2004): 165–168.
[15] Franceschini, Marco, Maurizio Massucci, Luciana Ferrari, Maurizio Agosti, and Chiara Paroli. "Effects of an ankle-foot orthosis on spatiotemporal parameters and energy cost of hemiparetic gait." *Clinical Rehabilitation* 17, no. 4 (2003): 368–372.
[16] Woo, Denise K., Giuseppe Militello, and William D. James. "Neoprene." *Dermatitis: contact, atopic, occupational, drug* 15, no. 4 (2004): 206–209.
[17] Benton, Dales. "Process for gelling an aqueous dispersion of neoprene." U.S. Patent 2,295,030, issued September 8, 1942.
[18] Mohammed, AL-KHALIDI ABDULRIDHA Murtadha. "Environmentally friendly recycling of metal cans and polymer bottles obtained from household waste."
[19] Geyer, R., J. R. Jambeck, and K. L. Law. "Production, use, and the fate of all plastics ever made. Sci Adv 3 (7): 1–5." (2017): 82.
[20] Rubin, Gustav, and M. Dixon. "The modern ankle-foot orthoses (AFO's)." *Bull Prosthet Res* 10, no. 19 (1973): 20–41.

any other skin conditions. The fabrication of the floating T-strap allows the easy addition to an existing AFO.[21]

Iglesias et al. found that an ankle brace with adjustable heel straps restricts inversion and eversion while allowing plantar flexion and dorsiflexion with an appropriate fit.[22] Nelson and Ronald stated that a compression strap restricts hyperextension and abnormal twisting of the ankle.[23] Stockings are worn to hold the leg in a particular position and provide heel lining.[24] The keel assists the patient to hold the foot at a proper stretched position to obtain the perfect footdrop.[25] Special shoes were thus designed for hemiplegic patients to control the footdrop.

Conclusion

Thus, the low-cost foot orthoses and footwear have been designed to rectify the gait anomaly of the foot drop in hemiplegic patients. It would help the patients to have an improved gait cycle without much effort and pain. The main purpose of devising the orthosis was to eliminate the circumduction or dragging gait of the patients and give them comfort and approximation towards a normal gait, thereby enabling these patients to feel better in their day-to-day movements.

Limitation

The limitation of the orthosis is that the entire elimination of circumduction motion would not be possible. A slight rectification can be made.

---

[21] Sherk, Kyle A. "The Development and Use of a Floating T-Strap on a Double Upright Metal AFO to Correct Coronal–Plane Pathologies and Reduce Skin Shear." JPO: Journal of Prosthetics and Orthotics 20, no. 1 (2008): 24–26.

[22] Iglesias, Joseph M., Tracy E. Grim, William K. Arnold, and Eric E. Johnson. "Ankle brace with adjustable heel strap." U.S. Patent 5,716,335, issued February 10, 1998.

[23] Nelson, Ronald E. "Ankle brace with compression straps." U.S. Patent 4,878,504, issued November 7, 1989.

[24] Kuhn, Jeffrey Andrew. "Custom ankle brace system." U.S. Patent 8,708,942, issued April 29, 2014.

[25] Spangler, Harry V. "Drop foot brace." U.S. Patent 5,382,224, issued January 17, 1995.

Future Scope

In the future, the orthosis can be designed with even better biomaterials for better comfort levels, and the design can be improvised to eliminate the circumduction motion.

CHAPTER NINE

3D BIOSENSORS IN MEDICAL DIAGNOSTICS: CURRENT STATUS AND FUTURE TRENDS

SATARUPA BISWAS, MOUMITA MUKHERJEE

Abstract

Biosensors are an important component of any biomedical device. Researchers all over the world are working towards the development of sensitive biosensors for application in advanced medical diagnostic systems in diseases with high mortality rates, especially malignancy, diabetes, and cardio-thoracic and vascular diseases (CTVD). It is well known that early detection and continuous monitoring are the primary requirement for the prognosis of affected patients. Modern biosensing technologies are mostly invasive and rely upon extracted blood samples from diseased persons for the specific biomarkers. However, the main concerns are the reduced sensitivity and very limited *in vivo* applications. Therefore, an urgent requirement is to develop new and improved diagnostic sensors. A 3D biosensor is a unique combination of nanotechnology, advanced biomaterials, and some relevant biochemical tools. The authors in this chapter will focus on the most recent advancements in the field of 3D biosensors for medical applications; special emphasis will be given to malignancy, CTVD, and diabetes.

The material selection and fabrication techniques are the fundamental factors on which 3D biosensors depend. Four types of materials are commonly used for 3D biosensor design and development: carbon-based, glass/silicon-based, metals, and polymers. Though carbon materials have shown significant advantages that include high conductivity, low electrical resistivity, easy functionalisation, and favourable mechanical properties, some serious disadvantages include the fact that they are non-biodegradable, and the limited data there is on their tolerance by healthy tissue. Similarly, glass or silicon are biocompatible and are known for having good optical

and mechanical properties, as well as for being cost-effective. However, these are non-biodegradable. In the case of metals, although their electrical and mechanical properties are excellent, they have poor biocompatibility properties and are, again, non-biodegradable. Of them all, polymers are highly biocompatible, but they are poor in their mechanical property. The first part of this chapter will throw some light on various aspects of material science and their suitability in the development of 3D biosensors. 3D biosensors provide several advantages in comparison to their flat counterpart, especially in terms of sensitivity and stability enhancement. The 3D electrode maximises the number of immobilised probes, as well as electron transfer that contributes to performance improvement. 3D biosensors offer improved implantable properties and are fundamental for continuous and real-time device monitoring. Innovative technologies result in flexible, high performance, biocompatible 3D biosensors platforms. For this, a new generation of 3D biosensors is emerging. 3D biosensors are associated with physicochemical phenomena that need to be considered fully to improve their overall performance. In this chapter, the capability of 3D biosensors to revolutionise disease detection and treatment follow-up for diseases with high mortality rates, as described earlier, will be explored.

Furthermore, in this chapter, an attempt will be made to study whether 3D biosensors can address the issues and limitations of ongoing biosensing technologies. Most of the tests have been *in vitro* biomarker detection, performed in medical laboratories, and simplified matrices were used, though this procedure results in poor sensitivity and selectivity of the sensors. The various limiting issues of 3D biosensors should first be addressed before being applied in real-life applications. This includes reproducibility, assay duration, and stability. State of the art fabrication technologies will be discussed in this chapter. The most recent advancement of 3D biosensors for clinical applications will be summarised in the second part of the chapter.

The new generation of devices aims to be implanted in the human body for real-time disease monitoring. Studies are ongoing on medical biosensors towards their miniaturisation. Planar surfaces show poor analytical performance and a low limit of detection. These are the direct consequence of a reduced surface area. Surface area, and surface-area-to-volume ratio are significant for enhancing the sensitivity of detection of protein biomarkers and point of care microfluidic tests. However, the response time of 2D biosensors is comparatively larger due to the lower limit of detection. All these associated technical phenomena will be addressed in this chapter,

which will be useful for biomedical researchers as well as associated medical and electronic industries.

1 Introduction

According to the Global Health Observatory data, provided by the World Health Organisation, 15.2 million deaths in 2016 occurred due to ischemic heart disease and stroke. Chronic obstructive pulmonary diseases and lung cancer are the next group of high-mortality diseases, causing 4.7 million deaths. The other chronic diseases, that killed 1.6 million people worldwide in 2016, are diabetes followed by dementia. These cardiothoracic and vascular diseases (CTVD), along with diabetes and cancer, were responsible for nearly 54% of deaths worldwide and are thus categorised as high mortality diseases. Popularly used diagnostic processes lack sensitivity and selectivity, which restricts them from producing believable results at an early stage of the disease, which further hinders regular monitoring of the health status of the patients (Ahmed et al., 2014). Focusing on early detection of high-mortality diseases and continuous monitoring is thus a critical need. Researchers all over the world, in the last three decades, have been trying to copy the efficiency of biological organisms in nature in recognising elements, and developing biosensors for diagnosis and continuous monitoring of cost-effective health conditions, to have faster response times and an easy readout of results with the initial analysis (Blais et al., 2004; Lynch and Loh, 2006). Moreover, these devices, similar to biosensors, the term introduced by Cammann, are analytical devices which couple a biological sensing element with a detector system, using a transducer that converts a biological response into an electrical signal (Malhotra et al., 2017). They usually estimate the levels of biological markers or any product associated with a chemical reaction and produce a signal that can be correlated with the concentration of a sample or analyte present in the reaction. Ideally, biosensors should be specific to the recognition molecule, unbiased of any changes in pH and temperature, and miniaturised for ease of use. Most of the successfully commercialised biosensors are electrochemical sensors, followed by optical sensors (Vo-Dinh and Cullum, 2000; Lynch and Loh, 2006). Implementation of biosensors in medical diagnostics ranges from screening or early detection of diseases to continuous monitoring of health and management of chronic diseases. The major steps being followed for the development of a complete biosensor are the fabrication of electrodes (the interface between the device and the sample); the immobilisation of biological receptors on the electrode surface, which can selectively detect compounds from the sample to be

tested; and the implementation of a transducer, which can convert the physical change after the reaction into electrical signals, directed to the readout circuitry. Biosensors are often categorised depending upon the biological substance used for detection. They can be divided into four groups depending on the working principle as: biocatalytic, i.e. enzyme-based biosensors (Rocchitta et al., 2016); bioaffinity, i.e. antibodies, antigens, and nucleic-acid used receptors (Byrne et al., 2009; Holford et al., 2012); microbe-based biosensors (Reshetilov et al., 2010); and Nanosensors, where sensors are made up of active nanoparticles to increase the sensitivity and specificity of the device (Su et al., 2017). However, most of these biosensors are based on immobilisation of the above-mentioned biological molecules on planar (2D) surfaces. Limitations of the 2D surface could be listed as:

1. Poor analytical performance in terms of the low dynamic range of detection, mostly due to the unstable immobilisation of recognition molecules. This limitation could be overcome by increasing the surface area of the electrodes. Increased surface-area-to-volume ratio would effectively increase the sensitivity of the sensor as more biomarkers detected would come in to contact with the recognition molecules in these surfaces (Barbosa and Reis, 2017).
2. The shear stress developed on the planar surface, due to the weight of the analyte, can restrict the bond between the analyte and the immobilised molecule, hence decreasing analytical performance.
3. The rigid substrate evokes foreign body response if used *in vivo*, which prevents its use in real-time monitoring of physiological conditions related to the diseases (Bertok et al., 2014).
4. Miniaturisation on a 2D silicon substrate has temperature dependence and high signal-to-noise ratio which leads to unsatisfactory results (Alpuim et al., 2011; Gao et al., 2011).

All of these challenges are now addressed by 3D biosensors.

3D biosensors have successfully addressed analytical performance limitations as they have a higher surface-to-volume ratio. The rough surfaces enable the biorecognition molecules to adhere to 3D topography that enhances contact with the analyte, eventually increasing the analytical response. The kinetics of the reactions are improved and the shear stress between the analyte and the receptors is also optimised (Rebelo et al., 2019). In general, the 3D biosensors provide enormous encouragement towards transforming the medical diagnostic system and possibly taking it to an entirely new level. With this new technology, implantable biocompatible biosensors seem achievable for continuous monitoring of chronic diseases.

This chapter will provide information about the new materials used for developing these 3D structures, their fabrication techniques, and application in medical diagnostic with a focus on cardiovascular diseases, coronary obstructive pulmonary diseases, cancer, and diabetes.

2 Common Materials for 3D Biosensors

There are broadly four types of materials which are generally used as the substrate for 3D biosensors. The selection of the material depends on several parameters to be optimised, based on the application of the biosensor. The substrate material should be compatible with the biorecognition molecule which acts as the receptor, the analyte to be tested, and also with the transducer (Zhang et al., 2000; Colombo et al., 2015; Vigneshvar et al., 2016). The substrates are mostly carbon-based structures, silicon-based structures, metals, or polymers.

2.1 Carbon-Based Materials

Carbon-based materials that are widely used as sensing materials are in the form of allotropes, like single-walled and multi-walled carbon nanotubes (CNTs), carbon fibres, graphite, glassy carbon, diamond and fullerene (McCreery, 2008). These materials exhibit high electrical conductivity, high stability, exceptional electrochemical performance, while being low cost due to their high availability. Due to such promising characteristics, they were utilised in the development of different biosensors (Tiwari et al., 2016). Fullerene, Graphene, and CNT/Graphene hybrid sensors have been used for the monitoring of nonenzymatic glucose for a diabetes diagnosis. 3-amino-capto-1,2,4-triazole functionalised C60-based gold nanocomposite, having a larger surface area, was used for non-enzymatic sensing of glucose (Sutradhar and Patnaik, 2017). Yang et al. developed hexadecyl tri-Me ammonium bromide functionalised graphene oxide/multi-walled carbon nanotubes, modified with glassy carbon electrode, as a novel system for the simultaneous detection of dopamine, ascorbic acid, uric acid, and nitrite. The combination of graphene oxide and MWNTs provided a larger surface area, good bio-compatibility, electrical conductivity and stability, high selectivity, and sensitivity (Yang and Li, 2014). A three-dimensional chitosan/vacuum-stripped graphene/polypyrrole interface with a hierarchical porous structure was fabricated as a free-standing and flexible electrochemical sensing electrode for dopamine detection, which exhibits unprecedented good selectivity, high sensitivity (632.1`A mM-1 cm-2), wide linear response range (0.1–200 `M), low detection limit (19.4 nM, S/N = 3) and

good sensing performance in human serum samples (Liu et al., 2014). A boronic-functionalised dopant, 4-N-Pentylphenylboronic Acid (PBA), was used to provide polypyrrole films with an enhanced affinity towards diols for sensing bacteria and other microorganisms (Dong et al., 2012). Hemin-graphene oxide-pristine carbon nanotubes complexes (H-GO-CNTs) were synthesised through the c - c interactions, and were used to construct a novel dual sensor for the detection of hydrogen peroxide, and the simultaneous detection of ascorbic acid, dopamine, uric acid, and tryptophan (Zhang et al., 2013). Recently, Hemanth et al. developed 3D pyrolytic carbon microelectrodes coated with bio-functionalised reduced-graphene oxide for the detection of glucose with two-fold increased sensitivity (Hemanth et al., 2018). The 3D architecture in most cases is fabricated through MEMS lithography and nano integration techniques (Greiner et al., 2013). Nanodiamonds are optically transparent in the UV/Visible and IR regions of the electromagnetic spectrum and have exceptional properties. Recombinant protein polymers were further used to increase the stability of these nanodiamonds and make them biocompatible (Zheng et al., 2017).

2.2 Silicon-Based Materials

Porous silicon-based (PSi) substrates have gained popularity due to their luminescence properties at room temperature. The porosity offers a large internal surface area that allows the bonding of active molecules over a large surface in a small volume, which effectively increases the efficiency of the device. PSi integrated devices, having layers with specific enzymes or with molecules with the specific target, allow for the realisation of label-free biosensors for DNA sensing (Rong et al., 2008) and quantification of triglycerides (Setzu et al., 2007). Optical transduction was mostly used, as the etch direction can modulate the refractive index of the PSi, which helps in designing the biosensors for different applications (Sailor, 2007). In recent advances, the PSi substrate is also used in electrochemical sensing through biomolecule immobilisation (Setzu et al., 2011). 3D bioprinting on silicon nitride substrates has also gained popularity in the field of medical diagnosis as immobilisation of aptamers, through laser patterning, was successfully achieved for the detection of biomolecules (Chatzipetrou et al., 2017). The 3D printing technology, along with silicon substrates, has great potential for use in the development of point of care devices. A study by Feng et al. implemented 3D silicon nanowires, producing electrode arrays, which effectively increased the surface area 11.35 fold, which could be used as glucose sensors at a low cost, but with high sensitivity, and a faster response time (Feng et al., 2014).

