# The Book of Teeth and Bones

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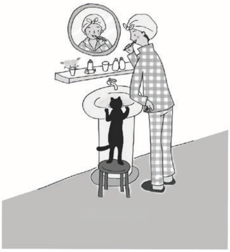


# INTRODUCTION

I have been engaged in education and research in medical materials (biomaterials) used for dental or medical treatments at a university. However, my family often say, “Daddy is poor at explaining things and is difficult to understand.” People talk about “nerd” or “geek”, but I seem to be fascinated by the charm of apatites and have shut myself in an ivory tower without realizing it. Formerly, I wrote a book titled “Chemistry of Apatites, Materials of Teeth and Bones”[1] in the hope of giving future dentists, doctors, and researchers knowledge about the material chemistry of “apatites” (primary components of teeth and bones: I will take time to explain them later). Recently, I have occasionally heard comments like, “I am using that book regularly,” which makes me happy in the back of my mind, but the book seems to be boring to regular people, and I have always wanted to do something about it.

Unfortunately, however, I got involved in education and research at college and, somewhere down the line, became preoccupied with words such as “principles of competition” and “self-assessment”. I became so wrapped up in daily life that I had no time to enjoy teaching or conducting research, let alone writing books catering to the interests of regular people. Fortunately, I retired and was blessed with the opportunity of visiting Garmisch-Partenkirchen, a German country town full of memories. While I was hiking through the mountains there, the plan for this book occurred in my mind.

After I visited the graves of a German couple, whom I owed a lot to in my student days, I thoroughly enjoyed hiking, which had long been my dream, for a full week (Fig. 1). I had previously been taken by the husband to the grave of his wife when she died, but since he drove me there, I had completely forgotten the location of the grave. After the husband also died, their relatives seem to have lived in their house for a period of time, but when I visited this time and asked their neighbors, no one knew about the couple. Fortunately, when I asked the city office, they kindly gave me the location, and I could finally visit the grave. The weather also happened to be beautiful, and, being gently wrapped in the bosom of calm nature, I could shed plaques that had deposited on my spirit over decades.

Here, I noticed that small children to aged couples enjoyed mountain hiking in their own styles, using cable cars and ropeways. Nothing is better for the mental and physical health of us humans.

Just at that time, the great earthquake struck the Tohoku District. My heart ached when I thought of the many lives that were lost and the people who were suffering, but the incident made me think all the more that it was time for me to write.

Concerning the unexpected damage caused by the accident at the nuclear power plant, the accumulation of isotopes in bone is closely related to the book I have just written. Since I studied technology, I do not insist that all nuclear power plants should be dismantled, but I think it is about time that we Japanese reconsider our lifestyles. In Germany, solar electric generators often catch my eye when I am on a train or walking in a town. Occasionally, nature suddenly bares its fangs and attacks humans, but it is intrinsically very gentle. Cherished in the bosom of nature, teeth and bones

Fig. 1 Landscape photo of Garmisch-Partenkirchen in Germany.

remain in mother earth for tens or hundreds of millions of years. This is the marvelous world of science.

Now, let's step into the world of apatites. I am sure it will boost your interest in dental materials, medical materials, and even dental practice and improve your understanding about the daily efforts of dentists, dental technicians, and hygienists, and about dentures and implants.

While attending, I added newly summarized PART II in the latter half for the people, who are interested in more detailed knowledge of apatites.

# PART I

# VALUABLE TALES OF TEETH AND BONES

# I-1. OH, I GOT IT! THE STORY OF DENTAL MATERIALS

## What I definitely want young people to know

I am sure you know that bones regenerate, but teeth do not, once they are damaged. This is exactly why we need dental materials. Of course, it would be best if we did not have to use them. Unfortunately, it is impossible to create artificial teeth exactly the same as natural teeth using today's dental materials and technology. So, I would advise you to pay attention to your teeth and routinely take good care of them if you do not want to regret the loss of your teeth in your old age. I hope this book helps you a little with protecting your teeth.

## Story of dentures

Are you not expecting too much from dentures? You must not think that dentures fit. If they fit, you are lucky. Our bodies change every day as we age. The environment in the mouth is also changing. Dentures become poorly-fitting even if they are originally made perfectly. If your glasses fit you poorly, you do not blame glasses shops or ophthalmologists, but you complain of the poor skills of the dentist or technician if your dentures fail to fit you. This is unfair. Of course, there seem to be differences in dexterity. There are dentists and technicians who can foresee gingival changes from their long experience and cleverly adjust dentures (Fig. 2). Good cooperation between dentists and technicians will make an even better job possible.

As vision deteriorates with age, the oral environment also changes and declines. If your dentures happen to be less than you expected, it may be necessary to accept them and learn to be content.

Fig. 2 Denture (By courtesy of Mr. T. Seo).

There is the term "pocket dentures (dentures that are deserted in pockets)" among dentists. This mocks dentures that have been made one after another but have been scarcely used and ended in pockets or drawers. If your first dentures do not fit you, you think that it is because of the poor skill of the dentist or technician and go to another dental clinic. But your new dentures also become unfit soon, and you go to another dentist, and while you keep going from one dentist to another, you end up with scores of unused dentures forgotten in your pockets or drawers. Such a comical story is actually happening. Since you can get dentures relatively inexpensively under health insurance, this occurs repeatedly.

But hold on a little! Are you not expecting too much from dentures? Unfortunately, current dentures do not transform with changes in the body. In a sense, it is the fate of artificial materials. The oral environment is changing slightly every day. Particularly, when you catch a cold or are not well, the gums change quickly. They also change slowly with aging. When you get old, they begin to change more markedly. If the alveolar bone stops receiving force, it is resorbed rapidly, accompanied by progressive retreat of the gingiva. Bone resorption progresses further if you stop eating hard food or lose teeth due to caries or pyorrhea. Hence, your dentures, on which you have spent time and money, soon become unfit.

The same thing happens even to expensive alloy dentures (metal dentures in technical terms). Metal dentures are considered more durable and have less effect on the taste of food than plastic dentures, but there is little difference in fitting. In addition, the part that corresponds to the gums is made of a polymer called polymethyl methacrylate (PMMA) in both plastic and metal dentures.

Then, what should we do? After all, I think it is best to keep new dentures made at appropriate timings. You can have the part of your denture that rubs against the gum planed, but its effectiveness is limited. It may instead change the balance of the denture and cause sores at other places. Although the use of a denture stabilizing material or tissue conditioner is an option, it is reported that overuse of a stabilizer is not recommended, because it increases the thickness of the part that rubs against the gums and induces bone resorption or tissue change under the area of its application. In the past, Japanese paper was once used as a tissue conditioner, but it is no longer in use, because bacteria are likely to attach to it and make dentures unclean. However, there are times when the body gets somewhat used to even dentures that do not fit well as they are used over a long time. Unlike inflexible artificial objects, the body seems to be somewhat friendly to foreign objects.

Formerly, attempts to make dentures using soft plastics have been made. Unfortunately, however, they were deformed rapidly and were not usable. Dentures are not easy to make. In Japan, there used to be special craftworkers called “denture masters”. They correspond to dentists and dental technicians in modern dentistry combined. They were mostly house craftworkers serving upper class people. A typical example of their craftsmanship is the wooden dentures (*mokusho gishi*) of Ieyasu Tokugawa, which were made of boxwood (Fig. 3)[1]. The artificial teeth were made of ivory or stone. The shape of the oral mucosa is reproduced so excellently that I wonder how they took impressions in the mouth. This wooden denture is considered to have superb adhesiveness to the oral mucosa.

I recommend that you take time to consult with your dentist, let him/her understand well about the condition of your mouth, and have dentures that fit you well made by patiently maintaining communication. It may be a good idea to review changes in your physical condition and oral environment before you complain about the skills of your dentists and technicians.

Fig. 3 Wooden denture (By courtesy of Dr. I Nakahara).

## Pitfalls of the 80-20 campaign

If you feel this way, it is best to keep your natural teeth as much as possible. “Having 20 natural teeth by the age of 80”. This is the motto of the 80-20 campaign. Recently, the idea of “minimal intervention” when treating dental caries as early as possible and preserving the teeth with minimum treatment without extracting them has become prevalent. Therefore, I recommend that you periodically visit your dentist, have dental scales and plaque removed, and have dental caries found in the earliest stage. Particularly, pyorrhea progresses without your knowledge. Those around 40 are already at risk.

Also, dentures are better stabilized in your mouth if you have more natural teeth remaining. Therefore, it is important to keep as many natural teeth as possible. However, the remaining teeth are also subject to unnatural forces in attaching and removing dentures, or the soft cement layer at the base of the lateral surface of the teeth is frictioned and wears off by the hooks called clasps, which keep dentures in position, and they are likely to develop caries and may eventually get extracted.

Also, if you develop dementia and become bed-ridden, this motto ceases to apply. Ironically, if you retain a few teeth in such a condition, they may interfere with cleaning of the oral cavity. If the teeth cannot be brushed, or the oral cavity cannot be cleaned well, oral bacteria proliferate, and, if they are aspirated into the lungs, they may cause pneumonia (Fig. 4).

## Story of implants

Recently, it has become easy to get dental implants (artificial dental roots) (Fig. 5). Since they look cosmetically favorable and do not have to be removed like dentures, everything seems wonderful. But, be careful!