2.3 Metal-Based Materials

Noble metals, like gold and platinum, have been used extensively in biomedical applications since the beginning of MEMS technology. Metals are preferred for their good electrical and mechanical properties, along with their ease of fabrication and functionalisation. Micro and nanopillars, tips, cavities, pits, grooves, etc., could be fabricated on metal surfaces through laser printing or simple lithography techniques. 3D gold nanoporous electrodes, having quinone oxidoreductase deposited on the surface of the electrode for sulphide detection, have shown remarkable results in detecting E. coli, even in wastewater (Liu et al., 2017). 3D metallic nanowires have been integrated as sensing elements, which showed greater affinity towards the biomolecules and hence increasing the sensitivity of the biosensor towards biomarkers (Lee and Watanabe, 2018). Katseli et al. demonstrated a single-step fabrication technique of 3D metal electrodes for electrochemical sensing. The fabricated device was tested for glucose detection with remarkable results (Katseli et al., 2019). Using additive technology in 3D printing, manufacturing of stainless-steel electrodes was faster and cheaper. These electrodes were then coated with gold for DNA sensing application (Loo et al., 2017), for detection of biomarkers (Ho et al., 2018), and for detection of phenols (Cheng et al., 2017). Metals like bismuth, nickel platinum, and iridium oxide could also be electroplated on these 3D printed surfaces for the detection of biomarkers and other bioactive molecules (Lee et al., 2017b; Ambrosi and Pumera, 2018; Ambrosi et al., 2016). Hence, conductive metal electrodes are believed to make electrodes suitable for a host of analytical applications in the development of diagnostic sensors. Moreover, 3D metal patterns and nanocomposites are now possible on flexible and stretchable substrates, which can be developed into wearable diagnostic sensors for continuous monitoring of physiological parameters (Xuan et al., 2018).

2.4 Polymer-Based Materials

The reasons behind polymeric devices gaining higher importance in biological sensing are their biocompatibility, flexibility, ability to mimic the natural environment, and incorporation of conductivity. Flexible biosensors are in high demand for building wearable devices that can monitor health conditions and provide patient care continuously while they are away from hospitals and medical facilities. Printed flexible sensors are already established for diagnostic purposes (Nag et al., 2019). 3D printing of electrodes on polydimethylsiloxane (PDMS) moulds was considered as one

of the solutions to this problem statement. Disposable electrochemical sensors were developed that could be mounted on special probes that reach directly to the cell or tissue to be diagnosed for malignancy (Ragones et al., 2015). 3D printed microcantilevers demonstrated mass sensitivity which was used to detect biomolecules combining with the immobilised reagents (Stassi et al., 2017). Stretchable electrodes designed with innovative fabrication techniques were achieved by Wei *et al.* which could be easily tuned according to the detection system (Wei et al., 2017). Highly sensitive pressure sensors were developed by Zhuo et al. on PDMS substrates which demonstrated a low detection limit, fast response/recovery speed, excellent durability, and good tolerance to temperature and pH (Zhuo et al., 2017). Hydrogel is another material extensively used by researchers for the development of flexible, wearable biosensors because of their similarity with the extracellular matrix of soft tissues. The hygroscopic property of the hydrogel substrate facilitates the diffusion of a larger amount of analyte towards the immobilised electrodes (da Silva et al., 2014). A sandwich-type biosensor for glucose detection and quantification was reported by Colombo et al. The enzymatic matrix of the biosensor was optimised for the detection of glucose oxidase (Colombo et al., 2015). Phenylboronic acid side chains were further incorporated into hydrogels to produce enzyme-free smart hydrogels which could detect the increase in glucose level, irrespective of the concentration of oxygen level in the blood (Guenther et al., 2010). Nanocomposite structures incorporated in hydrogels increase the surface area and hence the performance of the biosensors, as proposed by Kazemi et al. for cardiac markers detection (Kazemi et al., 2016).

3 3D Printing Technology

The ease of the operating principle and the ability to quickly fabricate complex 3D models allows the additive manufacturing technique to have a wide spectrum of applications. The advantage of 3D printing to create electrodes of different geometries is that it allows the technology to play a significant part in the field of biosensors. 3D printed sensors are now able to monitor some of the vital physiological parameters to be monitored in acute and chronic diseases, including blood pressure, heart rate, body motion, respiration rate, brain activity, and skin temperature (Ko et al., 2010; Zhang and Ning, 2012; Llandro et al., 2010). The complex geometry, along with the different shapes and sizes of electrodes, often helps in varying surface properties according to the need of the application. Researchers are mostly using 3D metal electrodes fabricated in helical-shaped and gauze-shaped 3D printing devices, while carbon-printed sensors

were mostly rectangular or disc electrodes. The sensors, printed through 3D additive technology, are either fabricated by integrating the sensor in the printed platform or by directly printing the sensing component (Patel et al., 2012).

The process of printing 3D objects usually begins with model designing within the CAD software and conversion into the Standard Triangle Language (STL) file format, which stores information of the 3D object surfaces as a list of coordinates of triangulated sections. The created 3D model is divided into several layers with 2D cross-sections, which is fed to the printer as the input. The 3D printer, through various techniques, then deposits a filament onto the print bed until the entire 3D object has been created.

The different techniques that are commonly used for the fabrication of sensors through the 3D additive technique are discussed in the subsequent paragraphs. The techniques, viz. fused deposition modelling (FDM), stereolithography (SLA), photopolymer inkjet printing, selective laser sintering (SLS), 3D inkjet printing, and direct laser writing (DLW), differ from each other in terms of the different parameters used, including the fabricated prototype, the required time to develop each prototype, the ability to process different raw materials, its repeatability, the resolution, and the accuracy, depending on the application of the sensor.

Fused diffusion modelling utilises thermoplastic polymeric materials extruded to print objects layer by layer from a heated nozzle onto a surface or platform, where it is cooled to below its thermoplastic temperature. Different materials, like acrylonitrile butadiene styrene, polycarbonate, a blend of both, and polylactic acid have been used for this technique (Mohamed et al., 2015). These polymers can be made conductive, which increases their applicability to integration with electronic circuits (Lee et al., 2017a). This technique, which has a fair amount of repeatability, has advantages because of the high speed of printing, producing durable and high-quality products. However, the porous structure for the binder provides weak mechanical properties of the product. So far, FDM has been used to fabricate sensors for detecting lactate (Rumley-Ouellette et al., 2017), cell toxicity (Cevenini et al., 2016), glucose (Song et al., 2018), DNA (Loo et al., 2017), bacteria (Connell et al., 2014) and also as an immunosensor (Pranzo et al., 2018).

Direct ink writing is similar to FDM as it also depends on the extrusion of ink through a fine deposition nozzle to form a 3D structure in the layer-by-

layer approach (Malek et al., 2017). Two different strategies are utilised in this technique, based on the ink type. One type is extrusion of low-viscosity ink that undergoes gelation via a chemical, photochemical, or noncovalent process (Smith et al., 2018). The second technique uses shear thinning of hydrogel ink, which possesses a viscoelastic response towards the applied pressure. Sodium alginate and gelatine are commonly used hydrogels (He et al., 2016). Although the devices produced have good accuracy and desirable surface finishes, they have fragile parts, require post-processing, and have poor mechanical strength. With this type of printing, the bionic ear (Mannoor et al., 2013), and the multifunctional bio-membrane (Low et al., 2017) have been developed with high resolution and improved sensitivity.

Stereolithography uses a photocurable polymeric resin. These polymers are cured into solids when exposed to light. Initially, curing was achieved with UV light until polymers curable with visible light were recently introduced. High intensity focused lasers or LED beams are employed, where the spot size of the light beam determines the printing resolution (Gross et al., 2014). Each layer of the object is printed as a point-by-point 2D cross-section and cured by the scanning focused beam onto a printing platform immersed in a photocurable tank that holds the liquid resin (Bose et al., 2013). Through this process, large parts could be built with good accuracy and surface finish, with simple scalability. However, the products do not show good mechanical properties and take longer to be fabricated, while the durability depends on moisture, heat, and chemical interaction. Despite the disadvantages, biosensors, like the DNA imaging sensor (Valentin et al., 2017), the bacteria sensor (Hinman et al., 2017), and the cellular sensor (Wang et al., 2018b) have been successfully implemented.

Multi-Jet modelling or photopolymer inkjet printing utilises multiple nozzles or multi-head printers with print heads similar to inkjet printers. The print head extrudes layers of photocurable resin or molten wax, usually with a second head printing support material to maintain the shape of the design until cured. After printing, the object is cured by UV irradiation or heat, and the support material can be removed by heating or dissolving it in a specific solvent (Hofmann, 2014). Although vulnerable to heat and humidity, and despite being less durable and relatively expensive, this technique has a different level of flexibility and allows different coloured photopolymers that provide control over accuracy and a smooth surface. It has so far been effectively used as a cell imaging sensor (Tappa and Jammalamadaka, 2018); as a cell-based sensor (for ATP sensing) (Rusling, 2018); as a physiological sensor (Wang et al., 2017); microfluidic devices (Munshi and Martin, 2016); and as an immunosensor (Pranzo et al., 2018).

Selective laser sintering utilises localised energy from a focused infra-red (IR) laser to sinter a finely powdered polymer into layers of solid. The IR laser scans through the surface of powder in the shape of each layer of the sliced 3D design. Thus, high energy laser sources, like CO2/Nd are used: YAG lasers are usually used to sinter powders (Williams et al., 2005). Based on temperature, SLS printing can be categorised into (a) solid-state sintering, where binding occurs at a temperature lower than the melting temperature and is usually used with polymers, like polycarbonate; and (b) full melting SLS, used for ceramics, where the sintering temperature is higher and is above the melting temperature (Shirazi et al., 2015). This technique provides high-resolution printing of complex structures with high strength in the least amount of time. This process applies only to printed metal parts. It has been used to develop a cell-density sensor (Zhang and Hoshino, 2018). Although not very popular in diagnostic sensors, this technique is commonly used for printing implants.

Direct laser writing utilises an ultrashort pulse of the femtosecond laser beam to cure a photosensitive resin material (Malinauskas et al., 2016). Two-photon absorption and polymerisation facilitate the fast fabrication of 3D scaffolds with high resolution (Marino et al., 2015). Since the process does not involve any localised overheating or UV toxicity, it is favourable for encapsulating living cells and biomaterials in 3D structures. This technique can use versatile substrate materials and hence is widely used in producing accurate high-resolution structures. This emerging high-resolution technique for 3D printing has been used for developing a piezoelectric acoustic sensor (Tiller et al., 2019), motion control and soft sensors (Ge et al., 2018), and a glucose sensor (Sharafeldin et al., 2018).

4 Application in High Mortality Diseases

High mortality diseases were monitored through the available biomedical instruments until the advent of biosensors. A combination of appropriate detection modes, materials, and biomarkers employed for disease detection determines the final performance of the biosensor. The selection of a specific biomarker depends on many factors. Importantly, among all available biomarkers, some are FDA-approved for clinical diagnosis, whereas others are still under investigation, awaiting clinical trial. Although the detection modes are similar for both 2D and 3D biosensors, the final analytical performance is better in 3D platforms, since the signal is amplified by the 3D architecture. These biosensors are often made wearable and do not require specially trained professionals for handling. Hence, the

technology promises early detection of the diseases and continuous real-time monitoring, which could effectively bring down the mortality rates of these diseases.

4.1 Cardiovascular diseases

Early diagnosis of cardiovascular risks is possible through the detection of metabolic ties in the body that indicate the onset of the disease. Myocardial infarction, the most common threat to people of various age groups, has many identifying markers, like cardiac troponin-I, cardiac troponin-T, creatine kinase-MB, and myoglobin. The level of these markers is helpful in the diagnosis of a heart attack. Thus, quantification of these markers could be done using electrochemical biosensors. When 3D biosensors are used, there is a manifold increase in the accuracy of quantification. Porous graphene electrodes could detect cardiac troponin-I (Kazemi et al., 2016) through electrochemical analysis. Implantable magnetic relaxation sensors were developed by Ling et al. to measure cumulative exposure to cardiac biomarkers (Ling et al., 2011). A carbon nanotube-based electrochemical immunosensor could successfully detect the cardiac troponin T marker (Gomes-Filho et al., 2013). Certain markers are considered to be cardiac risk factors. Hyperuricemia was reported to be positively associated with CVD in both sexes (Yang et al., 2015). LDL cholesterol, even at levels currently considered normal, was found to be independently associated with the presence and extent of early systemic atherosclerosis in the absence of major cardiovascular risk factors (Fernández-Friera et al., 2017), which could be detected by nanostructured hydrogel-based biosensors (Li et al., 2015). Triglycerides have been reported as a risk factor and could be detected with nanostructured composite electrodes through electrochemical biosensors (Rong et al., 2015).

4.2 Chronic Obstructive Pulmonary Disease (COPD)

COPD is an umbrella term that includes chronic lung diseases that cause airflow constraints in the lungs. Although many cases of COPD are considered to be treatable, early diagnosis is the key factor in their effective prevention and control (Csikesz and Gartman, 2014). The lung capacity of patients is commonly examined by spirometers for diagnosing COPD. However, sputum, consisting of mucin, water, epithelial cells of the airway mucosa, and salts might provide more accurate diagnostics and identification of bacterial infections (Price et al., 2009). The viscosity of sputum, regulated by its mucin (mucus glycoproteins) and water content,

was reported as an important marker for early and fast detection of COPD (Lopez-Vidriero and Reid, 1978). Microfluidic devices have thus been designed and developed. A portable dielectric biosensor was developed for rapid detection of viscosity variations in the saliva of COPD patients (Soltani Zarrin et al., 2019). Volatile organic compounds (VOCs) are distinctive of several respiratory diseases, including chronic obstructive pulmonary disease (COPD), asthma, lung cancer, pulmonary arterial hypertension (PAH), obstructive sleep apnoea syndrome (OSAS), tuberculosis (TB), cystic fibrosis (CF) and pneumoconiosis. Attempts were made to detect the VOCs from exhaled breath which could be the easiest non-invasive detection method for pulmonary diseases (Broza et al., 2018).

4.3 Cancer detection

Early diagnosis of cancer by biomarkers is the only key to the survival and treatment of cancer patients. Electrochemical biosensors have tremendous potential in cancer diagnosis owing to their advantages of ultra-sensitivity, high selectivity, low cost, quick readout, and simplicity (Cui et al., 2019). The electrodes used for analysis were mostly surface electrodes until 3D electrodes were recently introduced. A 3D DNA nanosphere-based photoelectrochemical biosensor was developed which combined multiple enzyme-free amplification for ultrasensitive detection of cancer biomarkers (Gao et al., 2020). Another 3D electric cell/Matrigel-substrate impedance sensing platform was developed for human hepatoma cells (HepG2) for the early detection of liver cancer (Pan et al., 2019).

A 3D micro/nanostructured graphene interface was fabricated by Wang et al. for impedance signal sensing in the single cancer cell. The 3D graphene bio-interface captured significantly more signals as compared to the 2D gold interface in sensing the sensitivity of a single-cell, demonstrating the increment in impedance signal of about 100% at the nodes of cell state change (Wang et al., 2018a). Implantable, long-term stable and biocompatible electrochemical biosensors were developed to detect biomarkers or metabolites in the malignant tissue or organ. The Sensing Cell Culture Flask is a platform that allows the integration of an electrochemical biosensor by simple adjustments during the post-processing steps of the chip fabrication (Kieninger et al., 2018).

4.4 Diabetes

Maintaining a regular blood glucose level is usually done by consulting clinicians, who have developed a series of blood glucose sensors. Glucose oxidase (GOx) oxidises glucose into gluconolactone in the presence of oxygen with the production of hydrogen peroxide and water. Therefore, detection of GOx and hydrogen peroxide have become the common markers for quantitative analysis of glucose levels in the blood (Milton et al., 2013; Chen et al., 2013). Carbon nanotube electrodes were later developed as pH sensing electrodes for the measurement of mediators, like GOx and glucose dehydrogenase pyrroloquinoline quinone (Harper and Anderson, 2010). Another type of electrode, based on charge transfer complexes, like conducting organic salt, tetracyanoquinodimethane, was developed to improve the performance of the sensors (Zhang and Li, 2004). Non-enzymatic glucose sensors were further developed for continuous glucose monitoring (Rodbard, 2017). With the advent of 3-dimensional electrodes, 3D porous graphene has been fabricated directly from insulation polyamide films by a commercial laser scribing system in the air, designated as laser-induced graphene for imparting flexibility, durability, and stability to the device (Lin et al., 2014). Moreover, to make the sensors low-cost and simple, while having good stability and selectivity, a paper-based microfluidic sensor was developed to determine if the glucose level is not only from the blood but also from sweat (Cao et al., 2020). 3D Ag nanoplates (NPs) with novel architecture have been synthesised on 3D nickel foam-graphene, which are able to demonstrate ultra-high glucose sensitivity, and ultra-fast response time with a good detection limit of 2 nM.