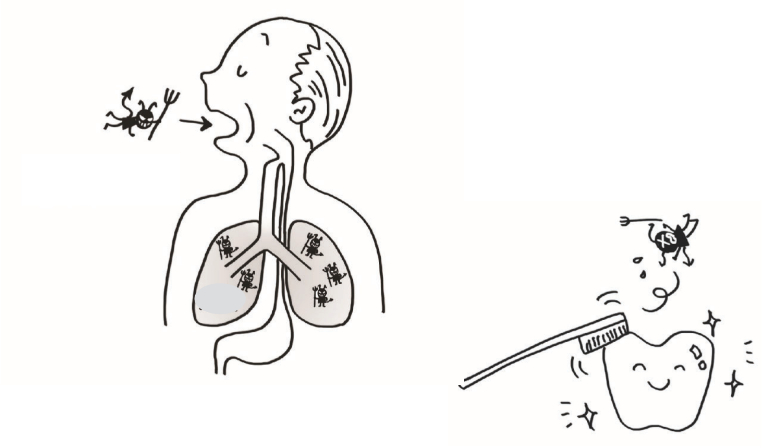
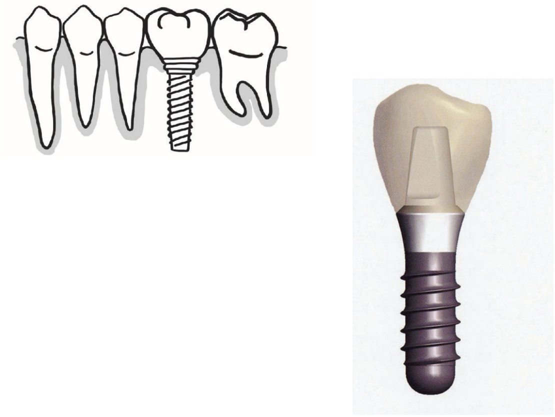


Fig. 4 Pneumonia caused by oral bacteria.



ITI® Dental Implant System

Fig. 5 Ti Implant (Photo: by courtesy of Taishin Boeki Co. Ltd.).

Not everybody is happy. If implants are set in the wrong direction, the bone that supports them is stressed and may be destroyed. Particularly, people with a small jaw are at risk. The roots of natural teeth curve smoothly along the jaw bone, but implants simply stand straight. So, in the worst case, the bone is penetrated all the way in making holes for implants. If implants are not placed exactly in the direction of occlusion, you may not be able to bite well, or the force of biting continues to work in the wrong direction, eventually making implants wobbly. In addition, if you get demented or bed-ridden, it becomes difficult to brush your teeth by yourself, and as your mouth gets unclean, bacteria are likely to proliferate. Therefore, implants are recommended to healthy people with well-developed jaws but not so much to those with a small and graceful mouth.

In fact, implants have a long history and date back to 700 B.C. In these days, implants seem to have been popular in Etruscan, Italy, and many relics of teeth bridged with gold remain. Probably, the gold bridges supported teeth that were lost due to pyorrhea, but the question of whether they actually worked or not is open (Fig. 6)[1, 2].

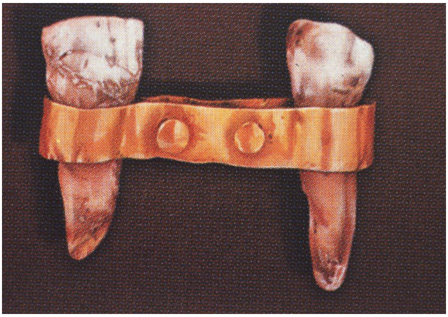


Fig. 6 Ancient Etruscan implant (By courtesy of The Gold Information Center, NY).

## Story of composite resin

In the past, dental filling materials were limited to cement and amalgam. Many of my teeth are also filled with amalgam. Amalgam is tough, because it is a metal (alloy of Hg and Ag), but as it contains Hg, it has recently been avoided as much as possible. Actually, Hg contained in amalgam is inorganic Hg, which is not as dangerous as organic Hg, and since it is alloyed, little Hg remains. So, we do not have to worry so much.

Composite resin has replaced amalgam from the viewpoint of safety and security. Composite resin is hard inorganic particles called silica cemented with plastic. It is particularly popular among young people, because its color can be adjusted to the natural color of teeth. However, since the particles are cemented with plastic, composite resin is bound to deteriorate. Fortunately, it has served well for more than 10 years in my mouth, but it may become fractured or detached in a few years. There are no such worries with metal filling materials.

Recently, ceramics are attracting attention because of both the color and strength. However, to my regret, the use of ceramics hikes the cost of treatment as it is not covered by health insurance.

## Story of tooth blackening

Taira no Atsumori, the kindachi (high-ranking noble) killed by Kumagai Naozane in the Battle of Ichinotani, a famous episode in the Tales of the Heike (1184), is considered to have applied tooth blackening. Tooth blackening appears to have been a habit restricted to high-ranking noble men in the Heian period. In the Edo period, it became popular among women, and a tooth blackening set (Fig. 7)[2] was part of the marital package.

This custom is considered to have been restricted to married women. Tooth blackening uses a non-aqueous solution prepared by mixing a powder called fushiko, which contains a large amount of tannic acid, with a brown liquid prepared by adding iron to vinegar (acetic acid). It smelled so awful that they made sure to rinse their mouth after applying it. However, from the viewpoint of dentistry, tooth blackening, which is like paint over the enamel of the teeth, is considered to have been effective for the prevention of dental caries because of the coating effect and anti-lytic effect of iron. In addition, tannic acid with its protein astringent and antibacterial actions seems to have contributed to the prevention of pyorrhea. This custom faded away by the beginning of the Showa period.

Fig. 7 Ohaguro (Tooth black)

(Picture: by courtesy of Dental History Library, Nihon University).

## Unexpectedly little-known constitution of teeth

If all teeth have erupted, there are 32 teeth in the mouth, but this is rare today. Most people have 28-30 teeth. First, 20 milk teeth grow, and they are replaced by permanent teeth by the age of about 20 years. That's all, and no third teeth come up. However, wisdom teeth may stay below the gum without erupting or morbidly come out sideways. Although rare, some teeth may remain unerupted. So, if you worry about the growth of your teeth, you should have X-rays taken and consult your dentist.

You may have healthy teeth extracted to correct the alignment of your teeth or lose teeth due to pyorrhea even without caries. These teeth used to be used simply as materials for clinical training of dental students, but studies to preserve them in a "tooth bank" until their use for regenerative therapy becomes a reality are in progress.

Teeth are supported by the gums, but they are not as rigidly fixed as implants. They are bound tightly by connective tissue called the periodontal membrane, which absorbs the impact of biting.

## White teeth?

When we see pictures of entertainers smiling with their white teeth exposed, we cannot resist their charm. But, be careful! Healthy teeth are not necessarily white teeth. I want to remind you that healthy teeth are those that allow you to bite and speak well. Of course, white teeth look clean, and teeth stained by tobacco tar are disenchanting. Recently, whiteners containing agents, such as hydrogen peroxide, have become easily available, but problems are concealed in the whitening technique. Occasionally, problems happen, such as that the teeth become white but are soon stained again, that metal crowns and fillings in the neighborhood are discolored, and that the tooth surfaces are roughened. Before you try whitening, you had better check with your dentist carefully.

## Why do lost teeth not regenerate?

Human teeth are called “lifetime teeth”, and once milk teeth are replaced by permanent teeth, we keep the same teeth to the end of our lives. Therefore, if we develop caries, teeth do not regenerate as bones do.

Fish, on the other hand, are called polyphyodonts, which means having many sets of teeth that are generated and replaced in succession. If humans were polyphyodonts like fish, we would not be troubled by caries, but we have only two sets of teeth, because the primordia of teeth called dental papillae disappear after they make teeth once. Recently, however, it has been reported that the gene that controls dental papillae are asleep and that having a third set of teeth may not be a dream if we succeed in waking up this gene. This story sounds great if it comes true.

If you observe the teeth of killifish, you will see that they have many teeth and that more teeth are waiting for their turn under them (Fig. 8)[3].

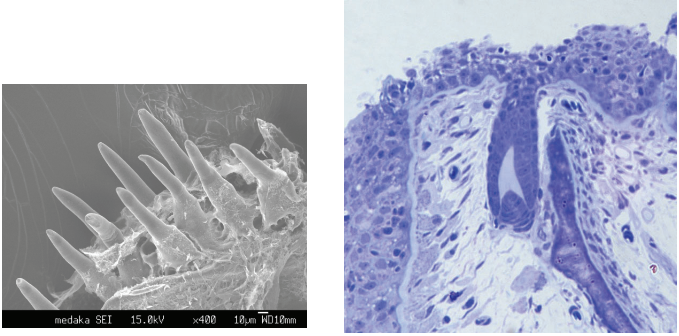


Fig. 8 Teeth of Killifish.

## Bones regenerate of themselves

Bones, on the other hand, are metabolized all the time, and new bone is generated while old bone is resorbed (Fig. 9). Osteoclasts cover the part of bone to be destroyed and lyse apatites by secreting acid (hydrogen ion). In contrast, osteoblasts diligently make bones using the calcium (Ca) and phosphorus (P) released from lysed bone. This balance is maintained superbly. Because of this ability of bone regeneration, fractures are repaired naturally by the body. Therefore, no particular artificial material is needed to repair fractures and small bone defects.

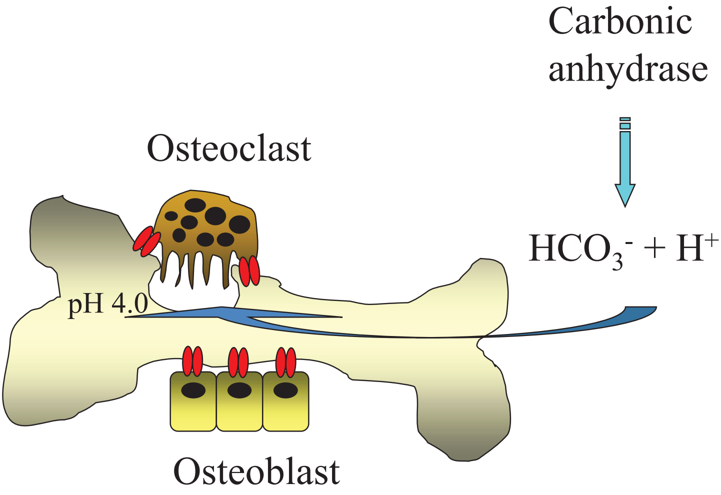


Fig. 9 Schematic model of bone metabolism.

However, the help of artificial materials is necessary to repair large areas or areas with low regenerative ability. The ability of bone regeneration declines with aging, and osteoblasts become less active as we get older. Tissue engineering and regenerative medicine aim to revitalize bones using young and fresh cells. I will discuss this later.