5 Conclusion

Although some 3D biosensors have translated to clinical use, most of them are still under investigation. Further exploration of signal processing, stability, reproducibility, durability, and the use of novel biomarkers would be required for the technology transfer to be successful. Real-time continuous measurement would require increased reproducibility and a decreased response cycle. Resetting the surface for calibration remains a major challenge to be addressed for point of care; wearables require continuous monitoring, while cancer and diabetes are more chronic diseases that require high sensitivity and specificity. Cardiovascular diseases require faster response time for early-stage investigation and prediction of these high mortality diseases. 3D electrodes show massive potential in solving the sensitivity, selectivity, and response time issues. Once the reaction kinetics

are well understood for the interfaces, most of the limitations could be addressed easily. Moreover, integration of the biosensing technology with tissue engineering approaches could be managed for translating the investigation in the laboratories to realistic products. Steps, like clinical trials and approval from the FDA or other competent authorities, need to be taken, along with the laboratory experiments to commercialise the products. We are just a few steps away from implementing these 3D biosensors into medical diagnostics.

References

Ahmed, M. U., Saaem, I., Wu, P. C., Brown, A. S., 2014. Personalized diagnostics and biosensors: a review of the biology and technology needed for personalized medicine. Critical reviews in biotechnology 34 (2), 180–196.

Alpuim, P., Correia, V., Marins, E. S., Rocha, J., Trindade, I., Lanceros-Mendez, S., 2011. Piezore- sistive silicon thin film sensor array for biomedical applications. Thin Solid Films 519 (14), 4574–4577.

Ambrosi, A., Moo, J. G. S., Pumera, M., 2016. Helical 3D-printed metal electrodes as custom-shaped 3D platform for electrochemical devices. Advanced Functional Materials 26 (5), 698–703. Ambrosi, A., Pumera, M., 2018. Self-contained polymer/metal 3D printed electrochemical platformfor tailored water splitting. Advanced Functional Materials 28 (27), 1700655.

Barbosa, A. I., Reis, N. M., 2017. Critical insight into the development pipeline of microfluidic immunoassay devices for the sensitive quantitation of protein biomarkers at the point of care. Analyst 142 (6), 858–882.

Bertok, T., Sediva, A., Vikartovska, A., Tkac, J., 2014. Comparison of the 2D and 3D nanostructured lectin-based biosensors for in situ detection of sialic acid on glycoproteins. International journal of electrochemical science/Electrochemical Science Group 9 (2), 890.

Blais, F., et al., 2004. Review of 20 years of range sensor development. Journal of electronic imaging 13 (1), 231–243.

Bose, S., Vahabzadeh, S., Bandyopadhyay, A., 2013. Bone tissue engineering using 3D printing. Materials Today 16 (12), 496–504.

Broza, Y. Y., Vishinkin, R., Barash, O., Nakhleh, M. K., Haick, H., 2018. The synergy between nanomaterials and volatile organic compounds for non-invasive medical evaluation. Chemical Society Reviews 47 (13), 4781–4859.

Byrne, B., Stack, E., Gilmartin, N., O'Kennedy, R., 2009. Antibody-based sensors: principles, problems, and potential for the detection of pathogens and associated toxins. Sensors 9 (6), 4407–4445.

Cao, L., Han, G.-C., Xiao, H., Chen, Z., Fang, C., 2020. A novel 3D paper-based microfluidic electrochemical glucose biosensor based on rgo-tepa/Pb sensitive film. Analytica Chimica Acta 1096, 34–43.

Cevenini, L., Calabretta, M. M., Tarantino, G., Michelini, E., Roda, A., 2016. Smartphone-interfaced 3D printed toxicity biosensor integrating bioluminescent "sentinel cells". Sensors and Actuators B: Chemical 225, 249–257.

Chatzipetrou, M., Massaouti, M., Tsekenis, G., Trilling, A. K., van Andel, E., Scheres, L., Smulders, M. M., Zuilhof, H., Zergioti, I., 2017. Direct creation of biopatterns via a combination of laser-based techniques and click chemistry. Langmuir 33 (4), 848–853.

Chen, C., Xie, Q., Yang, D., Xiao, H., Fu, Y., Tan, Y., Yao, S., 2013. Recent advances in electrochemical glucose biosensors: a review. Rsc Advances 3 (14), 4473–4491.

Cheng, T. S., Nasir, M. Z. M., Ambrosi, A., Pumera, M., 2017. 3D-printed metal electrodes for electrochemical detection of phenols. Applied Materials Today 9, 212–219.

Colombo, L., Baruzzi, A. M., Garay, F. S., 2015. Analysis and optimization of a hydrogel matrix for the development of a sandwich-type glucose biosensor. Sensors and Actuators B: Chemical 211, 125–130.

Connell, J. L., Kim, J., Shear, J. B., Bard, A. J., Whiteley, M., 2014. Real-time monitoring of quorum sensing in 3D-printed bacterial aggregates using scanning electrochemical microscopy. Proceedings of the National Academy of Sciences 111 (51), 18255–18260.

Csikesz, N. G., Gartman, E. J., 2014. New developments in the assessment of COPD: early diagnosis is key. International journal of chronic obstructive pulmonary disease 9, 277.

Cui, F., Zhou, Z., Zhou, H. S., 2019. Measurement and analysis of cancer biomarkers based on electrochemical biosensors. Journal of The Electrochemical Society 167 (3), 037525.

da Silva, L. P., Cerqueira, M. T., Sousa, R. A., Reis, R. L., Correlo, V. M., Marques, A. P., 2014. Engineering cell-adhesive gellan gum spongy-like hydrogels for regenerative medicine purposes. Acta biomaterialia 10 (11), 4787–4797.

Dong, X., Wang, X., Wang, L., Song, H., Zhang, H., Huang, W., Chen, P., 2012. 3D graphene foam as a monolithic and macroporous carbon electrode for electrochemical sensing. ACS applied materials & interfaces 4 (6), 3129–3133.

Feng, W.-C., Hsu, C.-W., Liu, H.-C., Wang, G.-J., 2014. A cost-effective and highly sensitive glucose biosensor based on a 3D silicon nanowire array electrode. In: 2014 Symposium on Design, Test, Integration, and Packaging of MEMS/MOEMS (DTIP). IEEE, pp. 1–5.

Fernández-Friera, L., Fuster, V., López-Melgar, B., Oliva, B., García-Ruiz, J. M., Mendiguren, J., Bueno, H., Pocock, S., Ibáñez, B., Fernández-Ortiz, A., et al., 2017. Normal ldl-cholesterol levels are associated with subclinical atherosclerosis in the absence of risk factors. Journal of the American College of Cardiology 70 (24), 2979–2991.

Gao, A., Lu, N., Dai, P., Li, T., Pei, H., Gao, X., Gong, Y., Wang, Y., Fan, C., 2011. Silicon- nanowire-based CMOS-compatible field-effect transistor nanosensors for ultrasensitive electrical detection of nucleic acids. Nano letters 11 (9), 3974–3978.

Gao, X., Niu, S., Ge, J., Luan, Q., Jie, G., 2020. 3D DNA nanosphere-based photoelectrochemical biosensor combined with multiple enzyme-free amplification for ultrasensitive detection of cancer biomarkers. Biosensors and Bioelectronics 147, 111778.

Ge, L., Dong, L., Wang, D., Ge, Q., Gu, G., 2018. A digital light processing 3D printer for the fast and high-precision fabrication of soft pneumatic actuators. Sensors and Actuators A: Physical 273, 285–292.

Gomes-Filho, S., Dias, A., Silva, M., Silva, B., Dutra, R., 2013. A carbon nanotube-based electrochemical immunosensor for cardiac troponin t. Microchemical Journal 109, 10–15.

Greiner, F., Quednau, S., Dassinger, F., Sarwar, R., Schlaak, H., Guttmann, M., Meyer, P., 2013. Fabrication techniques for multiscale 3D-mems with vertical metal micro-and nanowire integration. Journal of Micromechanics and Microengineering 23 (2), 025018.

Gross, B. C., Erkal, J. L., Lockwood, S. Y., Chen, C., Spence, D. M., 2014. Evaluation of 3D printing and its potential impact on biotechnology and the chemical sciences.

Guenther, M., Schulz, V., Gerlach, G., Wallmersperger, T., Solzbacher, F., Magda, J. J., Tathireddy, P., Lin, G., Orthner, M. P., 2010. Hydrogel-based piezoresistive biochemical microsensors. In: Electroactive Polymer Actuators and Devices (EAPAD) 2010. Vol. 7642. International Society for Optics and Photonics, p. 764227.

Harper, A., Anderson, M. R., 2010. Electrochemical glucose sensors—developments using electrostatic assembly and carbon nanotubes for biosensor construction. Sensors 10 (9), 8248–8274.

He, Y., Yang, F., Zhao, H., Gao, Q., Xia, B., Fu, J., 2016. Research on the printability of hydrogels in 3D bioprinting. Scientific Reports 6, 29977.

Hemanth, S., Halder, A., Caviglia, C., Chi, Q., Keller, S. S., 2018. 3D carbon microelectrodes with bio-functionalized graphene for electrochemical biosensing. Biosensors 8 (3), 70.

Hinman, S. S., McKeating, K. S., Cheng, Q., 2017. Plasmonic sensing with 3D printed optics. Analytical Chemistry 89 (23), 12626–12630.

Ho, E. H. Z., Ambrosi, A., Pumera, M., 2018. Additive manufacturing of electrochemical interfaces: simultaneous detection of biomarkers. Applied Materials Today 12, 43–50. Hofmann, M., 2014. 3D printing gets a boost and opportunities with polymer materials.

Holford, T. R., Davis, F., Higson, S. P., 2012. Recent trends in antibody-based sensors. Biosensors and Bioelectronics 34 (1), 12–24.

Katseli, V., Economou, A., Kokkinos, C., 2019. Single-step fabrication of an integrated 3D-printed device for electrochemical sensing applications. Electrochemistry Communications 103, 100–103.

Kazemi, S. H., Ghodsi, E., Abdollahi, S., Nadri, S., 2016. Porous graphene oxide nanostructure as an excellent scaffold for label-free electrochemical biosensor: Detection of cardiac troponin i. Materials Science and Engineering: C 69, 447–452.

Kieninger, J., Tamari, Y., Enderle, B., Jobst, G., Sandvik, J. A., Pettersen, E. O., Urban, G. A., 2018. Sensor access to the cellular microenvironment using the sensing cell culture flask. Biosensors 8 (2), 44.

Ko, J., Lu, C., Srivastava, M. B., Stankovic, J. A., Terzis, A., Welsh, M., 2010. Wireless sensor networks for healthcare. Proceedings of the IEEE 98 (11), 1947–1960.

Lee, C., Watanabe, H., 2018. A controlling method of nanowire array biosensor. In: 2018 IEEE 18th International Conference on Nanotechnology (IEEE-NANO). IEEE, pp. 1–3.

Lee, J.-Y., An, J., Chua, C. K., 2017a. Fundamentals and applications of 3D printing for novel materials. Applied Materials Today 7, 120–133.

Lee, K. Y., Ambrosi, A., Pumera, M., 2017b. 3D-printed metal electrodes for heavy metals detection by anodic stripping voltammetry. Electroanalysis 29 (11), 2444–2453.

Li, L., Wang, Y., Pan, L., Shi, Y., Cheng, W., Shi, Y., Yu, G., 2015. A nanostructured conductive hydrogels-based biosensor platform for human metabolite detection. Nano letters 15 (2), 1146–1151.

Lin, J., Peng, Z., Liu, Y., Ruiz-Zepeda, F., Ye, R., Samuel, E. L., Yacaman, M. J., Yakobson, B. I., Tour, J. M., 2014. Laser-induced porous graphene films from commercial polymers. Nature communications 5 (1), 1–8.

Ling, Y., Pong, T., Vassiliou, C. C., Huang, P. L., Cima, M. J., 2011. Implantable magnetic relaxation sensors measure cumulative exposure to cardiac biomarkers. Nature Biotechnology 29 (3), 273.

Liu, J., He, Z., Xue, J., Tan, T. T. Y., 2014. A metal-catalyst free, flexible, and free-standing chitosan/vacuum-stripped graphene/polypyrrole three-dimensional electrode interface for high-performance dopamine sensing. Journal of Materials Chemistry B 2 (17), 2478–2482.

Liu, Z., Ma, H., Sun, H., Gao, R., Liu, H., Wang, X., Xu, P., Xun, L., 2017. Nanoporous gold-based microbial biosensor for direct determination of sulfide. Biosensors and Bioelectronics 98, 29–35. Llandro, J., Palfreyman, J., Ionescu, A., Barnes, C., 2010. Magnetic biosensor technologies for medical applications: a review. Medical & biological engineering & computing 48 (10), 977–998.

Loo, A. H., Chua, C. K., Pumera, M., 2017. DNA biosensing with 3D printing technology. Analyst 142 (2), 279–283.

Lopez-Vidriero, M., Reid, L., 1978. Chemical markers of mucous and serum glycoproteins and their relation to viscosity in mucoid and purulent sputum from various hypersecretory diseases. American Review of Respiratory Disease 117 (3), 465–477.

Low, Z.-X., Chua, Y. T., Ray, B. M., Mattia, D., Metcalfe, I. S., Patterson, D. A., 2017. Perspective on 3D printing of separation membranes and comparison to related unconventional fabrication techniques. Journal of Membrane Science 523, 596–613.

Lynch, J. P., Loh, K. J., 2006. A summary review of wireless sensors and sensor networks for structural health monitoring. Shock and Vibration Digest 38 (2), 91–130.

Malek, S., Raney, J. R., Lewis, J. A., Gibson, L. J., 2017. Lightweight 3D cellular composites inspired by balsa. Bioinspiration & biomimetics 12 (2), 026014.

Malhotra, S., Verma, A., Tyagi, N., Kumar, V., 2017. Biosensors: principles, types, and applications. Int. J. Adv. Res. Innov. Ideas Educ 3 (2), 3639–3644.

Malinauskas, M., Žukauskas, A., Hasegawa, S., Hayasaki, Y., Mizeikis, V., Buividas, R., Juodkazis, S., 2016. Ultrafast laser processing of materials: from science to industry. Light: Science & Applications 5 (8), e16133–e16133.

Mannoor, M. S., Jiang, Z., James, T., Kong, Y. L., Malatesta, K. A., Soboyejo, W. O., Verma, N., Gracias, D. H., McAlpine, M. C., 2013. 3D printed bionic ears. Nano letters 13 (6), 2634–2639. Marino, A., Barsotti, J., de Vito, G., Filippeschi, C., Mazzolai, B., Piazza, V., Labardi, M., Mattoli, V., Ciofani, G., 2015. Two-photon lithography of

3D nanocomposite piezoelectric scaffolds for cell stimulation. ACS applied materials & interfaces 7 (46), 25574–25579.
McCreery, R. L., 2008. Advanced carbon electrode materials for molecular electrochemistry. Chemical reviews 108 (7), 2646–2687.
Milton, R. D., Giroud, F., Thumser, A. E., Minteer, S. D., Slade, R. C., 2013. Hydrogen peroxide produced by glucose oxidase affects the performance of laccase cathodes in glucose oxygen fuel cells: Fad dependent glucose dehydrogenase as a replacement. Physical Chemistry Chemical Physics 15 (44), 19371–19379.
Mohamed, O. A., Masood, S. H., Bhowmik, J. L., 2015. Optimization of fused deposition modeling process parameters: a review of current research and future prospects. Advances in Manufacturing 3 (1), 42–53.
Munshi, A. S., Martin, R. S., 2016. Microchip-based electrochemical detection using a 3-d printed wall-jet electrode device. Analyst 141 (3), 862–869.
Nag, A., Mukhopadhyay, S. C., Kosel, J., 2019. Printed Flexible Sensors: Fabrication, Characterization, and Implementation. Vol. 33. Springer.
Pan, Y., Hu, N., Wei, X., Gong, L., Zhang, B., Wan, H., Wang, P., 2019. 3D cell-based biosensor for cell viability and drug assessment by 3D electric cell/matrigel-substrate impedance sensing. Biosensors and Bioelectronics 130, 344–351.
Patel, S., Park, H., Bonato, P., Chan, L., Rodgers, M., 2012. A review of wearable sensors and systems with application in rehabilitation. Journal of neuroengineering and rehabilitation 9 (1), 21.
Pranzo, D., Larizza, P., Filippini, D., Percoco, G., 2018. Extrusion-based 3D printing of microfluidic devices for chemical and biomedical applications: A topical review. Micromachines 9 (8), 374.
Price, D., Crockett, A., Arne, M., Garbe, B., Jones, R., Kaplan, A., Langhammer, A., Williams, S., Yawn, B., 2009. Spirometry in primary care case-identification, diagnosis, and management of COPD. Primary Care Respiratory Journal 18 (3), 216–223.
Ragones, H., Schreiber, D., Inberg, A., Berkh, O., Kósa, G., Freeman, A., Shacham-Diamand, Y., 2015. Disposable electrochemical sensor prepared using 3D printing for cell and tissue diagnostics. Sensors and Actuators B: Chemical 216, 434–442.
Rebelo, R., Barbosa, A. I., Caballero, D., Kwon, I. K., Oliveira, J. M., Kundu, S. C., Reis, R. L., Correlo, V. M., 2019. 3D biosensors in advanced medical diagnostics of high mortality diseases. Biosensors and Bioelectronics 130, 20–39.
Reshetilov, A., Iliasov, P., Reshetilova, T., 2010. The microbial cell-based biosensors. Intelligent and Biosensors, 289–322.

Rocchitta, G., Spanu, A., Babudieri, S., Latte, G., Madeddu, G., Galleri, G., Nuvoli, S., Bagella, P., Demartis, M. I., Fiore, V., et al., 2016. Enzyme biosensors for biomedical applications: Strategies for safeguarding analytical performances in biological fluids. Sensors 16 (6), 780.

Rodbard, D., 2017. Continuous glucose monitoring: a review of recent studies demonstrating improved glycemic outcomes. Diabetes technology& therapeutics 19 (S3), S–25.

Rong, G., Ryckman, J. D., Mernaugh, R. L., Weiss, S. M., 2008. Label-free porous silicon membrane waveguide for DNA sensing. Applied Physics Letters 93 (16), 161109.

Rong, Q., Han, H., Feng, F., Ma, Z., 2015. Network nanostructured polypyrrole hydrogel/au composites as an enhanced electrochemical biosensing platform. Scientific Reports 5 (1), 1–8.

Rumley-Ouellette, B. J., Wahry, J. H., Baker, A. M., Bernardin, J. D., Marchi, A. N., Todd, M. D., 2017. In situ printing of conductive polylactic acid strain sensors embedded into additively manufactured parts. Struct. Health Monit.

Rusling, J. F., 2018. Developing microfluidic sensing devices using 3D printing. ACS Sensors 3 (3), 522–526.

Sailor, M. J., 2007. Color me sensitive: Amplification and discrimination in photonic silicon nanostructures. ACS Nano 1 (4), 248–252.

Setzu, S., Monduzzi, M., Mula, G., Salis, A., 2011. Porous silicon-based electrochemical biosensors. In: Biosensors-Emerging Materials and Applications. IntechOpen.

Setzu, S., Salis, S., Demontis, V., Salis, A., Monduzzi, M., Mula, G., 2007. Porous silicon-based potentiometric biosensor for triglycerides. physica status solidi (a) 204 (5), 1434–1438.

Sharafeldin, M., Jones, A., Rusling, J. F., 2018. 3D-printed biosensor arrays for medical diagnostics. Micromachines 9 (8), 394.

Shirazi, S. F. S., Gharehkhani, S., Mehrali, M., Yarmand, H., Metselaar, H. S. C., Kadri, N. A., Osman, N. A. A., 2015. A review on powder-based additive manufacturing for tissue engineering: selective laser sintering and inkjet 3D printing. Science and technology of advanced materials 16 (3), 033502.

Smith, P. T., Basu, A., Saha, A., Nelson, A., 2018. Chemical modification and printability of shear-thinning hydrogel inks for direct-write 3D printing. Polymer 152, 42–50.

Soltani Zarrin, P., Ibne Jamal, F., Roeckendorf, N., Wenger, C., 2019. Development of a portable dielectric biosensor for rapid detection of viscosity variations and its in vitro evaluations using saliva samples of

COPD patients and healthy control. In: Healthcare. Vol. 7. Multidisciplinary Digital Publishing Institute, p. 11.
Song, Y., Nesaei, S., Du, D., Gozen, A., Lin, Y., 2018. 3D printed wearable glucose sensors. In: Meeting Abstracts. No. 43. The Electrochemical Society, pp. 2482–2482.
Stassi, S., Fantino, E., Calmo, R., Chiappone, A., Gillono, M., Scaiola, D., Pirri, C. F., Ricciardi, C., Chiado, A., Roppolo, I., 2017. Polymeric 3D printed functional microcantilevers for biosensing applications. ACS applied materials & interfaces 9 (22), 19193–19201.
Su, H., Li, S., Jin, Y., Xian, Z., Yang, D., Zhou, W., Mangaran, F., Leung, F., Sithamparanathan, G., Kerman, K., 2017. Nanomaterial-based biosensors for biological detections. Arch J 3, 19–29. Sutradhar, S., Patnaik, A., 2017. A new fullerene-c60–nanogold composite for non-enzymatic glucose sensing. Sensors and Actuators B: Chemical 241, 681–689.
Tappa, K., Jammalamadaka, U., 2018. Novel biomaterials used in medical 3D printing techniques. Journal of functional biomaterials 9 (1), 17.
Tiller, B., Reid, A., Zhu, B., Guerreiro, J., Domingo-Roca, R., Jackson, J. C., Windmill, J., 2019. Piezoelectric microphone via a digital light processing 3D printing process. Materials & Design 165, 107593.
Tiwari, J. N., Vij, V., Kemp, K. C., Kim, K. S., 2016. Engineered carbon-nanomaterial-based electrochemical sensors for biomolecules. ACS Nano 10 (1), 46–80.
Valentin, T. M., Leggett, S. E., Chen, P.-Y., Sodhi, J. K., Stephens, L. H., McClintock, H. D., Sim, J. Y., Wong, I. Y., 2017. Stereolithographic printing of ionically-crosslinked alginate hydrogels for degradable biomaterials and microfluidics. Lab on a Chip 17 (20), 3474–3488.
Vigneshvar, S., Sudhakumari, C., Senthilkumaran, B., Prakash, H., 2016. Recent advances in biosensor technology for potential applications–an overview. Frontiers in bioengineering and biotechnology 4, 11.
Vo-Dinh, T., Cullum, B., 2000. Biosensors and biochips: advances in biological and medical diagnostics. Fresenius' journal of analytical chemistry 366 (6-7), 540–551.
Wang, K., Ho, C.-C., Zhang, C., Wang, B., 2017. A review of the 3D printing of functional structures for medical phantoms and regenerated tissue and organ applications. Engineering 3 (5), 653–662.
Wang, X., Liu, A., Xing, Y., Duan, H., Xu, W., Zhou, Q., Wu, H., Chen, C., Chen, B., 2018a. Three-dimensional graphene bio-interface with extremely high sensitivity to single cancer cell monitoring. Biosensors and Bioelectronics 105, 22–28.

Wang, Z., Kumar, H., Tian, Z., Jin, X., Holzman, J. F., Menard, F., Kim, K., 2018b. Visible light photoinitiation of cell-adhesive gelatin methacryloyl hydrogels for stereolithography 3D bioprinting. ACS applied materials & interfaces 10 (32), 26859–26869.

Wei, H., Li, K., Liu, W. G., Meng, H., Zhang, P. X., Yan, C. Y., 2017. 3D printing of free-standing stretchable electrodes with tunable structure and stretchability. Advanced Engineering Materials 19 (11), 1700341.

Williams, J. M., Adewunmi, A., Schek, R. M., Flanagan, C. L., Krebsbach, P. H., Feinberg, S. E., Hollister, S. J., Das, S., 2005. Bone tissue engineering using polycaprolactone scaffolds fabricated via selective laser sintering. Biomaterials 26 (23), 4817–4827.

Xuan, X., Kim, J. Y., Hui, X., Das, P. S., Yoon, H. S., Park, J.-Y., 2018. A highly stretchable and conductive 3D porous graphene metal nanocomposite based electrochemical-physiological hybrid biosensor. Biosensors and Bioelectronics 120, 160–167.

Yang, T.-Y., Fang, C.-Y., Chen, J.-S., Po, H. L., Chou, L.-P., Chiang, C.-Y., Ueng, K.-C., 2015. Association of serum uric acid with cardiovascular disease in Taiwanese patients with primary hypertension. Acta Cardiologica Sinica 31 (1), 42.

Yang, Y. J., Li, W., 2014. Ctab functionalized graphene oxide/multiwalled carbon nanotube composite modified electrode for the simultaneous determination of ascorbic acid, dopamine, uric acid, and nitrite. Biosensors and Bioelectronics 56, 300–306.

Zhang, G.-J., Ning, Y., 2012. Silicon nanowire biosensor and its applications in disease diagnostics: a review. Analytica Chimica Acta 749, 1–15.

Zhang, J. X., Hoshino, K., 2018. Molecular Sensors and Nanodevices: Principles, Designs and Applications in Biomedical Engineering. Academic Press.

Zhang, S., Wright, G., Yang, Y., 2000. Materials and techniques for electrochemical biosensor design and construction. Biosensors and Bioelectronics 15 (5-6), 273–282.

Zhang, W., Li, G., 2004. Third-generation biosensors based on the direct electron transfer of proteins. Analytical sciences 20 (4), 603–609.

Zhang, Y., Xia, Z., Liu, H., Yang, M., Lin, L., Li, Q., 2013. Hemin-graphene oxide-pristine carbon nanotubes complexes with intrinsic peroxidase-like activity for the detection of h2o2 and simultaneous determination for trp, aa, da, and ua. Sensors and Actuators B: Chemical 188, 496–501.

Zheng, T., Perona Martinez, F., Storm, I. M., Rombouts, W., Sprakel, J., Schirhagl, R., De Vries, R., 2017. Recombinant protein polymers for

colloidal stabilization and improvement of cellular uptake of diamond nanosensors. Analytical Chemistry 89 (23), 12812–12820.
Zhuo, B., Chen, S., Zhao, M., Guo, X., 2017. High sensitivity flexible capacitive pressure sensor using polydimethylsiloxane elastomer dielectric layer micro-structured by 3-d printed mold. IEEE Journal of the Electron Devices Society 5 (3), 219–223.

CHAPTER TEN

3D PRINTED SENSORS FOR MEDICAL HEALTHCARE APPLICATIONS

DR. GAURI M. SHUKLA

Abstract

Three-dimensional (3D) printing technology has been employed in device prototyping and fabrication for a variety of medical and research applications. In the medical field, 3D printing is utilised for the fabrication of diagnostic devices, tissues, organs, implants, external prostheses, anatomical models, surgical tools, etc. Further, 3D printing is also being used in the manufacturing of a variety of sensors. This technique facilitates the fabrication of customised sensors in a fast and low-cost manner compared to traditional fabrication techniques such as photolithography, screen printing, laser cutting, and contact printing. Quite a few sensing mechanisms can be easily incorporated in 3D printed structures. 3D printed sensors can be fabricated by directly printing the sensing platform, or the sensor can be integrated into the printed platform. This chapter provides a detailed review of various sensors developed for medical applications. Various 3D printing techniques used to fabricate these sensors are summarised in this chapter. Related sensing mechanisms, applications, and printing materials are also discussed, and 3D-printed microfluidic sensing platform for disease diagnosis is explored.

Introduction

Three-dimensional (3D) printing, commonly called additive manufacturing caters to a wide variety of medical and research applications for device prototyping and fabrication. It includes a group of manufacturing technologies competent enough to add materials layer-by-layer to fabricate 3D objects by digitally controlled processes. Additive manufacturing has a number of

benefits, such as minimal waste, cost reduction in terms of time, transportation, storage, and energy consumption; conversely, subtractive conventional methods rely on complex machinery and tools.[1] The very first requirement of 3D printing is the need to generate digital models via three-dimensional scanner or computer-aided design (CAD) software. Later, this 3D model is converted into an STL, file and by using this file, the 3D printer software cuts the object into a series of 2D cross-section layers. Lastly, the 3D object is produced in a layer-by-layer manner. A wide range of materials can be used, such as polymers, metals, composites and ceramics. These methods overcome the shortcomings of conventional fabrication for developing devices having complex geometric shapes, and for incorporating functionalities such as optical, chemical, electronic, electromagnetic, fluidic, actuation, thermal and acoustical.[2]

3D printing techniques can be compared using various existing parameters, for example: process material, time for printing, repeatability, resolution and accuracy. They are broadly categorised as material extrusion, vat polymerisation, material jetting, binder jetting, powder bed fusion, direct energy deposition, and sheet lamination.[3] The material extrusion method involves the deposition of molten thermoplastic filament via a nozzle head dispenser onto a platform which travels in horizontal and vertical directions. The fused deposition modelling (FDM) technique is based on this type. Acrylonitrile butadiene styrene (ABS), polylactic acid (PLA), polyamide (PA) polycarbonate (PC), polystyrene and polyethylene terephthalate (PET), etc., can be used as printing materials. The 3D structure is built via a layer-by-layer deposition in which subsequent layers are deposited once the previous layer cools down below its thermoplastic temperature.[4]

Direct printing is also based on the extrusion principle, in which the precise addition of highly viscous liquid materials is attained. A wide variety of materials can be printed, such as ceramics, polymers, and hydrogels. A variety of parameters need to be optimised, which include dimensions of the nozzle, the mass of the material, viscosity, writing speed and extrusion

[1] Manzanares Palenzuela, C. L. & Pumera, M. (Bio)Analytical chemistry enabled by 3D printing: Sensors and biosensors. *TrAC Trends Anal. Chem.* 103, 110–118 (2018).
[2] Xu, Y. *et al.* The Boom in 3D-Printed Sensor Technology. *Sensors* 17, 1166 (2017).
[3] MacDonald, E. & Wicker, R. Multiprocess 3D printing for increasing component functionality. *Science (80-.).* 353, (2016).
[4] Han, T., Kundu, S., Nag, A. & Xu, Y. 3D Printed Sensors for Biomedical Applications: A Review. *Sensors* 19, 1706 (2019).

speed. However, post-processing is necessary to achieve structural integrity through sintering, heat, and UV treatment and drying processes.

The vat photopolymerization method is based on a layer-by-layer hardening of liquid polymer using a light source. Among the two types of vat photopolymerization, stereolithography (SLA) is a method that uses a laser, whereas digital light processing (DLP) uses a laser or UV lamp to cure the liquid polymer. The laser spot diameter and the absorption properties of photocurable polymer govern the resolution of SLA. These techniques can generate structures with a high degree of accuracy and complex internal features, but the major drawback is that it is limited to the use of a single material. In material jetting, i.e. inkjet and PolyJet printing, a moving inkjet print head ejects the material onto a build area. However, multiple printing materials can be utilised in PolyJet printing. Binder jetting is based on bonding the layer of powder materials by ejecting special adhesives from the inkjet nozzle. Different materials such as ceramics, plaster, and sugar can be printed in this way. In laminated object manufacturing (LOM), sheets of material are bonded to form the solid 3D model. Powder-bed fusion-based techniques such as selective laser sintering (SLS) or selective laser melting (SLM) can print materials in powdered form. Polymers, metals, ceramics, and waxes can be printed using this method. They make use of thermal energy from a laser to fuse powder in a powder bed.

These techniques are extensively used in medical applications to create custom-made medical products and equipment. The medical applications of 3D printing can be categorised as tissue and organ fabrication; fabrication of prosthetics, implants, and anatomical models; and pharmaceutical research related to drug discovery and delivery.[5] Sensors are another area in which these techniques have made major contributions. With the advances in micro-machinery and micro-controller platforms, different varieties of sensors have been employed in manufacturing, aerospace, medicine, and biomedical devices and even in robotics. Traditionally, fabrication techniques such as photolithography, screen printing, laser cutting and contact printing are widely used in sensor fabrication. Conventional printing methods such as screen printing are being utilised to develop simple, small, and inexpensive sensors. But the inherent limitation is that the developed sensor is only two-dimensional. 3D-printed sensors involve fewer steps, require fewer manual interventions, and produce low waste compared to conventional techniques, e.g. photolithography for manufacturing of sensors. The sensing

[5] Ventola, C. L. Medical Applications for 3D Printing: Current and Projected Uses. *P T* 39, 704–11 (2014).

device prototype can be tailored to the sensor's end use. Also, a variety of materials can be used in 3D printing, more than in conventional techniques. A substantial amount of research is going on in 3D printed sensors which seek to exploit the various sensing mechanisms such as electronics, force, motion, optics, etc. It is easier to develop electronically, and force sensing modules via 3D printing. Other sensing mechanisms can be incorporated into 3D-printed structures using commercial components. Substrate board, electronic ink, and print processing techniques are vital constituents of 3D-printed sensors.