## This is how dental caries are made

It is rather difficult to recall the details of everyday activities in our early childhood, but I remember making great efforts to quickly pull out milk teeth that became loose by deliberately wiggling them or by tying a loose tooth to my toe with a thread to let it get pulled off while I was unconscious during sleep. In these days, I hated to go to see a dentist and would try to bear the pain even with a large hole of caries. I am still plagued by the chilling rasping sound of the electric engine (which has been replaced by the much quieter turbine) in my dreams.

Of course, I never knew that teeth are made of apatites, which are calcium phosphates, and that they are lysed by acid produced by bacteria that cause caries. My idea was that caries bacteria holding spears live in the mouth and that they dig holes in the teeth when I eat sweets. Still less did I even dream that enamel is a structure consisting of layers of robust hexagonal apatites. Today, however, knowledge about the prevention of caries has spread by improved dental education, and caries are becoming less prevalent year by year. This is wonderful. One thing that we must be careful about is that the children of parents indifferent about dental hygiene often have severe caries. After all, our world is full of sweets. I will talk about apatites in detail later in the technical sections.

Since primitive times, mankind appears to have been troubled by caries. Some of the teeth of our ancestors unearthed from their remains show large holes caused by caries or have worn so thin due to severe clenching that the pulp is exposed. Since they had no dentists in those days, I feel sorry for them, guessing that they suffered so much. However, I suppose common people began to have relatively easy access to dental consultation after the civilization and enlightenment of the Meiji period. Today, patients are lucky, because dentists are considered to exist in excess. Until the end of the Edo period, dental care was out of reach for average people.

Now, 3 factors are considered to be involved in dental caries, namely, tooth apatite, bacteria, and glucose (Fig. 10). The phenomenon sounds a little complicated, but, eventually, sugar decomposed by caries bacteria produces $H^+$ (hydrogen ions), and these $H^+$ ions lyse the host, i.e., apatites of teeth. In simple terms, caries are formed by the lysing of ceramics by acid. Therefore, in experiments, teeth immersed in carbonated water for a long period are lysed by acid and are eroded. We do not have to worry about carbonated drinks, because we immediately swallow them, but if a baby falls asleep holding a feeding bottle containing a lactic acid bacilli beverage in the mouth, the milk teeth are soon affected by caries. While the pH of water is 7.0, that of carbonated soft drinks is often about 2-4, even lower than the pH of the habitat of caries bacteria, which is 5-5.5.

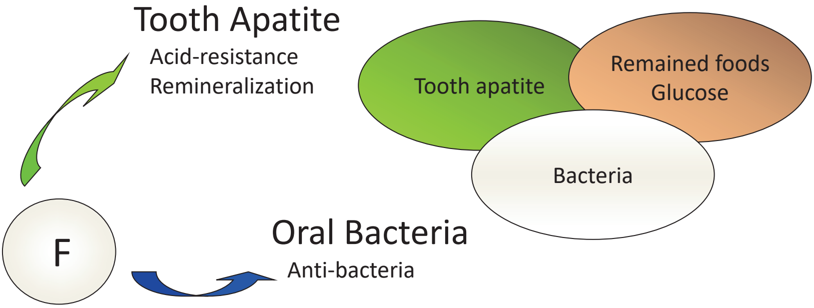


Fig. 10 Essential factors of dental caries.

Some of the Ca and P released as apatite is lysed may be deposited and crystalized again. This is called remineralization (recrystallization). When this process is studied in detail, crystals formed by redeposited Ca and P do not develop into crystals of the original enamel. Therefore, strictly speaking, once a tooth is affected by caries, it is never restored to the original condition. Furthermore, if a large hole has been made, repair using dental materials, such as composite resin, metal, and ceramics, is necessary.

In repairing teeth using such dental materials, how they should be attached to the tooth substance is a problem, because the oral cavity is a harsh environment that is constantly exposed to hot and cold foods and beverages. Fortunately, firm adhesives and cements have been developed one after another due to recent advances in adhesive dentistry. However, many of these materials are primarily made of macromolecules, and they are bound to deteriorate over a long period. In addition, their deterioration is accelerated in the severe environment of the oral cavity by the stress of temperature changes due to food and the force of occlusion. On the other hand, the durability of metals and ceramics themselves is sufficient, but as they are not adhesive, the above organic adhesives and cements are necessary for their use, and caries often develop again due to deterioration of these parts. Formerly, inorganic zinc oxide cement was widely used, but as it often caused recurrence of caries, it is mostly out of use today.

Fluorine (F) is known to be a silver bullet for the prevention and control of caries[4]. As I explain in the following sections on apatites, F has been shown to stabilize apatite crystals and to have an antibacterial activity. It is safe, because it is effective at a very low concentration. While water fluoridation is not practiced in Japan today, the WHO sets the safety criterion for fluoridation of tap water at 1 ppm ($10^{-6}$ g/g, g/mL in water solution). In direct application of fluorine to teeth, it is applied at 1,000 ppm or more, and while it is still a very low concentration, we must ask a dentist to do it. At a higher concentration, F is a dangerous drug that can lyse even glass and must be handled with caution. There are regions of the world in which natural water contains F at relatively high concentrations (10-50 ppm), and, in such regions, people may develop a disorder of tooth development called mottled tooth. In Japan, fluoridation of tap water was attempted in some areas, but as a high concentration of F was supplied to a water tank by mistake, causing mottled tooth, water fluoridation was given up on thereafter. However, please do not misunderstand. There is no reason to worry about mottled tooth, because it is a phenomenon that occurs in the period of tooth formation and is not observed in mature teeth. Attention is necessary in babies and children during the tooth replacement period. Also, in areas where natural water with a high F content is used, a disease called osteosclerosis may occur. Although its causal relationship with F has not been fully elucidated, the disease makes bones less flexible and more fragile.

## Risk of pyorrhea

The gums (periodontal tissue) have an amazingly delicate structure. The dental root and jaw bone are bound by connective tissue called the periodontal ligament (periodontal membrane) (Fig. 11), and teeth become loose if the periodontal ligament is damaged. Inflammation of the gums is an index of tooth instability. If the gums are red like an apple and swollen, we can imagine that the periodontal ligament inside is considerably damaged. Since inflammation is caused mostly by bacteria, it spreads rapidly without being restricted to a single tooth. That is a difference from caries, which is limited to the areas with holes. So, if you get pyorrhea, a considerable number of teeth may become loose at one time. People who had confidence in their teeth are shocked the most. Those who have boasted that they have no worries about caries or pyorrhea because they smoke and have their teeth coated and disinfected with nicotine may well suddenly suffer pyorrhea around the age of 40 years.

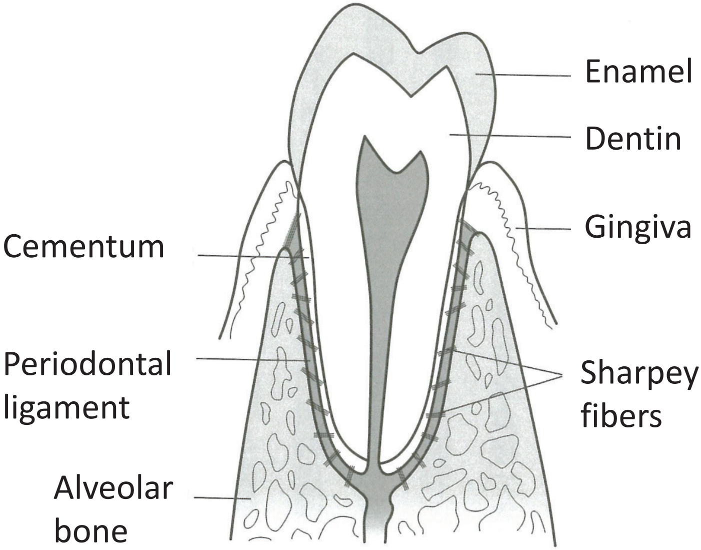


Fig. 11 Periodontal ligament of tooth.

In the periodontal ligament, many bundles of thin but very strong threads woven like ropes called Sharpey fibers are arranged perpendicularly to the periodontal ligament and tie the dental root and jaw bone sideways. Both ends of the bundles penetrate deep into the dental root and jaw bone and are firmly rooted there so they cannot be pulled out easily. It is like a suspension bridge supported by numerous ropes. This is why teeth are fixed firmly but can also absorb the impact of occlusion in a very sophisticated way. Current implants (artificial dental roots) do not yet have such an advanced function.

Sharpey fibers develop simultaneously with teeth and jaw bones. Therefore, if they are cut, they are not easy to repair. Studies to regenerate the periodontal ligament are being conducted, but teeth cannot be firmly supported unless cement at the dental root is regenerated. In addition, dental materials that can repair the periodontal ligament or Sharpey fibers have not been developed. This is why I recommend that you take good care of your remaining healthy teeth.

## Importance of tooth brushing

For these reasons, tooth brushing is very important for the prevention and control of pyorrhea as well as for the prevention of caries. If dental plaque or scale remains around teeth, it is likely to invite bacterial growth. Although we cannot drive bacteria completely out of the mouth, it is important not to give them a cozy dwelling place.

For this, I recommend that you brush your teeth as soon after meals as possible and, furthermore, after each meal. Food residues are the optimal source of nutrition for bacteria. They eat a lot, are vitalized, and become active. In addition, they release acid by decomposing sugars and cause caries by eroding apatites of the teeth. The waste produced by bacteria also causes inflammation in the gums. If food residues cannot be cleaned sufficiently by brushing alone, interdental brushes and dental floss are recommended. If you are busy, you do not have to spend a lot of time on this. Just take a few minutes to remove food particles. Using a small amount of toothpaste may be a good idea. You do not have to use a lot. It is the same thing as using some detergent to wash the dishes. As detergents are effective for removing tough stains, toothpaste is effective for brushing teeth.

Some people say, “I seldom brush my teeth, but my teeth are OK.” Some people are born with inherently strong teeth. However, many such people suffer pyorrhea when they get old. I warn you!