Among the various previously discussed 3D-printing techniques, FDM is essentially used to produce prototypes that involve electrochemical sensing mechanism. Although SLA and ink-jet printing develop devices with lower resolution, their usefulness in printing sensors has been recognized. Sensors catering to cell culture applications are typically printed by PolyJet and SLS processes. Various categories of sensors can be developed through 3D-printing technology to name just a few physical sensors, biosensors, and chemical sensors. This technology can be utilised to print sensing components, moulds for sensors, and platforms to incorporate sensors.[6] 3D printing can be applied to produce electrodes for recording electrophysiological signals, including ECG and EEG. Complex 3D geometries of these electrodes can allow for the recording of these signals in the presence of hair.[7]

3D printing technology has considerably revolutionised lab-based medical device development. With this technology, researchers can design multiple prototypes and can repeat the "design-test-redesign" cycle in a short period. In addition, it allows for the development of prototypes distinct to each application. Enzyme-based biosensors and immunosensors can also be fabricated using these techniques. The essential component of enzyme-linked immunosorbent assay (ELISA)-based detection is a 96-well plate. Such plates can be designed and developed using a 3D printing platform. This will open up the applicability of ELISA-based techniques towards miniaturisation and lab-on-a-chip platforms. Furthermore, by achieving a

[6] Ni, Y. *et al.* A review of 3D-printed sensors. *Appl. Spectrosc. Rev.* 52, 623–652 (2017).
[7] Salvo, P. *et al.* A 3D printed dry electrode for ECG/EEG recording. *Sensors Actuators A Phys.* 174, 96–102 (2012).

reduction in time and volume of reagents, the samples for testing of infectious diseases may be increased.[8]

3D-printing is popular in developing parts that can be incorporated in point of care diagnosis. Recent developments in smartphones have opened avenues for optical-based sensing. A smartphone can act as a potential spectrometer if the optical detection is in visible range.[9] A cartridge containing microfluidics is coupled to the smartphone with pre-optics to reduce the interference of ambient light.

Polymeric microfluidic devices can be obtained via inkjet printing, FDM, SLA, and two-photon polymerisation (2PP).[10] Microfluidic devices with microchannels larger than 400 μm can be easily printed via inkjet printing. To print fully enclosed microchannels of 10–100 μm, new support materials, and their removal processes need to be developed. SLA does not need support materials, but it is limited by the need to use UV curable resins. The 2PP method can generate precise and accurate 3D structures with high spatial resolution i.e. in the sub-100 nm range. This method makes use of solid resin in place of epoxy resin. The high cost of femtosecond lasers, positioning systems, and optics; no multi-material printability, and the time-consuming fabrication process are some of the limitations of the 2PP method. Also, removal of the material, which is not illuminated or photopolymerised, will be an issue in case of small, fully-enclosed structures. The FDM technique can print a variety of thermoplastic polymers, but is limited by resolution and surface finish.

Many reviews are present on different categories of sensors engaged in various applications. This chapter specifically focuses on a variety of sensors employed in medical healthcare applications. For simplicity, these sensors are categorised as enzymatic biosensors, microfluidic devices for disease diagnosis, lab-on-a-chip, DNA biosensing, smartphone-based biosensors and flex sensors. Microcantilever-based biosensors, capacitive tactile sensors, and optical components for label-free sensing are discussed under other sensors category.

---

[8] Singh, H. *et al.* Application of 3D Printing Technology in Increasing the Diagnostic Performance of Enzyme-Linked Immunosorbent Assay (ELISA) for Infectious Diseases. *Sensors* 15, 16503–16515 (2015).

[9] Lambert, A., Valiulis, S. & Cheng, Q. Advances in Optical Sensing and Bioanalysis Enabled by 3D Printing. *ACS Sensors* 3, 2475–2491 (2018).

[10] Waheed, S. *et al.* 3D printed microfluidic devices: enablers and barriers. *Lab Chip* 16, 1993–2013 (2016).

Enzymatic Biosensors

Direct-write 3D-printing techniques can create a microelectronics circuit on any substrate. This technique is extremely useful in the development of biosensors for specific applications. The sensor's circuitry needs to be tailored to achieve optimum detection range or limit. The direct-write technique was used by Yang et al. for the fabrication of microelectrodes arrays as a platform for electrochemical sensing.[11] The aerosol jet technology was utilised to fabricate microelectrodes with spacing of 30, 100, and 180 μm. Hydrogen peroxide and glucose were chosen as a model analyte to test the sensor's performance. The microelectrode was fabricated by dispensing Ag nanoparticle ink on the glass substrate using an ultrasonic atomizer with a nozzle exit diameter of 150 μm, atomizing flow rate of 25 sccm, and a sheath gas flow rate of 50 sccm. On top of these Ag layers, UV curable polymer ink was dispensed with the help of a jetting action which was cured immediately by a UV source (power was set to 30% of the maximum capability, 940 mW). The platen temperature was 80°C. The sensors were thermally sintered at 100^0C for 15 min and then cooled in air.

A prototype of a glucose dehydrogenase 3D-printed glucose sensor was fabricated possessing features and dimensions similar to the screen printed Zensor sensors.[12] The sensor was printed onto a mylar substrate using a conductive graphene polylactic acid filament. Travel speed, extruder temperature, and retraction distance of the printer were set to 30 mm/s of 220°C and 1.5 mm respectively. Recently, hydrogen peroxide detection was carried out by a 3D-printed graphene/polylactic (PLA) electrode.[13] With the help of fused deposition modelling, an electrode of total length 45 mm, and thickness of 2 mm having a basal circle of diameter 8 mm with a tail of 37 mm length was printed. A commercially available Black Magic 3D raw material filament was utilised for printing.

Compatibility of the digital light processing technique for photopolymerization of hydrogel was exploited to produce 3D shapes of hydrogels with

[11] Yang, H., Rahman, M. T., Du, D., Panat, R. & Lin, Y. 3-D printed adjustable microelectrode arrays for electrochemical sensing and biosensing. *Sensors Actuators B Chem.* 230, 600–606 (2016).

[12] Adams, A., Malkoc, A. & La Belle, J. T. The Development of a Glucose Dehydrogenase 3D-Printed Glucose Sensor: A Proof-of-Concept Study. *J. Diabetes Sci. Technol.* 12, 176–182 (2018).

[13] López Marzo, A. M., Mayorga-Martinez, C. C. & Pumera, M. 3D-printed graphene direct electron transfer enzyme biosensors. *Biosens. Bioelectron.* 151, 111980 (2020).

controlled water contents.[14] Biomolecules were trapped within acrylate-derivated polyethylene glycol (PEG) polymerisation. To achieve a high biomolecule loading capacity, the length of the PEG chain (700 Da) was optimised. The printing ink comprised cresol red, PEG-700-DA, and Irgacure 819 (UV−visible photoinitiator) prepared in ethanol. Different biomolecules like peroxidase glucose oxidase and anti-brain natriuretic peptide monoclonal antibodies were subsequently mixed in this solution. Both enzyme and antibody-based assays were tested that ensured the compatibility of 3D printing for the production of biosensing components.

The FDM technique can be utilised for 3D-print reactions by integrating reactive substances into the raw materials before printing.[15] The authors developed the 3D object of a 48-well plate without a bottom supporting plate using a commercial FDM-type 3D printer, with the iron oxide nanoparticles-incorporated PLA filaments. A mixture of Fe_2O_3 or Fe_3O_4 nanoparticles were added in PLA pellets into a commercial filament maker with an extrusion temperature of 160°C to produce nanoparticles incorporated PLA filaments. The developed plate was able to catalyse the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) by hydrogen peroxide (H_2O_2). Using this plate, glucose detection was then demonstrated by combining the catalytic reactions of the iron oxide nanoparticles-incorporated multi-well plate and the H_2O_2 generated from glucose oxidation with glucose oxidase (GOx). In another study, FDM-printed bioreactors were immobilised with glucose oxidase and lactate oxidase for the detection of glucose and lactate, respectively, in rat-brain microdialysate.[16] The printing was done with ABS plastic filaments. A 0.4 mm copper nozzle was used, which was maintained at 260°C, and the printing speed was in the range 10 to 100 cm^3/h. The platform was preheated to 80°C.

[14] Mandon, C. A., Blum, L. J. & Marquette, C. A. Adding Biomolecular Recognition Capability to 3D Printed Objects. *Anal. Chem.* 88, 10767–10772 (2016).
[15] Su, C. K. & Chen, J. C. Reusable, 3D-printed, peroxidase mimic–incorporating multi-well plate for high-throughput glucose determination. *Sensors Actuators, B Chem.* 247, 641–647 (2017).
[16] Su, C. K., Yen, S. C., Li, T. W. & Sun, Y. C. Enzyme-Immobilized 3D-Printed Reactors for Online Monitoring of Rat Brain Extracellular Glucose and Lactate. *Anal. Chem.* 88, 6265–6273 (2016).

A 3D-printed batch injection analysis cell was employed for glucose detection from artificial serum using paper-based enzymatic reactors.[17] The 9×9×3.5cm) cell was printed by FDM using ABS polymer. Electronic micropipette and screen-printed electrodes required for electrochemical detection were coupled to this cell through a rectangular opening.

The holder for the paper-based sensor is printed via SLA. A paper-based printed sensor for the measurement of butyrylcholinesterase activity from serum samples was fixed inside the 3D printed device.[18] Butyrylthiocholine as an enzymatic substrate was loaded on paper, and an electrochemical sensor modified with a Carbon Black and Prussian Blue nanocomposite was engaged for butyrylcholinesterase activity measurement.

The 3D printed holder was printed by stereolithography using white methacrylate. The device consisted of two parts: a base (13x25x4 mm^3) and a collector (13x25x0.2 mm^3) in which the sensor was placed, and the electrodes accessible through the circular collector hole (diameter 8 mm).

Microfluidic Devices

For personalised healthcare diagnostic applications, microfluidic arrays incorporating necessary complex processes onto simple, portable, and economical platforms are crucial. For printing polylactic acid-based microfluidic immunoarray, a 3D-Fused deposition modelling printer was used. The temperatures of the heated platform and extruder were set to 60°C and 230°C, respectively. The layer height was 200 mm. The extruder-travelling speed and extruding speed were maintained at 80 mm/s and 40 mm/s respectively. Screen-printed carbon sensors were fixed into the 3D-printed immunoarray using silicone glue. The developed immunosensor could detect three cancer biomarker proteins: prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), and platelet factor-4 (PF-4) in serum within 35 min.[19] Furthermore, the same group demonstrated a 3D printed unibody with an optically clear electrochemiluminescence

[17] Dias, A. a. *et al.* Paper-based enzymatic reactors for batch injection analysis of glucose on 3D printed cells coupled with amperometric detection. *Sensors Actuators B Chem.* 226, 196–203 (2016).
[18] Scordo, G., Moscone, D., Palleschi, G. & Arduini, F. A reagent-free paper-based sensor embedded in a 3D printing device for cholinesterase activity measurement in serum. *Sensors Actuators, B Chem.* 258, 1015–1021 (2018).
[19] Kadimisetty, K. *et al.* Biosensors, and Bioelectronics immunoarray. *Biosens. Bioelectron.* 77, 188–193 (2016).

microfluidic array having 5 reagent reservoirs emerging into a common microfluidic serpentine channel.[20] The serpentine channel was 3D-printed having dimensions 1.2(L) x 0.15(W) cm and 350 μm (D). The channel was connected to a 32-microwell array for the simultaneous detection of multiple proteins.

A microfluidic device was fabricated by Tang et al. for multiplexed detection of prostate cancer biomarker proteins PSA and PF-4.[21] The Unibody device comprised three reagent reservoirs of 125 ± 5 μL volume. Equal volume empty chambers were placed in between the reservoirs to separate the solutions and prevent mixing followed by an efficient 3D network passive mixer (210 ± 10μL) and an optically transparent detection chamber (30 ± 2μL) containing poly-L-lysine coated glass slide immobilised with antibodies for quantifying chemiluminescence using a CCD camera. The device was printed by a stereolithographic printer using clear transparent methacrylate-based resin (FormLab GCPL02).

A 3D-printed continuous flow system was coupled to disposable screen-printed electrodes for the rapid detection of hepatic oval cells.[22] Oval cell marker antibodies were immobilised on chitosan film present onto multiwall carbon nanotube (MWCNT) electrodes. The flow cell was printed using a DLP printer using an acrylate-based photopolymer. 3D-printed thin-layer flow cell integrated with a flat screen-printed flow-field shaped solid electrode was developed for the detection of Pb^{2+}.[23] A complete flow cell consisted of an outlet, an inlet, an electrode housing, and a saddle-shaped

[20] Rusling, J. F., Kadimisetty, K., Malla, S., Bishop, G. W. & Satterwhite-Warden, J. E. Low-cost 3D-printed biosensor arrays for protein-based cancer diagnostics based on electrochemiluminescence. *BIODEVICES 2016 - 9th Int. Conf. Biomed. Electron. Devices, Proceedings; Part 9th Int. Jt. Conf. Biomed. Eng. Syst. Technol. BIOSTEC 2016* 1, 17–22 (2016).

[21] Tang, C. K., Vaze, A. & Rusling, J. F. Automated 3D-printed unibody immunoarray for chemiluminescence detection of cancer biomarker proteins. *Lab Chip* 17, 484–489 (2017).

[22] Damiati, S. *et al.* Embedded disposable functionalized electrochemical biosensor with a 3D-printed flow cell for the detection of hepatic oval cells (HOCs). *Genes (Basel).* 9, (2018).

[23] Sun, Q. *et al.* A New Electrochemical System Based on a Flow-Field Shaped Solid Electrode and 3D-Printed Thin-Layer Flow Cell: Detection of Pb 2+ Ions by Continuous Flow Accumulation Square-Wave Anodic Stripping Voltammetry. *Anal. Chem.* 89, 5024–5029 (2017).

working area that was printed with a PolyJet 3D printer using acrylate-based polymer materials.

A new 3D printed device was fabricated for electrochemical detection. The integration of removable, reusable electrodes with 3D printed microfluidic devices made from proprietary acrylate-based polymer material was demonstrated.[24] The detection of enzymatic activity in cells and tissue was demonstrated using a bio-electrochemical sensor.[25]

A DMS cast chip was obtained from a polymer master, fabricated by 3D stereolithography using proprietary rigid white polymer. The chip holder was also 3D printed by ABS. This sensor was validated for the detection of biomarker alkaline phosphatase secreted by colon cancer cell lines. In other electrochemical biosensors, 3D-printed capillary channels were incorporated to guide and constrain the clinical sample for the detection of liver cancer cells.[26] The microfluidic chamber, being 1.5 × 1 × 7 mm, was printed with the help of co-polyester polymer filament.

Using the SLA technique, the fluidic device which was made of clear plastic to allow electrochemiluminescence (ECL) measurements having ports for electrodes, was fabricated.[27] To examine stored blood components through the plate reader, 3D-printed fluidic devices were developed which will help to reduce the occurrence of storage lesion.[28]

Gowers et al. demonstrated a 3D-printed microfluidic analysis system which could be coupled with FDA-approved clinical microdialysis probes.[29] Removable needle-type integrated biosensors for glucose and Lactate were

[24] Erkal, J. L. *et al.* 3D printed microfluidic devices with integrated versatile and reusable electrodes. *Lab Chip* 14, 2023–2032 (2014).
[25] Ragones, H. *et al.* Disposable electrochemical sensor prepared using 3D printing for cell and tissue diagnostics. *Sensors Actuators, B Chem.* 216, 434–442 (2015).
[26] Damiati, S. *et al.* Acoustic and hybrid 3D-printed electrochemical biosensors for the real-time immunodetection of liver cancer cells (HepG2). *Biosens. Bioelectron.* 94, 500–506 (2017).
[27] Bishop, G. W., Satterwhite-Warden, J. E., Bist, I., Chen, E. & Rusling, J. F. Electrochemiluminescence at Bare and DNA-Coated Graphite Electrodes in 3D-Printed Fluidic Devices. *ACS Sensors* 1, 197–202 (2016).
[28] Chen, C., Wang, Y., Lockwood, S. Y. & Spence, D. M. 3D-printed fluidic devices enable quantitative evaluation of blood components in modified storage solutions for use in transfusion medicine. *Analyst* 139, 3219–3226 (2014).
[29] Gowers, S. A. N. *et al.* 3D Printed Microfluidic Device with Integrated Biosensors for Online Analysis of Subcutaneous Human Microdialysate. *Anal. Chem.* 87, 7763–7770 (2015).

included in this device. They were inserted via the openings made on the top wall of the microchannel positioned in 3D-printed electrode holders. ULTRA 3SP, a 3D printer, was employed to print the microfluidic chip consuming "ABS 3SP White" resists. To print the needle holders with rigid and soft parts, an Objet260 Connex 3D printer was used. The materials involved in printing were VeroWhitePlus (RGD835) and TangoBlack (FLX973).

The disposable and real-time bacteria detection system was developed by Yeh et al.[30] The developed 3D-printed bio-sensing microfluidic "RoationChip" comprised two layers: a sample injecting inlet, four sample wells and a pair of mechanical latches were a part of the top layer, while sample flowing channels, four reagent wells and the locking latch on the edge were present on the bottom layer. Mechanical latches were designed to achieve vertical chip assembly and in-plane rotation. This chip was printed by a PolyJet 3D printer using transparent material analogous to PMMA (polymethyl methacrylate) and a soluble support material (SUP706). To avoid leakage between the two layers of the chip, a layer of transparent waterproof silicone grease was screen-printed on the contact surfaces. A lubricating thin layer of FC-40 fluorinates oil was subsequently added to the contact surfaces.

The microfluidic device was developed for influenza hemagglutinin detection where paramagnetic particles (MPs)-based isolation of hemagglutinin (HA) was carried out followed by the indirect detection of the isolated compound with CdS quantum dots (QDs) labels.[31] This microfluidic device for isolation and detection of HA–QDs was fabricated using three-dimensional (3D) printing. A polylactide material was extruded at a temperature of 210°C on a heated surface of 40°C to fabricate a chip having dimensions [x, y, and z] of 42.64/14.95/4.87 mm. In another study, a 3D microfluidic magnetic preconcentrator was developed by Park et al. It was devised to detect Escherichia coli O157: H7 in large-volume sample solutions with the help of a commercial ATP luminometer.[32] Specific

[30] Yeh, P. C., Chen, J., Karakurt, I. & Lin, L. 3D Printed Bio-Sensing Chip for the Determination of Bacteria Antibiotic-Resistant Profile. *2019 20th Int. Conf. Solid-State Sensors, Actuators Microsystems Eurosensors XXXIII, TRANSDUCERS 2019 EUROSENSORS XXXIII* 126–129 (2019).
DOI:10.1109/TRANSDUCERS.2019.8808229

[31] Krejcova, L. *et al.* 3D printed chip for electrochemical detection of influenza virus labeled with CdS quantum dots. *Biosens. Bioelectron.* 54, 421–427 (2014).

[32] Park, C. *et al.* 3D-printed microfluidic magnetic preconcentrator for the detection of the bacterial pathogen using an ATP luminometer and antibody-conjugated magnetic nanoparticles. *J. Microbiol. Methods* 132, 128–133 (2017).

antibody-conjugated magnetic nanoparticles (Ab-MNPs) were first mixed with a sample containing E. coli O157:H7.

Subsequently, bacteria-Ab-MNP complexes formed in the mixture were preconcentrated in a 3D microfluidic magnetic preconcentrator using a permanent magnet. The presence of these bacterial cells was detected by measuring ATP in the cells using the ATP luminometer. E. coli O157: H7 in samples containing 10% of blood was assessed using this device. This preconcentrator comprised three functional units as follows: 1) an array of pillars to filter out very large particles having diameter 400 μm and height 500 μm with varying spacing between the pillars from 600 μm to 1000 μm; 2) a trapezoidal chamber for preconcentration of bacteria-Ab-MNP complexes from the samples; and 3) a particle collector to collect bacteria-Ab-MNP complexes from the preconcentrator, being 2 mm (W), 3 mm (L) and 1.5 mm (H). The device is printed using a DLP 3D printer using a photo-curable resin acrylate-based photopolymer.

Unibody Lab on a Chip

SLA 3D printers can be utilised to produce a Unibody lab-on-a-chip (ULOC). This device can incorporate features such as compactness, robustness, reproducibility, and, like microfluidic configuration, less consumption of reactants. Comina et al. used a consumer-grade 3D printer to develop a prototype of the Unibody lab-on-a-chip.[33] Proprietary resin comprising modified acrylate oligomer and monomer in a mixture with an epoxy monomer, a photoinitiator and additives were utilised for printing, while exposure time, printing speed and the vertical separation between exposed layers were set at 7s, 2 cm/hour and 50 μm, respectively. This prototype was probed for H_2O_2 and glucose detection, which included passive, active transport and fluorescence, and colorimetric readout.

A miniaturised bioluminescence sensing system is needed for sensitive and continuous monitoring of ATP having an integrated microfluidic chamber for the handling of biological samples. Efforts were made by Santangelo et al. to fabricate 3D-printed microfluidics chips coupled with silicon photomultipliers (SiPMs) for ATP detection, which would lower reactant consumption and increase solution delivery close to the SiPM to improve

[33] Comina, G., Suska, A. & Filippini, D. Low-cost lab-on-a-chip prototyping with a consumer-grade 3D printer. *Lab Chip* 14, 2978–2982 (2014).

detection efficiency.[34] The developed system showed a detection range of ATP comparable to that of a bench-scale commercial bioluminescent reader.

The SLA-based manufacturing of unibody lab-on-a-chip (ULOC) systems incorporating bio functional materials are a challenge, as there is a limitation of the choice of processable material. Credi et al. have demonstrated the fabrication of highly integrated biologically active ULOC devices using multi-material SLA printing.[35] They have developed a novel, bioactive, photocurable polymer, which exploited the covalent functionalization of a methacrylate-based biocompatible resin with a lateral biotin pending group. After printing, the performance of the functional biotin at or near the surface of bioactive resin was directly measured by carrying out a standard colorimetric 4-hydroxyazobenzene-2′-carboxylic acid (HABA)/avidin assay, and a fluorescence-based assay with fluorescein isothiocyanate labelled avidin, based on the strong biotin/avidin affinity.

DNA Biosensing

Electrochemical DNA biosensing application was evaluated with the help of helical-shaped stainless-steel electrodes.[36] The SLM method was utilised to print these electrodes. In this method, a high-intensity laser beam was used to fuse a fine metal powder which was precisely scattered on the printing stage in a layer-by-layer manner as per the design. Later, these 3D-printed electrodes were modified to obtain a DNA biosensing interface.

A portable quantitative polymerase chain reaction (qPCR) device was fabricated for the diagnosis of infectious diseases.[37] The device casing (1.6 mm thick), the holder for the heating element, the fan and cooling system, and the structure of the light path for fluorescence reading are made of 3D fused-filament deposition parts. A 1.75 mm black ABS filament was used

[34] Santangelo, M. F., Libertino, S., Turner, A. P. F., Filippini, D. & Mak, W. C. Integrating printed microfluidics with silicon photomultipliers for miniaturized and highly sensitive ATP bioluminescence detection. *Biosens. Bioelectron.* 99, 464–470 (2018).

[35] Credi, C., Griffini, G., Levi, M. & Turri, S. Biotinylated Photopolymers for 3D-Printed Unibody Lab-on-a-Chip Optical Platforms. *Small* 14, 1–8 (2018).

[36] Loo, A. H., Chua, C. K. & Pumera, M. DNA biosensing with 3D printing technology. *Analyst* 142, 279–283 (2017).

[37] Mulberry, G., White, K. A., Vaidya, M., Sugaya, K. & Kim, B. N. 3D printing and milling a real-time PCR device for infectious disease diagnostics. *PLoS One* 12, 1–18 (2017).

for the 3D print with a 0.4 mm nozzle, and a 0.2 mm layer height. The circuit board for the electronic controls was fabricated with a 3D CNC milling device.

3D printed prototypes of a paper-based syringe test and a magnetic bead-based well test for malaria diagnosis using aptamer-tethered enzyme capture (APTEC) assay were developed.[38]

Smartphone-based Biosensors

Recent progress in smartphones has enabled them to become an integral part of analytical chemistry instrumentation. These smartphone-based devices exploit either optical or electrochemical sensing mechanism. Roda et al. developed a disposable mini cartridge coupled with a smartphone or tablet for lactate determination in oral fluid and sweat.[39] The device comprised three components, namely a disposable analytical cartridge, a mini dark box, and a smartphone adapter. It is made of black and transparent acrylonitrile-butadiene-styrene (ABS). The analytical cartridge, being 42 mm × 28 mm, comprises two reaction chambers with dimensions of 4 mm in diameter, and a depth of 5 mm for sample and control, along with small 4 mm-diameter discs of the nitrocellulose membrane for immobilisation of lactate oxidase and horseradish peroxidase. This analytical cartridge was enclosed in a mini dark box during the measurement which was further coupled to a smartphone adapter. The adapter was fitted onto the smartphone to properly position the dark box containing the embedded smartphone camera. The adapter contained a plano-convex plastic lens (with a diameter of 6 mm, and a focal length of 12 mm), to focus the image of the reaction chambers of the cartridge onto the smartphone camera.

The 3D-printed accessories comprised a cartridge of 8 cm length, 2.5 cm width and 1 cm thickness, and a smartphone adaptor with a plano-convex lens of 6mm diameter and a cartridge-insertion slot.[40] The cartridge housed a nitrocellulose strip on which a lateral flow immunoassay was performed

[38] Dirkzwager, R. M., Liang, S. & Tanner, J. A. Development of Aptamer-Based Point-of-Care Diagnostic Devices for Malaria Using Three-Dimensional Printing Rapid Prototyping. *ACS Sensors* 1, 420–426 (2016).
[39] Roda, A. *et al.* A 3D-printed device for a smartphone-based chemiluminescence biosensor for lactate in oral fluid and sweat. *Analyst* 139, 6494–6501 (2014).
[40] Zangheri, M. *et al.* A simple and compact smartphone accessory for quantitative chemiluminescence-based lateral flow immunoassay for salivary cortisol detection. *Biosens. Bioelectron.* 64, 63–68 (2015).

for the detection of salivary cortisol. The cartridge also comprised a 25 µl reservoir for polyclonal anti-peroxidase–cortisol conjugate solution, two separate 100 µl reservoirs for chemiluminescent substrate, and PBS and an inlet for sample injection. The cartridge was printed in two pieces to introduce the strip and reagents. A lid offered an optical window for signal acquisition and a grid for evaporation of the absorbent pad. The device is printed using thermoplastic black and yellow acrylonitrile butadiene styrene (ABS) polymers.

Similarly, smartphone-based biosensors for point-of-care detection of L-lactate in the oral fluid were developed.[41] The smartphone accessory device comprised a mini cartridge, a mini dark-box, and a cover-like adapter that was fabricated using 3D printer technology. A black acrylonitrile-butadiene-styrene (ABS) polymer was utilised for printing this device. A mini cartridge housed a paper functionalised with polyelectrolytes, all the reagents, and enzymes in the reaction chamber. The positioning of the reaction chamber in front of the camera at the correct focal distance was achieved with the help of the adapter and the mini dark box. A flash diffuser made of polydimethylsiloxane (PDMS) membrane and a plano-convex plastic lens was placed in the adapter.

Strain/Flex Sensor

Skin-mountable and wearable strain sensors that are highly stretchable, flexible, durable, biocompatible, and lightweight are drawing attention to real-time monitoring of human activities. Such sensors can potentially be applied to personalised health-monitoring, human motion detection, human-machine interfaces, and soft robotics.[42] These sensors can be fixed on the skin or clothing, causing lesser levels of discomfort. To fabricate strain sensors in a highly compliant and extensible elastomeric material, an embedded 3D printing technique was developed.[43] In this technique, a viscoelastic ink was directly extruded through a deposition nozzle into an elastomeric reservoir. Carbon-based resistive ink was used to make a

[41] Calabria, D. *et al.* Smartphone-based enzymatic biosensor for oral fluid L-lactate detection in one minute using confined multilayer paper reflectometry. *Biosens. Bioelectron.* 94, 124–130 (2017).
[42] Amjadi, M., Kyung, K.-U., Park, I. & Sitti, M. Stretchable, Skin-Mountable, and Wearable Strain Sensors and Their Potential Applications: A Review. *Adv. Funct. Mater.* 26, 1678–1698 (2016).
[43] Muth, J. T. *et al.* Embedded 3D printing of strain sensors within highly stretchable elastomers. *Adv. Mater.* 26, 6307–6312 (2014).

resistive sensing element, where the reservoir serves as a matrix material. The void space is filled by a capping layer during the translation of the nozzle through the reservoir. The reservoir and filler fluid were co-cured after printing to form a monolithic part, while the embedded conductive ink stayed fluid. Leigh et al. demonstrated a 3D printed flex sensor.[44] The authors developed an "exo-glove" comprising a main body of 3D printed PLA and strips of carbomorph embedded over each finger, which were able to detect resistance changes upon movement of the finger (Figure 1). Carbomorph was a composite made of a thermoplastic matrix of polycaprolactone and conductive Carbon Black filler.

[44] Leigh, S. J., Bradley, R. J., Purssell, C. P., Billson, D. R. & Hutchins, D. a. A Simple, Low-Cost Conductive Composite Material for 3D Printing of Electronic Sensors. *PLoS One* 7, e49365 (2012).

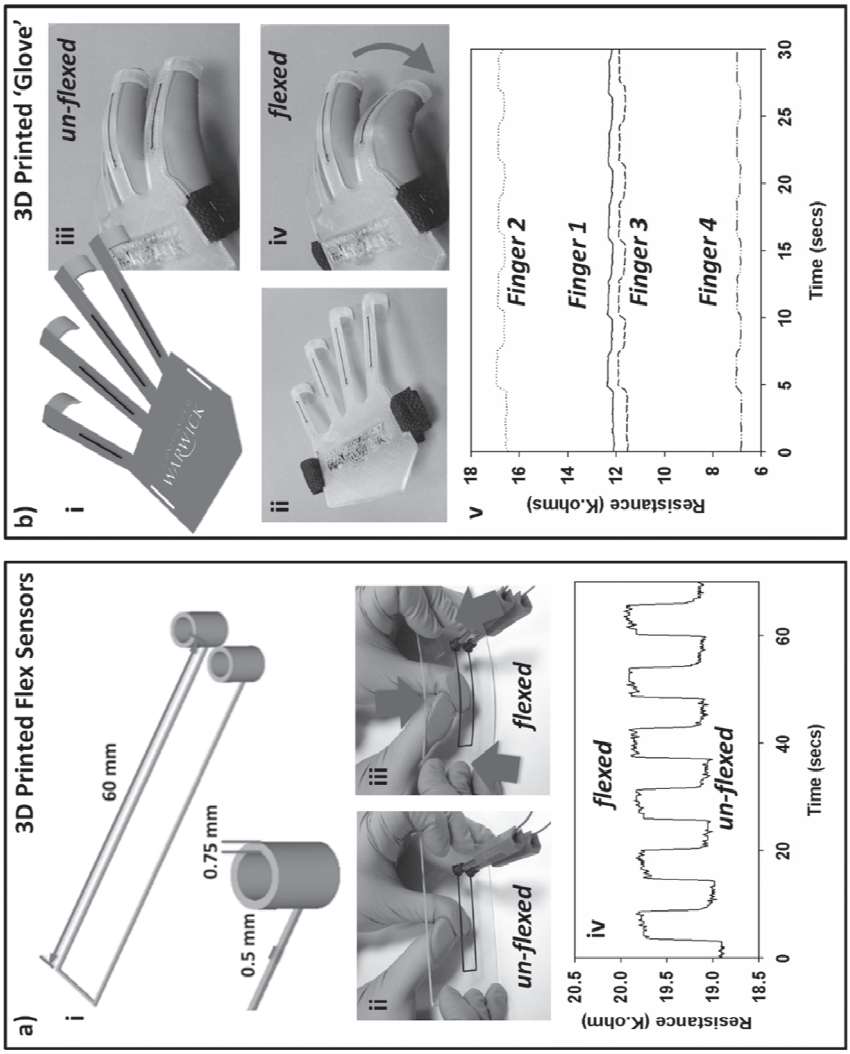

Figure 1: (a) 3D-printed flex sensor (ai) corresponding CAD design, (aii) printed sensor which is unflexed, (aiii) printed sensor in flexed position, (aiv) change in resistance of printed sensor during unflexed and flexed positions, b) 3D-printed glove, bi) corresponding CAD design, (bii & biii) the printed glove in unflexed position, (biv) the glove in a flexed position and (bv) change in resistance due to flexing of each finger. (Reprinted with permission from ref. 44. **Copyright:** © 2012 Leigh et al. Open-access).

Other Sensors

3D printed mass sensitive microcantilever (MC) based biosensors were developed and tested using a standard immunoassay protocol.[45] The array of MCs was printed using a mixture of bisphenol A ethoxylate diacrylate (BEDA), acrylic acid (AA), photoinitiator, and reactive orange 16 dye. The role of AA was to provide a controlled number of functional groups for immobilisation of the biomolecules covalently onto the polymer. The MC array structure was printed using a DLP printer where the layer thickness was chosen to be 25 μm, with an exposure time of 0.8 s per layer.