## Story of the toothbrush and toothpaste

There is an old saying, “A samurai uses a toothpick even when he is starving,” which depicts a poor and starving samurai pretending to be full and compliments the attitude of the samurai class valuing and showing off the honorable poverty and dignity of keeping up appearances. As this saying indicates, the toothpick was used as a simple tool for cleaning the interdental gaps. Today, toothpicks are only used to clean dental crowns, but tassel toothpicks were often used as an equivalent of the toothbrush. Tassel toothpicks are made by crushing one end of a willow or bamboo stick into a tassel. They seem to have been popular in the Edo period and to have been used to clean and massage the tongue as well as to clean the teeth. In addition, they were also used to apply tooth blackening. Pig and horse hair used to be used as bristles of the tooth brush, but nylon fiber is the mainstay today. Recently, electric toothbrushes are widely available.

As mentioned above, toothpaste is not always necessary, but it is effective for improving the time efficiency of tooth brushing and facilitating tooth cleaning for the same reason as the use of detergents. Toothpaste contains ingredients aimed at preventing caries, periodontal disease, and deposition of scales. They include moistening agents, such as glycerin and sorbital, cleaning agents, such as inorganic silicic acid and calcium carbonate, foaming agents, such as sodium lauryl sulfate, flavoring agents, such as menthol and mint, and pharmacologically active ingredients such as sodium fluoride (caries prevention) and isopromethyphenol (periodontal disease prevention). In the past, people brushed their teeth by applying salt to the toothbrush or tassel toothpick. This practice seems to have served a dual purpose of cleaning the teeth and killing bacteria.

## What is osteoporosis?

When we talk about bones, we imagine a fracture, a plaster cast, and walking on crutches. If we tolerate these inconveniences, the broken bone is fused and heals in a month or so. When we think about it, it is an amazing process, but we take it for granted and do not give it much thought. However, from the viewpoint of material science, this is a surprising phenomenon—the body repairs itself by releasing an adhesive agent with exactly the same composition as the damaged tissue, which no artificial adhesive can match. This is what bone regeneration is all about, but it may be difficult to imagine osteoblasts diligently and perseveringly making bone.

Fingers, for example, are flexible, with the joints separated shortly after birth. So, we have to be careful in handling babies. Their bodies grow slowly thereafter and are completed when they are around 15 years old. The growth stops at the age of about 20 years, and the body continues to degrade thereafter. Bone resorption is accelerated from the age of about 40 years, if early, and bones become porous in time. This is the condition called osteoporosis. Bone resorption also occurs in the oral cavity. Particularly, the jaw bones seem to be resorbed rapidly after the teeth are lost, and less force begins to be applied to the jaws. This is one reason that your dentures soon become poorly fitting despite the money and effort you have spent on their preparation.

Bone is metabolized daily, and the balance of bone formation and bone resorption is usually maintained. However, bone resorption is accelerated after the 40s, particularly, in women. Bone resorption also progresses in men after the 50s. The interior of bone (cancellous bone), in particular, gradually becomes porous and fragile. Minerals (apatites) and an organic substance (collagen) that constitute bone are also resorbed, and bone becomes less flexible, more breakable, and hollow. This is the beginning of osteoporosis. Since this process progresses over a long period, its signs are unnoticed for some time. When we get older, we become more likely to stumble, and, one day, we may find ourselves unable to stand up due to a broken bone. This is how you begin to need biomaterials such as an artificial hip. Such patients are increasing rapidly with aging of the population. One way to prevent fracture is the measurement of bone density in regular health check-ups. Textbooks of physiology show the balance of bone metabolism. We take in Ca and P every day, excrete them if they are present in excess, and supply them to the body from bones if they are deficient. Microscopically, this process is explained by the functions of osteoblasts and osteoclasts. This is why textbooks of nutrition and newspaper ads of dietary supplements recommend an intake of Ca at 600 mg/day and P at 300 mg/day.

## Bone is a storehouse of minerals

Thus, bone is a storehouse of minerals (inorganic substances necessary for the body). Other than Ca and P, which are primary components of bones and teeth, bone contains many important microelements. For example, Mg is very important as a catalyst which makes chemical reactions rapidly progress in the body. Fe is involved in the transport of oxygen as an element indispensable to sustain our life that constitutes the central nucleus of hemoglobin in red blood cells. Zn and Si are also essential trace elements.

On the other hand, there are elements that should not be taken into the body such as Hg, Ni, and Pb. In addition, dangerous elements, such as radioactive $^{137}$Cs, $^{131}$I, and $^{90}$Sr, which were released in recent accidents in nuclear power plants, are inevitably absorbed from the air and through food and accumulate in bone.

Ca may be deficient even in people with no osteoporosis if they have renal failure, a condition in which the kidney does not function normally. Bone is known to be a storehouse of important minerals, such as Ca, but the kidneys are constantly adjusting their balance in the body. The kidney is a horsebean-shaped organ the size of a fist. There are about 2 million microscopic units called nephrons within the two kidneys combined, and they remove waste in the body (metabolic end products) by filtering them with about 180 L of water every day. Nephrons resorb 99% of water and important minerals and nutrients, and excrete only unnecessary waste as about 1.8 L of urine per day. The orders of these activities are transmitted by the para-thyroid hormone (PTH), and the mineral balance is maintained by these delicate functions. However, minerals leak in renal insufficiency and become deficient. This, in turn, tips the balance of bone and causes Ca deficiency. Presently, artificial kidneys are supporting the lives of kidney insufficiency patients. Kidney transplantation is an alternative treatment, but we still rely on artificial kidneys because of a shortage of donors.

Incidentally, in the 1960s, only a few artificial kidneys were available in the world, so a body called the “black committee” is rumored to have been organized in the United States to evaluate which patients should be given priority for treatment. There was discussion over who should be treated first, young or old, women or men, rich or poor, but the committee is said to have been dismissed, being unable to reach any conclusion about the preference of human lives. It is an episode that is related to the essence of medical care. Thereafter, the number of artificial kidney machines increased drastically, and as the treatment began to be covered by health insurance in Japan, many patients began to benefit from the technology. However, current artificial kidney machines simply remove waste and are not equipped with a sophisticated resorptive function. I wish that the creation of nephrons from cells, such as iPS (induced pluripotent stem) cells, would soon become a reality and replace the current artificial kidney machines. I am anxious to see which is realized sooner, tooth regeneration or kidney regeneration.

## Story of astronauts

We often hear on the news that astronauts who spend a long time in a space station can no longer walk by themselves, or their bones are markedly thinned, when they return to Earth. It seems that bone metabolism cannot be maintained normally without mechanical stress. Electric stimuli also seem to affect bone formation. Bone formation is reportedly facilitated in negatively charged areas and inhibited in positively charged areas. Also, in polarized bones, bone formation is reported to be facilitated on the north pole side, probably because osteoblasts are stimulated and activated for more vigorous bone formation. At any rate, bone may be weakened unless it receives some stimulation.

## "Mixed crystals" made of two or more substances

Human bones and teeth are made of hydroxyapatite, which is calcium phosphate, but, strangely, seashells are made of calcium carbonate. Large amounts of carbonic acid are dissolved in seawater and the freshwater of lakes and rivers, so it is easy to understand that calcification is likely to occur if Ca is present. However, why did mammals choose calcium phosphate as the primary component of their skeletons? According to one theory, mammals that advanced to land from the ocean established a system using P as the main component and chose bone as its storehouse, and this idea is widely accepted. To be sure, P is a primary component of ATP, which is the main source of energy and an element indispensable for life.

Here is an interesting story. Mixed crystals of hydroxyapatite and calcium carbonate are occasionally found near the seashore (Fig. 12). I also used to wonder so much about why calcium carbonate and hydroxyapatite are crystalized as a mixture. I continued to frantically search literature for the solution of the enigma of why human bones are made up of hydroxyapatite. Surprisingly, however, I happened to find the answer in a dictionary of minerals. As birds leave droppings on rocks of calcium carbonate covered with seashells and coral on the seashore, Ca, released as the bird droppings dissolve the calcium carbonate, and P in the droppings are recrystallized as hydroxyapatite. Certainly, hydroxyapatite is less soluble than calcium carbonate, so this hypothesis is persuasive, but the enigma remains unsolved. I will touch on this point some more in the later sections on “apatites”.

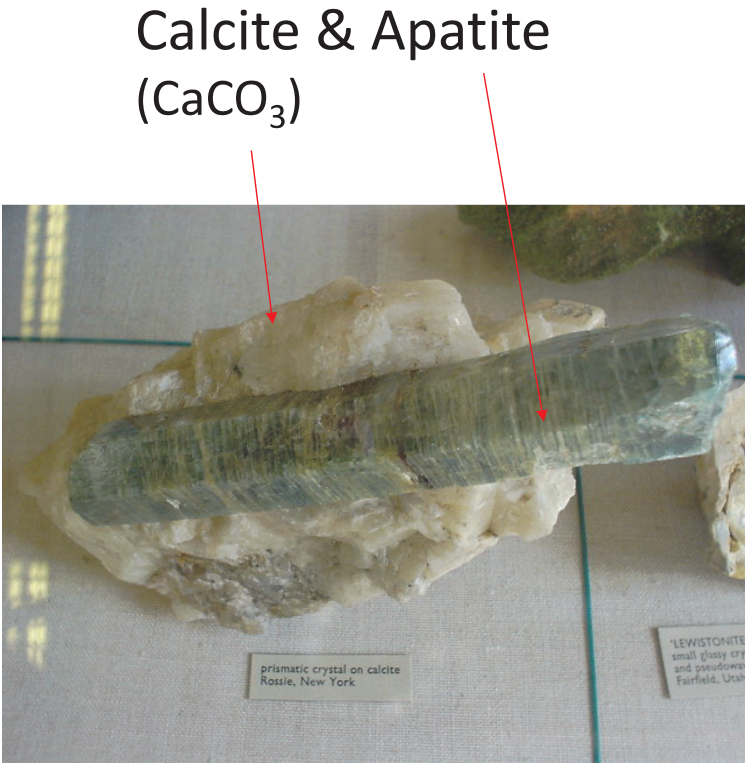


Fig. 12 Mixed crystal of Calcite and apatite.