A new class of instrumented cardiac microphysiological devices was fabricated via multi-material 3D printing. Six functional inks made of soft materials exhibiting properties such as piezoresistivity, high-conductance, and biocompatibility were developed. They helped in integrating soft strain gauge sensors within micro-architectures which could help in the self-organisation of physio-mimetic laminar cardiac tissues.[46] Each device encompassed multilayer cantilevers made of a base layer, an embedded strain sensor, and a tissue-guiding layer; electrical interconnects for readout; and eight independent wells. The tissue-guiding layer helped in the self-assembly of engineered physio-mimetic laminar tissues from neonatal rat ventricular myocytes and human-induced pluripotent stem cell-derived cardiomyocytes. This device assisted tissue culture and non-invasive analyses of tissue contractile strength over several weeks. Drug studies could also be conducted inside a controlled incubator environment. Six materials are patterned consecutively by direct ink writing, multi-material 3D printing. In the first step, a sacrificial layer of 0.5 μm dextran thin-film was printed. In the second step, dilute thermoplastic polyurethane (TPU) was used to print a 3 μm thick cantilever base. A 6.5 μm thick strain sensor loop was printed using TPU filled with carbon black nanoparticles and a 1.5 μm TPU wire cover was printed in the next two steps. In the next step, 20 μm tall and 60 μm wide PDMS microfilaments were added in overlapping lines. Electrical leads and contacts were subsequently printed using a high-conductivity silver particle-filled, polyamide ink. Finally, an insulating layer to cover lead wires, wells to contain cells and media were printed using PDMS, polylactic acid (PLA), or acrylonitrile butadiene styrene (ABS).

---

[45] Stassi, S. *et al.* Polymeric 3D Printed Functional Microcantilevers for Biosensing Applications. *ACS Appl. Mater. Interfaces* 9, 19193–19201 (2017).
[46] Lind, J. U. *et al.* Instrumented cardiac microphysiological devices via multi-material three-dimensional printing. *Nat. Mater.* 16, 303–308 (2017).

The 3D printed thermo-responsive hydrogel was combined into a capacitor circuit to formulate a multifunctional and mechanically compliant skin-like sensor. N, N-dimethyl acrylamide (DMA) monomer and hydrophobic n-octadecyl acrylate (C-18), along with other reagents were mixed to prepare PDMA-C18 hydrogel for 3D printing.[47] The extrusion cartridge was filled with the hydrogels along with a temperature controller. The hydrogels were first melted at 45°C for about an hour. The homogenous, low-viscosity, and transparent hydrogels were then extruded through a flat tip needle (0.41 mm diameter), with an extrusion speed of 6 mm/s. The temperature of the platform was maintained at 10°C. Two layers of hydrogels were printed with a rotation angle of 90°, and the lateral centre-to-centre spacing of about 0.6 mm.

The 3D-printing method was exploited to obtain direct, template-free fabrication of stretchable capacitive sensors with interdigital and double-vortex designs that were used for tactile and electrochemical-sensing purposes.[48] Solidworks 2016 was utilised to design the electrodes. The length and width of the inter-digital electrodes based on carbon nanotubes (CNT)-PDMS were selected as 100 mm and 15 mm respectively. The length, width, and pitch were set to 100 mm, 30 mm, and 1 mm for a double-vortex design based on CNT-Ecoflex. These electrodes were printed on glass substrates using a home-built 3D printer. The nozzle's inner diameter and printing speed were set at 400 μm and 12 mm/s, respectively.

The printing layer height and total conductive pattern height were 400 μm and 1 mm, respectively. The printing ink comprised multi-walled carbon nanotubes (MWCNTs) in PDMS or MWCNTs in Ecoflex part A with other additives for printing. Double-vortex sensors based on CNT-Ecoflex showed a detection limit as low as 1μM for NaCl aqueous solution.

The optical particle characterisation device was printed using stereolithography.[49] A hydrodynamic focusing chamber was used to direct the particles for their counting and analysis by embedded optical fibres. The

[47] Lei, Z., Wang, Q. & Wu, P. A multifunctional skin-like sensor based on a 3D printed thermo-responsive hydrogel. *Mater. Horizons* 4, 694–700 (2017).
[48] Li, K. *et al.* 3D printed stretchable capacitive sensors for highly sensitive tactile and electrochemical sensing. *Nanotechnology* 29, 185501 (2018).
[49] Hampson, S. M., Rowe, W., Christie, S. D. R. & Platt, M. 3D printed microfluidic device with integrated optical sensing for particle analysis. *Sensors Actuators, B Chem.* 256, 1030–1037 (2018).

intensity of occluded light through the fibre was proportional to the size of the particles, which enabled the identification of particles of various sizes.

The 3D-printed Equilibrium-Dialysis Device was fabricated, and an experiment was carried out to measure the binding affinity of zinc metal ion for human serum albumin.[50] The base-plate was printed with a rigid material (VeroClear) having 12 wells lined by 0.6 mm of Tangoblack, whose rubber-like properties ensured a watertight seal in the well. Using a PolyJet printer, the print-pause-print approach was adopted for the integration of membranes directly into the windows of the membrane holder, 3.3 mm wide and 32.8 mm long, printed with Verowhite material for rigidity and a thin layer of Tangoblack, which was printed directly in the middle of the Verowhite.

A 3D-printing technology, specifically stereolithography, can be employed to fabricate high-quality optical components for label-free biosensing. Hinman et al. printed prisms by stereolithography which were deployed in a Kretschmann configuration for plasmonic sensing of bacterial cholera toxins.[51] Photoactive resin comprised of methacrylate oligomers, a methacrylate monomer, and photoinitiator(s) were used for printing these prisms.

Conclusion

The 3D printing method displays advantages over the traditional fabrication technique in terms of cost, time, and the material of printing. These techniques enable researchers to rapidly design and test the sensor's prototypes. 3D-printed sensors can be developed by direct printing of the sensing platform, or the sensor can be integrated into the printed platform. Customised moulds to fit sensors or accessories to incorporate commercial sensors can also be manufactured. Microfluidic sensing platforms can be developed for the diagnosis of diseases. By developing a sensor cartridge and accessories that can be coupled to smartphones, it will be possible to realise user-friendly diagnostic devices for innumerable POC applications.

[50] Pinger, C. W., Heller, A. A. & Spence, D. M. A Printed Equilibrium Dialysis Device with Integrated Membranes for Improved Binding Affinity Measurements. *Anal. Chem.* 89, 7302–7306 (2017).
[51] Hinman, S. S., McKeating, K. S. & Cheng, Q. Plasmonic Sensing with 3D Printed Optics. *Anal. Chem.* 89, 12626–12630 (2017).

In the future, many more sensing mechanisms can be incorporated using this 3D technique.

CHAPTER ELEVEN

CHALLENGES OF 3D BIOPRINTING

DR A.J. VANISREE

Abstract

The emerging dynamic field of 3D bioprinting, which is an additive manufacturing process, has great potential in the fields of education, engineering, medicine, and in the arts. Research over the last decade has led to the possibility of the bioprinting of biocompatible materials—combining cells with related components into a composite 3D printing of functional tissues and organs—and is regarded as a revolutionary, high-throughput art. This exciting technique does, however, face various potential challenges. This chapter will categorise the types of challenge and discuss the nature of such challenges.

In general, the challenges can be viewed as: i. skill; ii. financial; iii. legal; and iv. psychological impact. Currently, the printing process is seen to be very slow, and speeding up of the building architectures is, therefore, essential. For production to reach commercially acceptable levels, faster printing and scaling up the process is required. The bioprinter technology also needs to improve resolution, in addition to speed; it should also be compatible with a wider spectrum of biocompatible materials, so that the higher resolution will enable better interaction and control in the 3D microenvironment. Financially cost-effective customisation, increased environmental sustainability, reduced consumption of, and manufacturing efficiency of, materials are perceived as difficult tasks. Concerning the legal perspective, it is at an embryonic stage, and there are risks of litigation with regard to how 3D technology is being used. Much effort is needed to resolve ethical challenges in 3D bioprinting, as with many other facets of bioengineering, like equality and safety. Finally, 3D-printed organs may raise controversial concerns over moral and ethical issues about the origin

of the biomaterials and organs being used. The procedure can pose a threat by exacerbating society's already existing socio-psychological problems.

If the challenges that have been listed are seriously addressed, this exhilarating technology could potentially become an active and effective process to help contribute to the welfare of humankind.

A category of an additive manufacturing process called three-dimensional printing, crafts 3D entities by a digitally controlled deposition of consecutive layers, using bio-inks and bio cartridges. The latest developments in the design and fabrication of 3D constructs illustrate the potential of the technology in fulfilling the need for patient-specific devices. The United States Food and Drug Administration (FDA) has approved several such devices for clinical use that include patient-matched implants, surgical guides, and dental bridges, etc.[1,2]

However, the construction of living cellular materials has faced substantial impediments, especially the transition from synthetic to biologically-functional materials. In addition to this biological component, the technology faces other challenges. All of these issues being encountered can be categorised under four types. i. skills-based; ii. financial; iii. legal; and iv socio-psychological. Each category of potential hindrance, if analysed and appreciated individually, would result in successful management, followed by efficacious implementation of the upcoming technology.

Skills-based

As mentioned earlier, the evolutionary conversion of synthetic materials—e.g. ceramics, metals, and plastics—into biologically-functional materials is subjected to significant obstacles. In particular, the maintenance of control over micro- and macro-scale mechanical features, the achievement of physiological heterogeneity, the expansion of cells and vasculature are currently viewed as strong obstacles to the progress of the technology. Essential elements, such as lymphatics, innervation, number, and diversity

[1] Kruth, Jean-Pierre. "Material increase manufacturing by rapid prototyping techniques." *CIRP annals* 40, no. 2 (1991): 603–614.
[2] Heller, Timmy B., Ray M. Hill, and Abdalla F. Saggal. "Apparatus for forming a solid three-dimensional article from a liquid medium." U.S. Patent 5,071,337, issued December 10, 1991.

of supporting cells for the construction of large tissues and organs are also seen as being very complex challenges.

Furthermore, the time required for both assembly and maturation of a perfused vascular network all through the whole tissue constructs is, ironically, longer than the survival of the cell type being used. Let us consider the skill-based challenges under crucial parameters that govern the process and outcome.

Tissue bioprinting strategies

The various levels of skill demanded in 3D bioprinting technology need to be contemplated under different crucial factors, such as cell viability, surface resolution, and the materials used for 3D printing. The major technologies utilised in deposition and patterning of 3D constructs are inkjet, micro-extrusion, and laser-assisted printing.[3,4,5]

The scope of this chapter is not to describe the techniques being used, but to discuss the challenges that are being encountered, and the paper seeks to directly examine the limitations of the techniques.

The main drawback of inkjet printing is that it needs the required materials in liquid form for the initial droplet formation and deposition. This would then result in the formation of the solid 3D structure. Cross-linking of chemicals leads to changes in the pH, and UV exposure is demanded following the initial deposition in order to maintain the structural organisation and its functionality. However, the procedure (the cross-linking) was identified to affect chemical and material properties and was also found to be toxic to cells. Another challenge that lies in this mode of printing is the need to achieve biologically-relevant cell densities using

[3] Barron, J. A., P. Wu, H. D. Ladouceur, and B. R. Ringeisen. "Biological laser printing: a novel technique for creating heterogeneous 3-dimensional cell patterns." *Biomedical microdevices* 6, no. 2 (2004): 139–147.
[4] Guillemot, Fabien, A. Souquet, S. Catros, B. Guillotin, J. Lopez, M. Faucon, B. Pippenger et al. "High throughput laser printing of cells and biomaterials for tissue engineering." *Acta biomaterialia* 6, no. 7 (2010): 2494–2500.
[5] Guillotin, Bertrand, Agnès Souquet, Sylvain Catros, Martí Duocastella, Benjamin Pippenger, Séverine Bellance, Reine Bareille, et al. "Laser-assisted bioprinting of engineered tissue with high cell density and microscale organization." *Biomaterials* 31, no. 28 (2010): 7250–7256

inkjet printing, as the high concentration of cells might affect the hydrogel cross-linking.[6]

Micro-extrusion printing is an affordable, non-biological 3D-printing mode. Unfortunately, the percentage of cell viability following microextrusion bioprinting is lower when compared to inkjet bioprinting: this is due to the shear tensions imposed on cells in viscous fluids. The dispensing pressure exerts more effect on cell viability than the diameter of the nozzle.[7] Even if the viability issue could be addressed by adopting low pressures and using nozzles with a larger diameter, a great loss of resolution and print speed could be encountered.

Laser-assisted bioprinting (LAB) is another bioprinting method, and though less common than inkjet or micro-extrusion bioprinting, it is widely employed in tissue and organ-engineering applications. The limitations of this technique as compared to earlier techniques—i.e. cell densities, viability and even resolution—have been mostly addressed, thus making the laser-based technique potentially very useful in any clinical situation.

Nevertheless, LAB also poses certain challenges. The high resolution of the technique demands rapid gelation kinetics to attain high shape fidelity which will, in turn, affect the overall flow rate. Each type of cell and hydrogel individual ribbons needs to be prepared, which is a time-consuming process, and even more so for multiple cell types/materials. Further, accurate targeting and positioning of the cells are considered major tasks which need statistical cell printing.[8] As vaporisation of the metallic laser-absorbing layer is involved during printing, contamination by the presence of metallic residues is often encountered. The cost of the system is also a major concern.

---

[6] Skardal, Aleksander, Jianxing Zhang, and Glenn D. Prestwich. "Bioprinting vessel-like constructs using hyaluronan hydrogels crosslinked with tetrahedral polyethylene glycol tetracrylates." *Biomaterials* 31, no. 24 (2010): 6173–6181.

[7] Nair, Kalyani, Milind Gandhi, Saif Khalil, Karen Chang Yan, Michele Marcolongo, Kenneth Barbee, and Wei Sun. "Characterization of cell viability during bioprinting processes." *Biotechnology Journal: Healthcare Nutrition Technology* 4, no. 8 (2009): 1168–1177

[8] Guillotin, Bertrand, and Fabien Guillemot. "Cell patterning technologies for organotypic tissue fabrication." *Trends in biotechnology* 29, no. 4 (2011): 183–190.

3D bioprinting materials

The bioprinting of functional tissues and organs is an attempt to address the challenges faced in the process of achieving the desired biomechanical properties from the biomaterials.

Bio-inks

Hybrid constructs, made of both synthetic and natural materials, that can assure control over mechanical, geometrical, structural, and biochemical properties are available. For instance, extracellular matrix-derived hydrogels enable the build of well-defined cell-laden constructs by inducing gelation of bio-inks. These constructs, however, are weak in their mechanical properties. There is a need for extra cellular matrix (ECM)-mimicking materials that provide robust mechanical strength. Further, the time-scale for the degradation of the scaffold should match the scheduling of host remodelling of a construct, which should neither be delayed nor be inhibitory to native tissue formation, nor support the premature failure of the construct. The process has yet to explore factors governing the spatial hierarchies of materials and dynamics of tissue fabrication.

Cell sourcing and biocompatibility

The foremost challenge faced in the case of 3D-bioprinting materials is to attain an adequate population of cells that will not lead to the elicitation of an immune response by the transplanted cells. Biocompatibility in bioprinting means that the construct should provide a well-regulated contribution to the biological and functional aspects. In other words, the construct should be interactive and supportive in facilitating endogenous, molecular or signalling systems which are considered desirable for any biomaterial.[9]

It is well known that implanted biomaterials can elicit inflammation and inhibit wound-healing processes, and the fibrotic condition can efficiently demarcate the region between the host and biomaterials. As a consequence, patients can experience painful tissue distortion and censored nourishment which can lead to the failure of the construct. Generally, in 3D bioprinting,

[9] West, Jennifer L., and Jeffrey A. Hubbell. “Polymeric biomaterials with degradation sites for proteases involved in cell migration.” *Macromolecule*s 32, no. 1 (1999): 241–244.

terminally differentiated primary cells are harvested from the patients which tend to de-differentiate on expansion *in vitro*.