(By courtesy of Natural History Museum, London)

## Story of orthodontics

Recently, we often see young people receiving orthodontic treatment (Fig. 13). If you patiently undergo this treatment, which usually takes 2-3 years, you will have an astonishingly beautiful teeth alignment. In the early days, there seems to have been many failures because of inadequate application of force, but, now, you can have orthodontic treatment with a sense of security. Formerly, the attachment of the brackets (metal pieces that support orthodontic wires), which were worn over a long period, was weak, and they occasionally became detached or displaced, but the attachment force has become more than sufficient due to the improvement of the adhesive agent. Ironically, the attachment has become so strong that even enamel may be damaged in removing the brackets after the end of treatment, although it is rare. It would be best if there were brackets that could be attached and detached anytime as freely as electric magnets, but it does not seem easy to achieve. Since brackets are usually made of metal, they look unattractive. Although transparent plastic brackets have also been used, they are not strong enough. Orthodontic treatment is mostly applied to those in their teens and 20s, but it can be performed also in those in their 50s.

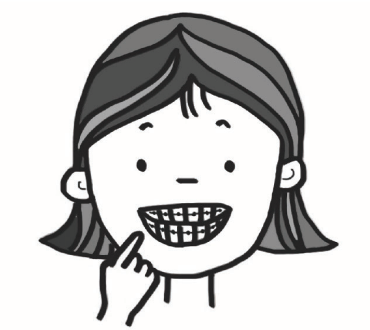


Fig. 13 Orthodontics.

## Metal allergy

Allergy seems to be a very complex biological response, and its causes are often difficult to identify. Particularly, the relationship between dental metal and generalized allergy is even more difficult to clarify. However, I will present some of the many cases that have been reported to the present (Fig. 14).

Generally, allergies are likely to occur in soft parts of the body and often cause itchy reddening and swelling in areas such as the oral mucosa, face, and hands. It is impossible to calmly watch the ongoing process of antibodies attacking the antigens, and once you happen to scratch the itchy areas, before you know it, the itch and reddening begin to grow worse and worse.

Fig. 14 Metal allergy (By courtesy of Dr. N Kou).

I have heard about cases in which the cause of allergies was dental metal filled in or crowned on teeth and that the symptoms were resolved completely when all dental metal was replaced with ceramics. Metals, such as Ni and Cr, are dissolved into the mouth, although in minute amounts, and as they circulate in the whole body, they cause allergies, but this mechanism has not been made entirely clear. Some researchers consider that even precious metals, such as gold (Au) and platinum (Pt), can cause allergies. Recently, patch tests, i.e., applying stickers containing various metals to the skin and examining the reactions to evaluate the sensitivity to them, have begun to be performed easily. Since metal allergies vary widely among individuals, I recommend for you to consult with specialists.

## Regeneration of teeth and bones

Regeneration evokes the image that something that has been lost, like the tail of a lizard, and grows again. In humans, perhaps because they have excessively evolved, the regenerative capacity seems to be low. Still, broken bones are fused, and the skin is constantly metabolized and renewed. However, this does not apply to the teeth and heart. So, stem cells with a very high regenerative potential, such as ES cells (embryonic stem cells) and iPS cells (induced pluripotent stem cells), are recently attracting attention.

Since ES cells are derived from fertilized eggs, there is the ethical problem of violating the sanctity of life. In addition, the use of cells from other persons is associated with the possibility of rejection at transplantation. In contrast, iPS cells were created from mouse fibroblasts first in the world in 2006 by the group of Professor Yamanaka of Kyoto University. Since they can be prepared by introducing genes into the patient’s own somatic cells, such as skin cells, they can be cultured readily at any time and can be transplanted with less possibility of rejection. However, a defect of iPS cells is that they are difficult to use for the treatment of people with inherited diseases. In addition, since viruses that can induce cancer are used in culturing iPS cells, concern over the safety remains. Recently, methods not using viruses are being studied. For this achievement, Professor Yamanaka was awarded the Nobel Prize in Medicine.

To the present, teeth have been considered not to regenerate, but tooth regeneration is no longer a dream due to the advent of ES cells and iPS cells. It may become possible in the near future to regenerate teeth by culturing the iPS cells of ameloblasts and odontoblasts in a test tube and grafting the resultant tooth primordium to the site of the lost tooth.

Interesting reports have also been made by gene studies. After teeth are completed, the genes that have controlled the development of the teeth are not considered to disappear; they simply fall asleep. Therefore, some researchers suggest that teeth may be regenerated by waking up sleeping genes. It is a very exciting prospect.

## Story of age determination and forensic dentistry

Unlike soft tissues, such as the skin and heart, hard tissues, such as teeth and bones, remain for tens of millions of years after our death. This is because they are made of an inorganic substance called hydroxyapatite, which remains in the earth without decaying similar to stones. For this reason, we can guess the times and lifestyles of early humans, such as the Pithecanthropus and Neanderthal, from their remains.

Presently, the age can be determined precisely using isotopes. $^{14}$C (carbon with an atomic weight of 14), an isotope heavier than usual carbon $^{12}$C (carbon with an atomic weight of 12), is used. While a human is alive, $^{14}$C enters and leaves the body, and the ratio between $^{14}$C and $^{12}$C is maintained at a constant level. However, as metabolism stops after death, no more $^{14}C$ enters the body, and the $^{14}C$ remaining in the body is progressively disintegrated and lost. The time needed for the original amount to be reduced to half is called the half-life. Since the half-life of $^{14}C$ is about 13 years, the time of death of a person can be estimated from the half-life by measuring the remaining $^{14}C$. After the introduction of this isotopic method for age determination, fake fine arts and crafts are reported to have been largely swept from the market. Similarly, we can also determine the age of teeth and jaw bones.

Even more interesting is forensic dentistry. Dental examination is often made to identify those who have died a solitary death, those murdered, and those killed in natural disasters. Since charts of dental treatments are filed at the dental clinics that have treated the deceased, we can immediately check which teeth were affected by caries and what dental materials were used to treat them. Therefore, unidentified bodies are likely to be identified by comparing the conditions of their teeth with the charts. Of course, the alignment of teeth and the shape of the jaw also help with the identification. Unfortunately, however, forensic dentistry is helpless if the person has been so healthy that he/she has never visited a dentist.

# I-2. EXPERT INFORMATION ABOUT TEETH AND BONES THAT HELPS A LITTLE

## What are apatites? Mysterious world of apatites

There was a period when "Celebrities bet their lives on their teeth" was a popular phrase. If a smiling face showing off shining white teeth is projected on the TV screen, we are tempted to sigh with wonder. My profession being what it is, the view of perfectly aligned dentition makes me think, "I am sure he/she can speak fluently and masticate well!" However, a second look often reveals that they are not natural teeth but are artificial teeth with metal cores covered by ceramics called metal bonds.

Yes, they are. If you have a broken bone, it may be fixed temporarily using a splint and metal bolts, but they are mostly removed after the fracture heals. Of course, artificial bone is used semi-permanently if there is a large defect such as in the hip bone. In contrast, after a tooth decays or is lost, it must be filled with dental materials (because it does not regenerate).

Also, astronauts have difficulty in standing on their own feet on their return from a long stay in space. Or, even if you are not an astronaut, you will have trouble after you have stayed in a hospital for several weeks. Walking just a few meters can be difficult, because not only your heart and muscles but probably also bones are weakened. When you are healthy, you pay no attention, but if you get sick and weak, even walking becomes a toil. What, then, are teeth and bones made of? We used to be told by our teachers and parents to eat a lot of fish bones to take in Ca and make our teeth and bones strong, but I did not know what it really meant. It was after I began to work at the dental department 45 years ago that I first leaned the term “apatites”. In those days, few even experts in dentistry or orthopedics knew well about “apatites”. I will tell you that the essence of teeth and bones is apatites.

Apaties consist of calcium phosphate[5-8]. There are similar hard parts of living things, such as seashells, coral, and eggshells, but they are a little different; they are made of calcium carbonate. Teeth and bones are made of calcium phosphate rather than calcium carbonate, presumably because when living things, which were created in the ocean, first climbed onto the land, they chose phosphate (P) instead of carbonate as the material of teeth and bones, and, what a surprise, this phosphate was destined to play very great roles in sustaining life. P is a vital component of sources of energy for life such as ATP.

Now, I will talk a little about the chemistry of apatites. The human body consists of about 60% water, 36% organic substances, and 4% minerals. Most of the minerals exist as apatites, which are chemicals that constitute hard tissues such as bones and teeth[4]. The term “apatites” comes from the Greek term meaning “delude”. Its chemical formula is generally $A_{10}(BO_4)_6C_2$ (O: oxygen), and various elements occupy the positions of A, B, and C. Most of the elements in the periodic table are said to be able to replace them, indicating the difficulty of characterizing apatites, hence this name. There are a large number of apatites on the Earth, and the apatite that constitutes teeth and bones is hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ with an $OH^-$ ion replacing C. Hydroxyapatite is a form of calcium phosphate and has many siblings (Table 1), which are all crystals consisting of ions. Crystals are solids made up of atoms (ions) arranged with the same pattern periodically repeated in a three-dimensional space. In contrast, amorphous solids, such as glass, are called non-crystalline materials.

**Table 1 Calcium phosphate family.**

| | Chemical name | Chemical formula |
|---|---|---|
| HAp | Hydroxyapatite | $Ca_{10}(PO_4)_6(OH)_2$ |
| TCP | Tricalcium phosphate | $Ca_3(PO_4)_2$ |
| DCPD | Dicalcium phosphate dihydrate | $CaHPO_4 \cdot 2H_2O$ |
| OCP | Octacalcium phosphate | $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ |
| 4CP | Tetracalcium phosphate | $Ca_4O(PO_4)_2$ |

The crystalline structure of hydroxyapatite was determined in 1964 (Fig. 15)[9-11]. It was clarified superbly with considerable effort by the simultaneous use of X-ray diffraction and neutron diffraction analyses. Analysis was very difficult, because the position of $OH^-$ was only 0.3Å (=0.03 nm = $0.03\times10^{-9}$ m) away from its position in stable crystal. Incidentally, $F^-$, which is known to be the specific agent for the prevention of dental caries (Fig. 16), is located exactly in the stable position[12], resulting in stability and resistance to the dissolution of the crystal. The crystal of hydroxyapatite is hexagonal, but as each third of it is identical, it is sufficient to show only the rhombic third of the crystal. Also, by this way, it is more convenient for its comparison with other crystals such as cubical crystals. $Ca^{2+}$ ions are present at two different positions. One is called columnar Ca, which has a chimney-like structure and is mobile.

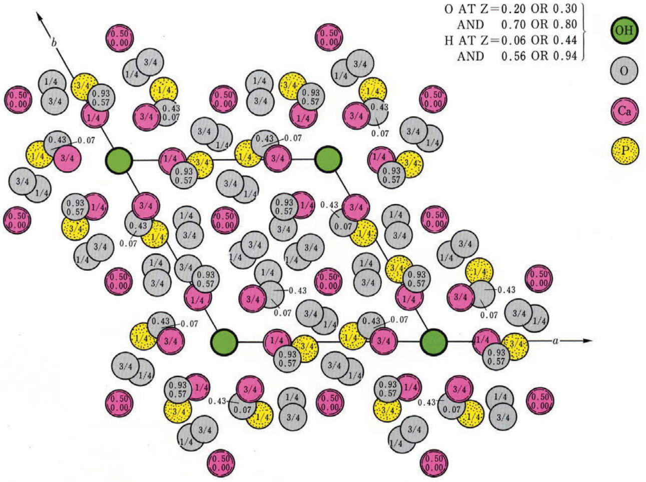


Fig. 15 Crystal structure of hydroxyapatite.

This Ca is considered to be lost easily when the tooth is affected by caries. The other is called screw axis Ca, which is located at each corner of a triangle (Fig. 17)[1, 10]. This diagram shows only part of the ions, and, more precisely, Ca, P, and OH ions are packed tightly as in the computer graphic image shown in the right. The computer graphic image faithfully represents the crystal based on the ion radius of each atom viewed from directly above along the c-axis.

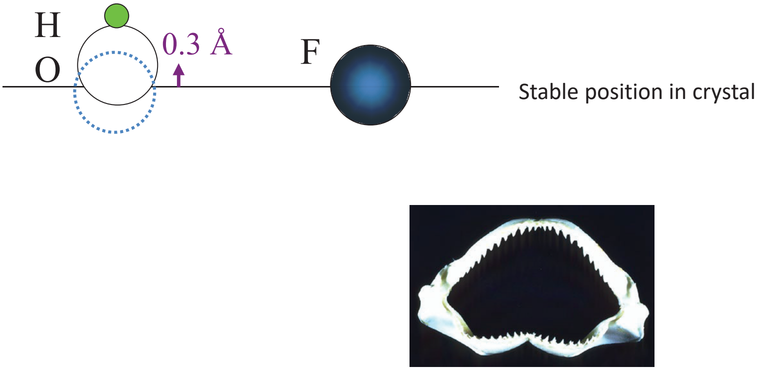


Fig. 16 F substitution into hydroxyapatite and shark teeth with fluoridated hydroxyapatites.

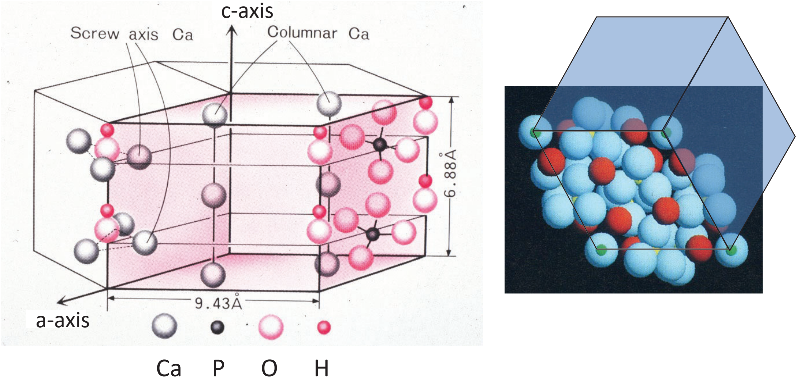


Fig. 17 Crystal model and computer graphics of hydroxyapatite.

The lattice parameters of hydroxyapatite are a=b=9.43 Å, c=6.88 Å; and the theoretical density is $\rho=3.15$ g/cm$^3$. Real biological apatites are more complicated, and Ca, P, and OH are replaced with various trace elements (Fig. 18)[13]. Therefore, in teeth and bones, a few percent of $PO_4^{3-}$ is substituted with $CO_3^{2-}$, in particular. Such apatites are called carbonate apatites[5]. Minute quantities of $CO_3^{2-}$ are considered to be taken into the body in the formation of apatites due to chemical equilibration, because some amount of $CO_2$ gas is inevitably dissolved in body fluid as $CO_3^{2-}$ in the process of inspiration of oxygen and expiration of carbon dioxide.

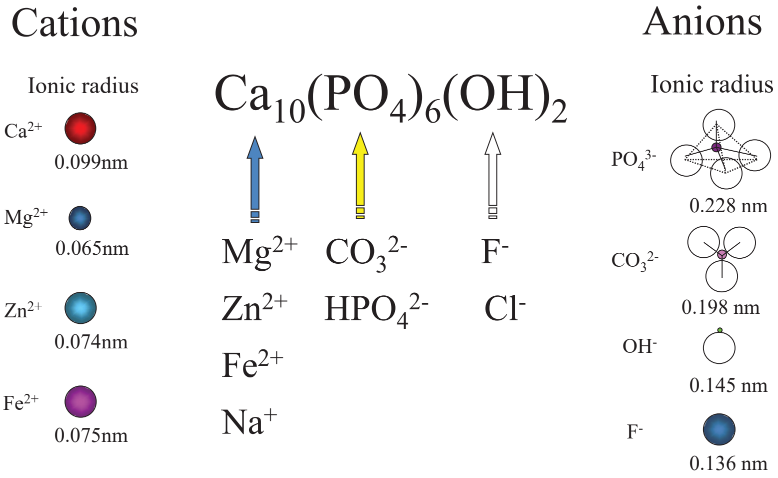


Fig. 18 Substitution of trace elements into hydroxyapatite.

By examination using a scanning electron microscope (SEM) and transmission electron microscope (TEM), the structure of the enamel of teeth (Fig.19)[1] and even the arrangement of atoms in the ultrastructure of its crystals (Fig. 20) [1] are visualized. This photograph allows the estimation of the crystal lattice constant of apatites.

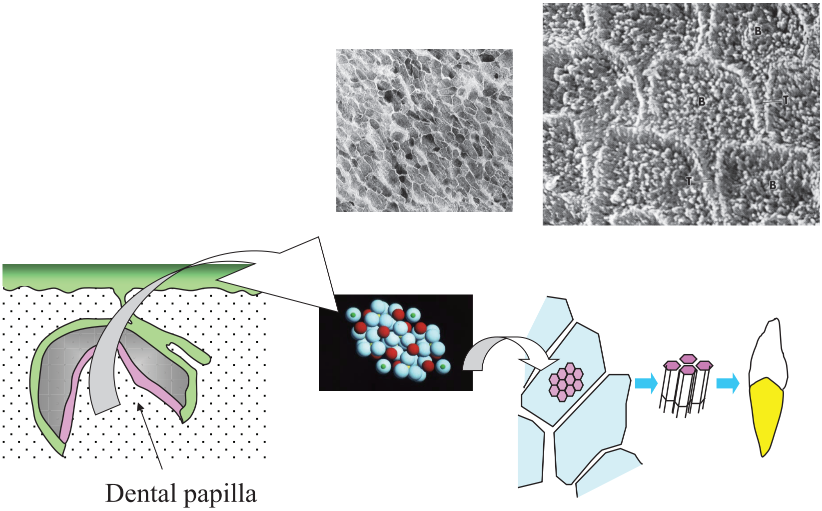


Fig. 19 Formation of tooth (Photos: by courtesy of Prof. T Ichijo).

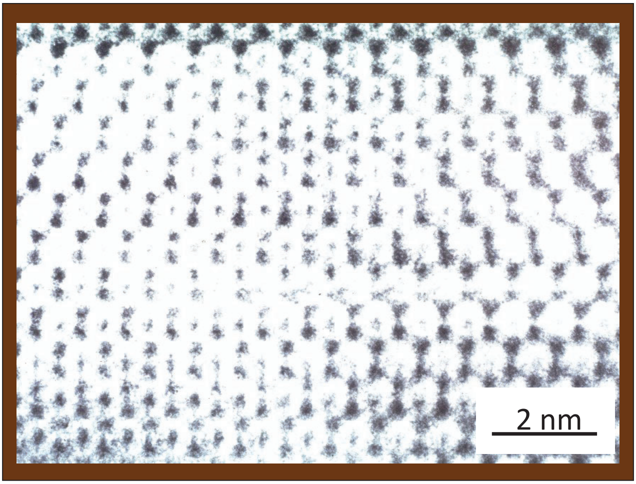


Fig. 20 TEM photo of hydroxyapatite at atomic level (Photo: by courtesy of Prof. T Ichijo).

## Tooth structure

Unlike bones, after completion of the permanent dentition, healthy teeth are not restored once they are affected by caries or periodontal disease, and teeth do not grow again once they are lost. Since milk teeth are replaced by permanent teeth, humans are called diphyodonts, except that molars come out only once. In contrast, fish, in which teeth are renewed continually, are called polyphyodonts. In humans, a total of 20 milk teeth grow from the age of 6 months to early childhood and serially drop out from the age of about 6 years and are replaced by permanent teeth. Eventually, with the addition of 8-12 molars, a set of 28-32 teeth is completed. The tooth can be divided from the appearance into the crown and root. The crown is covered by enamel and mostly exposed in the oral cavity. The root is wrapped in cement and is buried in the jaw bone. The number of roots is 1 in the incisors and canines, but the premolars and molars are single-rooted, double-rooted, or multirooted.

More than 95% of enamel is made of ionic crystals of high crystallinity hydroxyapatite and does not contain proteins such as collagen. The remaining only 5% consists of non-collagenous protein and water[4]. Crystals of hydroxyapatite are arranged regularly (orientation) and extend to the surface. Enamel is very hard and can withstand the occlusal force during mastication and protect the dentin and pulp inside like a shell.

On the other hand, 60-70% of the dentin protected by the enamel is composed of crystals of low crystallinity hydroxyapatite somewhat resembling those of bone. The remaining 30-40% consists of organic collagen. In the dentin, many thin tubes a few microns in diameter, called dental tubules, extend from the interface with the enamel vertically toward the interior and eventually reach the pulp cavity.