Degradation kinetics and by-products of the materials are key aspects that require further research. Scaffolds employed in 3D bioprinting undergo degradation *in vivo*, while secretion of proteases by the embedded cells and production of ECM will begin to outline the new tissue.[10] The challenge faced in this post-printing is the mismatched rate of degradation of appropriate biomaterials used for a particular tissue with the rate of replacing ability by the cellular components. The by-products of the degradation are also of great concern since they affect the biocompatibility of the degradable material, and should be quickly cleared from the system. If this is not done effectively, undesirable effects, such as inflammation, contraction, or swelling would occur that might deform the construct.

The multipotent stem-cell population is widely employed in the bio-fabrication of mesodermal-derived tissues. Still, their inclination towards senescence *in vitro* following a series of multiple expansion is being experienced,[11] which is also found to be affected by age.[12,13] It is crucial to keep in mind that the method of induced pluripotent stem cells (iPSCs), used for treating macular regeneration, was withheld in 2014[14,15] after the

[10] Williams, David F. "On the mechanisms of biocompatibility." *Biomaterials 2*9, no. 20 (2008): 2941–2953.
[11] Smith, Cynthia M., Alice L. Stone, Robert L. Parkhill, Robert L. Stewart, Mark W. Simpkins, Anatoly M. Kachurin, William L. Warren, and Stuart K. Williams. "Three-dimensional bio-assembly tool for generating viable tissue-engineered constructs." *Tissue engineering* 10, no. 9–10 (2004): 1566–1576.
[12] Jones, Nicola. "Science in three dimensions: the print revolution." *Nature News* 487, no. 7405 (2012): 22.
[13] Chang, Carlos C., Eugene D.Boland, Stuart K.Williams, and James B.Hoying. "Direct write bioprinting three-dimensional bio-hybrid systems for future regenerative therapies." *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 98, no. 1 (2011): 160–170.
[14] Fedorovich, Natalja E., Ives Swennen, Jordi Girones, Lorenzo Moroni, Clemens A Van Blitterswijk, Etienne Schacht, Jacqueline Alblas, and Wouter JA Dhert. "Evaluation of photo crosslinked lutrol hydrogel for tissue printing applications." *Biomacromolecule*s 10, no.7 (2009): 1689–1696.
[15] Visser, Jetze, Benjamin Peters, Thijs J. Burger, Jelle Boomstra, Wouter JA Dhert, Ferry PW Melchels, and Jos Malda. "Biofabrication of multi-material anatomically shaped tissue constructs." *Biofabrication 5*, no. 3 (2013): 035007.

identification of two genetic variants in a patient's iPSCs.[16]

Tissues under 3D bioprinting

The potential of the technology is evident in the construction of clinically relevant tissues, such as bone, cartilage, the skin of various geometries and sizes.

Bone:

Calcium phosphate ceramics (bibasic, hydroxyapatite, tri-calcium phosphate) are widely used to develop 3D bone-like structures.[17] Using a modified inkjet printer and composites of calcium-phosphate-collagen, bone scaffolds are fabricated. Hard scaffolds are considered appropriate for 3D bioprinting but the limitation is seen in terms of the failure of recapitulation of the cellular constituents. Though 3D bioprinting could create bone structures with interconnected porosity (a desirable feature), the construction of scaffolds demands certain conditions, such as appropriate solvents and temperatures that are highly detrimental to the cells. Non-uniform cell distribution and the lack of a proper attachment further complicate the limitation. The scaffolds also need to fulfil the need for anatomical accuracy in the case of bone injuries.[18]

The reader shall recollect the mention of limitation of vascularity in 3D bioprinted tissues. The diffusion is possible for 150µm, beyond which achieving vasculature becomes highly difficult. The good news about the bioprinting of cartilages is that these tissues are avascular and aneural in

---

[16] Censi, Roberta, Sander Van Putten, Tina Vermonden, Piera Di Martino, Cornelus F. Van Nostrum, Martin C. Harmsen, Ruud A. Bank, and Wim E. Hennink. "The tissue response to photopolymerized PEG-p(HPMAm-lactate) -based hydrogels." *Journal of Biomedical Materials Research* Part A 97, no. 3 (2011): 219–229.
Guvendiren, Murat, Hoang D. Lu, and Jason A. Burdick. "Shear-thinning hydrogels for biomedical applications." *Soft matter* 8, no. 2 (2012): 260–272.

[17] Smith, Cynthia M., Joseph J. Christian, William L. Warren, and Stuart K. Williams. "Characterizing environmental factors that impact the viability of tissue-engineered constructs fabricated by a direct-write bio-assembly tool." *Tissue engineering* 13, no. 2 (2007): 373–383.

[18] Bose, Susmita, Sahar Vahabzadeh, and Amit Bandyopadhyay. "Bone tissue engineering using 3D printing." *Materials Today* 16, no. 12 (2013): 496–504. Cartilage.

nature, with a comparatively low density of chondrocytes.[19] Hence, the scaling of cartilage is possible; yet it is a challenge to build functional cartilage owing to the zonal structure and the heterogeneity. Native cartilage is heterogeneous, with different cells and cell arrangements having different constituents of extracellular matrix and distribution.[20] This property confers tensile properties that enable the structure to resist the shear forces during articulation.[21,22]

Skin:

Fabrication and bioprinting of skin tissue are widely demonstrated by various groups.[23,24] Nevertheless, mimicking natural skin and its properties remains a challenge as it is highly elusive as far as the integrity of skin, sweat glands, and hair follicles are concerned,[25] but achieving a stratified, three-layered skin structure—namely epidermis, dermis, and hypodermis—along with the vascularisation and pigmentation of the constructs is not an easy task.[26]

---

[19] Buckwalter, J. A., and H. J. Mankin. "Articular cartilage: tissue design and chondrocyte-matrix interactions." *Instructional course lectures* 47 (1998): 477–486.
[20] Hunziker, E. B., T. M. Quinn, and H-J. Häuselmann. "Quantitative structural organization of normal adult human articular cartilage." *Osteoarthritis and Cartilage* 10, no. 7 (2002): 564–572.
[21] Coruh, Atilla, and Yalcin Yontar. "Application of split-thickness dermal grafts in deep partial–and full–thickness burns: a new source of auto–skin grafting." *Journal of Burn Care & Res*earch 33, no. 3 (2012): e95-e101.
[22] Leon-Villapalos, Jorge, Mohamed Eldardiri, and Peter Dziewulski. "The use of human deceased donor skin allograft in burn care." *Cell and tissue bank*ing 11, no. 1 (2010): 99–104.
[23] Koch, Lothar, Andrea Deiwick, Sabrina Schlie, Stefanie Michael, Martin Gruene, Vincent Coger, Daniela Zychlinski, et al. "Skin tissue generation by laser cell printing." *Biotechnology and bioengineering* 109, no. 7 (2012): 1855–1863.
[24] Michael, Stefanie, Heiko Sorg, Claas-Tido Peck, Lothar Koch, Andrea Deiwick, Boris Chichkov, Peter M. Vogt, and Kerstin Reimers. "Tissue-engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skinfold chamber in mice." *PloS one* 8, no. 3 (2013): e57741.
[25] Higgins, Claire A., James C. Chen, Jane E. Cerise, Colin AB Jahoda, and Angela M. Christiano. "From the Cover: Feature Article: Microenvironmental reprogramming by three-dimensional culture enables dermal papilla cells to induce de novo human hair-follicle growth." *Proceedings of the National Academy of Sciences of the United States of America* 110, no. 49 (2013): 19679.
[26] Hsu, Ya-Chieh, Lishi Li, and Elaine Fuchs. "Emerging interactions between skin stem cells and their niches." *Nature medicine* 20, no. 8 (2014): 847.

Neural tissue and brain:

Very limited work has been done on neural tissue bioprinting. A group has shown a successful proliferation and migration of murine neural stem cells bioprinted on collagen with a VEGF-loaded fibrin disk.[27] 3D-printed Schwann cells implanted in mice were shown to function by Owes et al., although it is not possible to draw a definite conclusion about the performance as the sample size was very small.[28]

The brain is, among other things, a three-dimensional complex tissue that is composed of many layers. Additional complexity remains in the neuronal network between those layers. A 3D brain-like structure was created using layered 3D bioprinting.[29] Following several days of culturing, confocal microscopy revealed penetration of axons between layers with distinct cell populations. Nevertheless, the ability to build a fully–functional, 3D-bioprinted brain construct has yet to be acquired.

Pancreas:

There have been very few pre-clinical trials for bioprinting the pancreas, as the pancreatic B cells cannot easily survive *in vitro*. Marchioli et al., 2015, used alginate scaffolds in mice. In this attempt, however, a challenge was encountered by the team: over a period of seven days, a complete loss of functionality of the construct was exhibited by the bioprinted cells.[30]

---

[27] Lee, Yeong-Bae, Samuel Polio, Wonhye Lee, Guohao Dai, Lata Menon, Rona S. Carroll, and Seung-Schik Yoo. "Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture." *Experimental Neurology* 223, no. 2 (2010): 645–652.

[28] Owens, Christopher M., Francoise Marga, Gabor Forgacs, and Cheryl M. Heesch. "Biofabrication and testing of a fully cellular nerve graft." *Biofabrication* 5, no. 4 (2013): 045007.

[29] Lozano, Rodrigo, Leo Stevens, Brianna C. Thompson, Kerry J. Gilmore, Robert Gorkin III, Elise M. Stewart, Marc in het Panhuis, Mario Romero-Ortega, and Gordon G. Wallace. "3D printing of layered brain-like structures using peptide modified gellan gum substrates." *Biomaterials 67 (2015): 264–273.*

[30] Marchioli, Giulia, Leon van Gurp, P. P. Van Krieken, Dimitrios Stamatialis, Marten Engelse, C. A. Van Blitterswijk, M. B. J. Karperien, et al. "Fabrication of three-dimensional bio-plotted hydrogel scaffolds for islets of Langerhans transplantation." *Biofabrication 7*, no. 2 (2015): 025009

Legal aspects and regulations:

As mentioned in the initial paragraph of this chapter, 3D-bioprinted materials are approved by the FDA in the USA for clinical use. It should be mentioned, however, that the obtained approvals were based on the same guidelines which are applicable to conventional medical products. It is thus necessary to revamp the existing guidelines which would facilitate the demands of, and rising use of, the newly-evolved 3D-bioprinting technologies.

The 21st Century Cures Act[31] describes the eligibility of the design of regenerative medicine modes, such as cell therapies, tissue engineering products, and tissue products that lead to the desirable modification of cells. However, viewing this as a serious concern, the FDA[32] released guidelines for 3D-printing manufacturers to assist the stakeholders of 3D-bioprinting products. This is crucial to maximising the regularised applicability of the technology.

Policy formations and funding future developments should be meticulously discussed and structured among scientists from different backgrounds. For instance, in the UK, 3D bioprinting involves collaboration among the Engineering and Physical Sciences Research Council (EPSRC), the Biotechnology and Biological Sciences Research Council (BBSRC), the National Centre for the Replacement Refinement and Reduction of Animals in Research (NC3Rs) and the Medical Research Council (MRC). This is of serious concern, as there are still debates that try to tackle the questions of collaboration, viable and appropriate regulatory protocols, and fabrication as they do not seem to be suitable for clinical trials.[33,34,35] 3D-printed

[31] Bonamici, S. H. R.34 – 21st Century Cures Act Senate Report 114–146 (United States Congress, 2016).
[32] Di Prima, Matthew, James Coburn, David Hwang, Jennifer Kelly, Akm Khairuzzaman, and Laura Ricles. "Additively manufactured medical products–the FDA perspective." *3D printing in medicine* 2, no. 1 (2016): 1–6.
[33] Wang, Xiaohong, Yongnian Yan, and Renji Zhang. "Recent trends and challenges in complex organ manufacturing." *Tissue Engineering* Part B: Reviews 16, no. 2 (2009): 189–197.
[34] Vermeulen, Niki. "From Virus to Vaccine: Projectification of Science in the VIRGO Consortium. Collaboration across Health Sciences and Care. Penders, BartVermeulen, Niki, Parker, John N., editors." (2015).
[35] Mittra, James, Joyce Tait, Michelle Mastroeni, M. L. Turner, J. C. Mountford, and Kevin Bruce. "Identifying viable regulatory and innovation pathways for regenerative medicine: a case study of cultured red blood cells." *New biotechnology* 32, no. 1 (2015): 180–190.

products do need to pass the "morality test", i.e. it should be accepted that it is morally acceptable to patent the process or the product. An ethical question arises if the process can be considered as a skill or a technology that can generate profits through the modes of therapy that are selected or adopted.[36] It is worth mentioning that the FDA, in the USA, remains uncertain about categorising the bioprinted products, which thus affects its decision about issuing approvals.[37]

Socio-Psychological:

Using 3D bioprinting technology, it could be possible to increase the patient's lifespan. At the same time, it is essential to apprise the beneficiaries of the reality of the attempts at rejuvenation, as 3D-printing technology is not the answer to immortality. Since ancient times, attempts have been made to find the elixir for life; the most famous Chinese alchemical book, written ca. 581–682 CE, Essential Formulas of Alchemical Classics, attributed to Sun Simiao, contains discourses on the creation of elixirs for immortality, and there is still a great deal of interest in the topic.[38] Considering the high cost of the technique, there might be a heavy line of demarcation between patients with different economic backgrounds, and this may have a psychological impact on them. The foremost ethical concerns associated with 3D bioprinting are about equality, safety and human enhancement.[39]

According to Gartner Inc., 3D-bioprinting technology will lead to production of customised anatomical parts that can have widespread appeal in markets and high demand for devices.[40] Society needs to be equipped to confront the future emergence of 3D bioprinting, and hence the foreseeable imbalances in the labour market and society as well. At present, the

---

[36] Li, Phoebe H. "3D bioprinting technologies: patents, innovation, and access." *Law, Innovation, and Technology* 6, no. 2 (2014): 282–304.
[37] Varkey, Mathew, and Anthony Atala. "Organ bioprinting: a closer look at ethics and policies." *Wake Forest* JL and Poly 5 (2015): 275.
[38] Glick, Thomas F., Steven Livesey, and Faith Wallis. Medieval science, technology, and medicine: an encyclopedia. Routledge, 2014.
[39] Pavlovich, Slaviana. "Should society encourage the development of 3D printing, particularly 3D bioprinting of tissues and organs." *Int. J. Sci. Technol.* Res 5 (2016): 41–46.
[40] Lucas Mearian. "Bio-printing Human Parts Will Spark Ethical, Regulatory Debate." Computerworld. 29 Jan. 2014. Web. 08 Apr. 2016. <http://www.computerworld.com/article/2486998/emerging-technology/bio-printing-human-parts-will-spark-ethical--regulatory-debate.

establishment of higher education and professional training systems for specialists in 3D printing is at an embryonic stage, hence the market could experience a dearth of employees with professional competence which, in turn, could limit the impact that this technology has in improving, and having a positive impact on, society.

Undergoing treatment using 3D printing by simply purchasing the materials of interest and incorporating them into the body may further diminish the line between the virtual world and reality in this computer era, the consequences of which are unpredictable. It has been predicted that about 10 per cent of the people in the developed world will be wearing 3D-printed objects in or on their bodies within three to four years.[41] There is also the possibility of a loss of age-old medical professionals as they will be transformed into organ-craftsmen.

It is further suggested that 3D bioprinting demands immediate attention in addressing various philosophical questions about personal identity, cultural and religious differences, and thus demands risk-benefit analysis.[42]

Cost-effectiveness:

The affordability of 3D bioprinting technology is an obvious barrier to its implementation. The substantial burden of cost is due to an imbalance between the cost of scaling and commercialisation. Further tissue design, manufacturing, cell sourcing, and fabrication are viewed as hurdles by industry. The need for highly personalised materials is still considered more crucial, and is a very serious concern. This is because, from an industrial point of view, only designing applications for widespread use is cost-effective. Thus, personalised 3D bioprinting demands upfront costs (e.g. for device training and set-up) in addition to the lack of manpower, which is also costly.

Nevertheless, despite the challenges discussed in this chapter, refinement, and advancement of smart technology could make this exhilarating technology effective in addressing the health issues of mankind.

---

[41] Alec. "Futurist Ray Kurzweil Predicts That 3D Printing Will Become Common in Daily Life in the 2020s." 3ders.org. N.p., 18 Dec. 2015. Web. 08 Apr. 2016. <http://www.3ders.org/articles/20151218-futuristray-kurzweil-predicts-that-3d-printing-will-becomecommon-in-daily-life-in-2020s.html>.

[42] Vijayavenkataraman, Sanjairaj, W. F. Lu, and J. Y. H. Fuh. "3D bioprinting–an ethical, legal, and social aspects (ELSA) framework." *Bioprinting* 1 (2016): 11–21.