The pulp is soft tissue filling the pulp cavity. It consists of cells, fibers, matrix, blood vessels, and nerves, is composed of about 25% organic substances and about 75% water, and is surrounded by dentin except in the apical part. The pulp supplies nutrition to the dentin, controls the sensation of the tooth, and has functions of protection and repair against stimuli.

The surface layer of the root dentin is covered by thin cement and is made of low crystallinity hydroxyapatite closely resembling that of bone but, having no blood vessels, it has little regenerative potential unlike bone. In cement, a strong connective tissue called Sharpey’s fibers, which bind the tooth with the alveolar bone, runs through the periodontal membrane to the interior and firmly fix the tooth.

## Bone structure

Bone as a hard tissue is renewed daily by constantly repeating remodeling. From the viewpoint of biodynamics, bone tissue plays the important role of supporting our bodies under gravity. In addition, it also plays the role of a storehouse of various minerals indispensable for sustaining life.

Similar to teeth, bone consists of hydroxyapatite, which is a type of inorganic calcium phosphate, and organic collagen. Histologically, bone is classified into compact bone and cancellous bone (Fig. 21). Since bone is made of living cells, it has a rich supply of blood vessels. Compact bone consists of columns of bone aggregation surrounding blood vessels called osteons or haversian systems. Osteoblasts, which eventually become osteocytes, are involved in bone formation. On the other hand, osteoclasts, which are multinucleated cells, are involved in bone resorption by phagocytizing bone.

Microscopically, inorganic substances, which account for 60-70% of bone, consist of ionic crystals of hydroxyapatite, but actual biological apatites are carbonate apatites that contain a wide variety of ions including trace amounts of $Mg^{2+}$, $Fe^{2+}$, $CO_3^{2-}$, $HPO_4^{2-}$, and $F^-$ as well as $Ca^{2+}$, $PO_4^{3-}$, and $OH^-$.

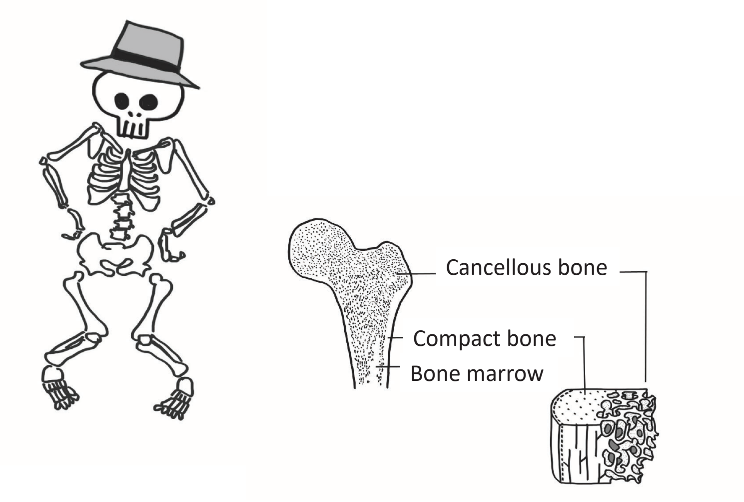


Fig. 21 Schematic images of bone structure.

A widely supported hypothesis is that biological apatites undergo nucleation in the membrane of granular cell secretion called the matrix vesicle with a diameter of about 500 nm, gradually grow as crystals, and eventually break through the membrane, and initial bone synthesis occurs as collagen fibers cling around them. In this process, materials including Ca-ATP, Mg-ATP, and alkaline phosphatase are considered to play important roles.

Bone is a storehouse of minerals and contributes to mineral homeostasis of the body. The adult human body contains about 1,100 g of Ca and maintains the Ca balance by ingesting about 1 g of Ca daily. Particularly, 99% of Ca in the human body is contained in bone tissue. Concerning calcium metabolism in adults, 1,000 g of Ca are present in bones in a stable condition, and only 4 g are exchangeable. The remaining about 1% is present in body fluids and tissues other than bones. About 250 mg of Ca are present in plasma (Fig. 22)[14].

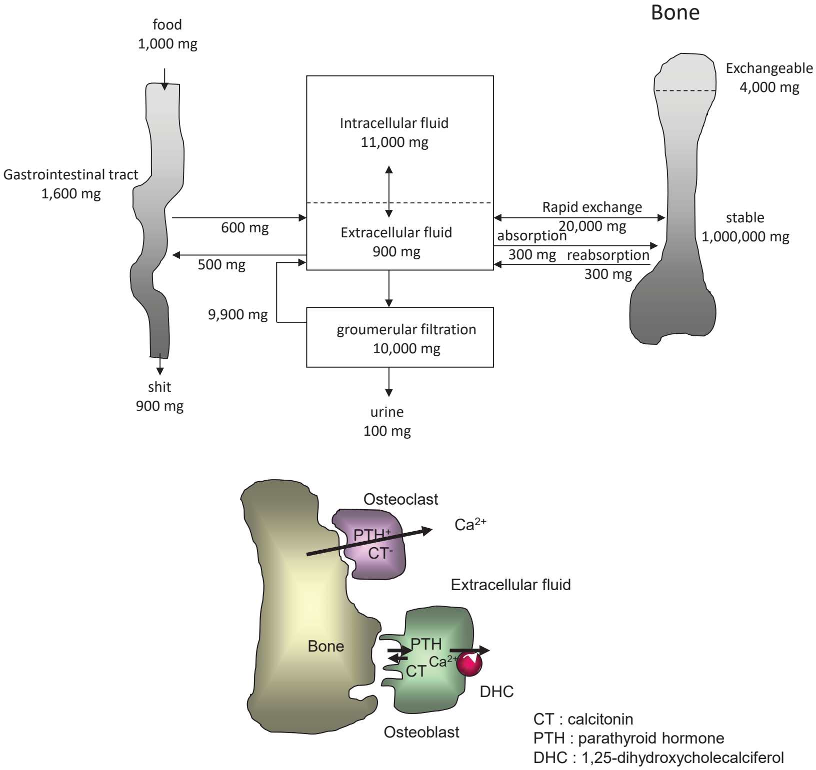


Fig. 22 Ca metabolism (adult/day) with an illustration of Ca regulation in human body.

Ca necessary for maintaining normal functions of the body, such as blood clotting, muscle contraction, and nerve function, is ionized free $Ca^{2+}$. Three hormones, i.e., vitamin $D_3$ (1,25 dihydroxycholecalciferol DHC), thyroid hormone (parathyroid hormone: PTH), and calcitonin (CT), are primarily involved in the adjustment of Ca metabolism. DHC has been recently advertised widely on TV and newspapers. PTH acts to have Ca released from bone, and CT, in contrast, acts to inhibit bone resorption. These actions are related to the control of the $Ca^{2+}$ permeability by osteoclasts and osteoblasts.

Concerning Mg, about 20-30 g is present in the body, and most of it is contained in the bones, muscles, liver, and heart. It is present in a free form or is adsorbed on protein and inorganic substances, but part of it is substituted in the crystals of bone apatites. Humans maintain the Mg balance by ingesting about 200-300 mg of Mg daily. However, the amount of Mg stored in the body is not increased markedly by eating a large amount of Mg. For example, there are data about the Mg balance in Japanese young women that the amount of Mg stored in the body was 58 mg with an intake of 220 mg of Mg but increased to 71 mg with an intake of 400 mg. These data suggest the presence of a limitation in Mg uptake by bone apatites.

Some bone diseases are caused by overall secondary failure of metabolism. Osteosclerosis affects patients with metastatic tumors, lead poisoning, or hypoparathyroidism and promotes Ca deposition in bones. It is also caused by increased uptake of F into bone, and intake of Mg is considered effective as a treatment. Osteomalacia is a disease represented by rickets, in which the amount of inorganic substances deposited in a unit amount of bone matrix is deficient. Osteoporosis is an abnormality commonly observed as a result of increased bone resorption or reduced bone formation characterized by a decrease in bone substance while the ratio between minerals and the bone matrix remains normal. In this disease, both inorganic apatites and organic collagen are generally reduced, and bones become markedly porous. It is observed frequently in postmenopausal middle-aged women, in particular, and is considered to be related to hormone imbalance. Deliberate intake of Ca is important.

## Story of collagen, the source of resilience

Collagen in teeth and bones is Type I similar to collagen that makes up our skin[15, 16]. Generally, a collagen molecule consists of 3 α chains, which are polypeptide chains with a molecular weight of about 100,000. If I am allowed to go into detail, the α chain is made up of two α1 chains and one α2 chain. The ends of both types of chains are N-peptide ($-NH_2$) and C-propeptide (-COOH), and these terminal peptides are closely involved in antibody reactions. Collagen is a called structural protein and is involved in the maintenance of the shape of the body and accounts for about one-third of all proteins in mammals. To the present, 27 types of collagen have been discovered. Collagen in the skin, tendons, and bones is Type I, and that in cartilage and hyaline bodies is Type II (Table 2).

Collagen molecules make up filaments, filaments are bound into fibers, and fibers are bound into fiber bundles (Fig. 23). Collagen is synthesized continuously by bone-making osteoblasts. Although artificial synthesis of some sequences of collagen has become possible, unfortunately, complete artificial collagen molecules have not been created. Complexes formed by collagen and hydroxyapatite make bones resilient.

## Table 2 Types of collagen.

| Type | chain | distribution |
|---|---|---|
| I | α1(I), α2(I) | skin, tendon, bone et al. |
| II | α1(II) | cartilage, vitreous |
| III | α1(III) | skin, muscle et al. |
| IV | α1(IV), α2(IV) | basement membrane |
| V | α1(V), α2(V), α3(V) | interstitial tissues |
| VI | α1(VI), α2(VI), α3(VI) | interstitial tissues |
| VII | ? | Binding fiber |
| VIII | α2(VIII) | endothelial cell |
| IX | α1(IX), α2(IX), α3(IX) | cartilage |
| X | α1(X) | cartilage |
| XI | α1(XI), α2(XI), α3(XI) | cartilage |

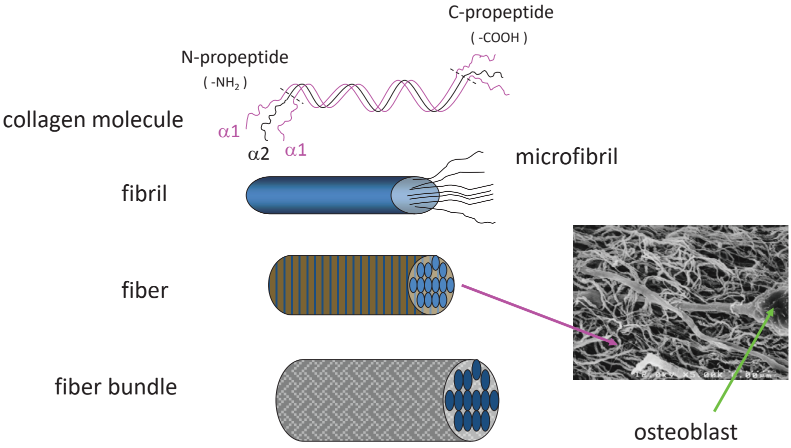


Fig. 23 Structure images of collagen molecule and fibers.

## Story of hyaluronic acid

Recently, hyaluronic acid is attracting attention as a substance that makes joints smooth. Hyaluronic acid is the primary component of joint fluid (also called synovial fluid), and a composite of hyaluronic acid and protein covers the surface of articular cartilage and serves as a lubricant that helps the joint move smoothly.

The volume of joint fluid is usually only a few milliliters, but joints can be smoothly extended and flexed, because the surface of articular cartilage covered by joint fluid is smooth and glides freely. The friction coefficient of articular cartilage covered by joint fluid is reportedly 0.02, which is difficult to achieve artificially by current technology. Incidentally, the friction coefficient of a snow-covered road is 0.5-0.35, and that of a frozen road is 0.2-0.1 or less, so you can see how runny joint fluid is.

Hyaluronic acid (Fig. 24) is a substance produced in chondrocytes and is called mucopolysaccharide. Its molecular weight is greater than 1 million. Therefore, if you drink it, it is not absorbed in its original form through the intestinal wall or transported directly to joints. So, please carefully evaluate the benefit of its intake as a drink by yourself.

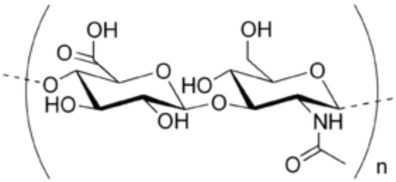


Fig. 24 Chemical structure of hyaluronic acid.

## Story of muscles, tendons, and ligaments

Muscles are made of highly contractile muscle cells and connective tissue that binds muscle cells together. Muscle cells are thin fibrous cells 10-100 μm in diameter and 35 cm long at the maximum and are also celled “muscle fibers”. ATP is needed for contraction of muscle fibers.

There are 3 types of muscles, and bone smooth muscle is called striated muscle, because stripes (striae) are observed under a microscope. This type of muscle can be moved voluntarily, but, unfortunately, it fatigues. Visceral muscle has no stripes and is called smooth muscle. It cannot be moved voluntarily unlike skeletal muscles. The digestive tract, blood vessels, bladder, etc., are made of this muscle. Myocardium, the muscle that constitutes the heart, is striped, but cannot be moved voluntarily. So, it has characteristics between those of bone smooth muscle and visceral muscle (Fig. 25).

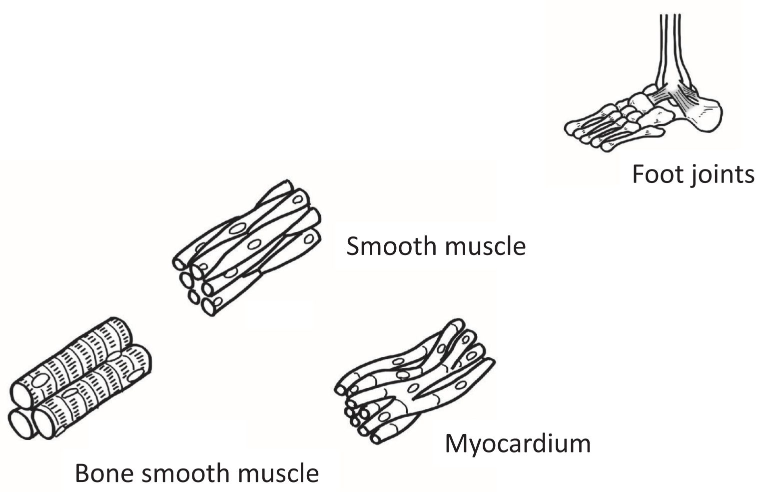


Fig. 25 Smooth muscle, bone smooth muscle (striated muscle) and myocardium.

Bone smooth muscle moves the skeleton as the name indicates. To move the skeleton, bone smooth muscles must be attached to bones. However, soft muscles cannot attach firmly to bones as they are, and tendons are present to mediate between bone smooth muscles and bones. While muscles are made of muscle cells and connective tissue, tendons are made of connective tissue alone, so they do not contract. If you stretch them beyond their limits, they may break. Rupture of the Achilles tendon, which connects the gastrocnemius muscle in the calf to the heel, is the best-known example.

Ligaments are structurally similar to tendons and are made of connective tissue, but its density is even higher than in tendons. The role of ligaments is to provide connections between bones rather than between muscles and bones.

## Story of gout and false gout

Gout is known as a “rich man’s disease”, perhaps because it occurs in many males in their 30s to 40s who enjoy eating and drinking. Gout is a disease characterized by recurrent attacks of intense pain in the joints of the feet, hands, knees, etc., caused by deposition of uric acid as a result of excess intake of animal protein, particularly nucleic acid. Uric acid is precipitated as needle-like crystals unlike phosphates, and they cause stinging pain. The affected joints become so sensitive that even a gentle wind can induce pain, hence the Japanese term “tsufu”, literally meaning pain on wind.

There is a similar condition called “pseudogout”. In this disease, pain is caused as crystals of pyrophosphate or hydroxyapatite instead of uric acid are deposited.

# I-3. KNOWLEDGE ABOUT DENTAL MATERIALS THAT HELPS A LITTLE

## Variations in dental materials

A variety of materials are used for filling or crowing teeth or making dentures if teeth are lost[17, 18]. They are generally classified into metal, plastic, ceramic, and their composites similarly to industrial materials. These materials have different characteristics and are used for appropriate objectives at appropriate sites.

Metals are highly extensible and not fractured even when they are bent or twisted to an extent. Particularly, gold is very spreadable, so it used to be spread into foil, packed into holes in teeth (cavities), and stretched by pressing to seal cavities. Usually, alloys of precious metals (Au, Ag, Pt, etc.), which do not rust, are used. There used to be a trend of crowning incisors with gold to show off gilded teeth as a symbol of wealth, but, today, it is used exclusively in the molars, which are usually concealed, by valuing the aesthetic aspect. Metals are much more durable than composite resin and last a lifetime if handled carefully. However, the cement used to adhere metal crowns and inlays to teeth and seal the gaps is not so durable. Inorganic cement, which used to be used widely, is gradually dissolved by saliva and water in the mouth. However, some senior dentists still prefer to use an inorganic cement called zinc phosphate cement with a history of more than a century. Indeed, the crowns mounted on my molars have served me for more than 50 years owing to this zinc phosphate cement. I am grateful to the dentist who treated me for adjusting the thickness of the cement so skillfully and preventing the infiltration of water. However, organic cement, which is widely used recently, is bound to decompose as the destiny of a macromolecular substance. As a result, food residues are deposited in the gaps created after the decomposition of cement, cavity-forming bacteria proliferate, and the teeth become susceptible to caries again (secondary caries).

Presently, a wide variety of filling materials are available. Formerly, however, there was only an inorganic cement called silicate cement, and caries were inevitably filled with this material. However, as mentioned above, it gradually decomposed over a long period of time. In addition, although silicate cement was satisfactory in aesthetics when used in incisors, it was not strong enough when it was filled in molars. Amalgam, which is a metal, was durable, but it could not be used in incisors and was used exclusively in molars. Even when amalgam was used, thin margins were often fractured over a long period of time, food residues deposited, and bacteria infiltrated with saliva, causing secondary caries. Since amalgam is prepared by kneading silver alloy with mercury, the problem of mercury contamination was raised, and amalgam has been mostly abandoned in Japan. Presently, compounds of organic and inorganic materials called composite resins are usually used. After repeated improvements, the present composite resins are made of an inorganic filling material called a filler surrounded by hard resin (plastic), and some composite resins have a filler content of even 80-90%. Therefore, they sufficiently bear the occlusal force of the molars. In addition, they are very easy to use for treatment and are not decomposed by saliva. However, they are not as durable as metal or ceramic inlays, because the adhesive called matrix, which covers the filler, is organic resin. In addition, since composite resins themselves do not have much adhesiveness to the tooth substance, an adhesive must be used to attach them to teeth. However, this adhesive is not so durable and is considered to last for only about 3-5 years, so our usual stance is that we treat again if the composite resin is fractured or detached. Of course, composite resin prosthetics last for 10 to 20 years if they are used carefully.

## Story of metals

Gold has been used since ancient times as ornaments and currency. Its glitter is considered to have something that fascinates our mind. You probably know that it has recently been used as a material of electronic devices in cell phones. Chemically, gold has the lowest ionization tendency and is, therefore, the least soluble and very stable. In addition, it has excellent extensibility, and 1 g of gold is said to be able to be extended linearly to about 3,000 m. Therefore, gold has been used as gold foil, which decorates the famous Golden Pavilion and Hideyoshi’s gilded tea house. Stories about gold seem inexhaustible. The tale of Archimedes revealing that an alleged gold crown was a fake from the difference in specific gravity is also well-known.

One reason that gold appeared from the dawn of civilization is that it is exposed on the earth in an unbound form such as lumps and dust and can be used directly without refinement.

Pure gold is regarded as 24 carats (K), and the hardness of gold is adjusted by mixing other metals. Gold used as accessories, such as necklaces and pendants, is usually 18-20 K. Instances of mismanagement where the quality of gold currency was deteriorated by mixing silver or copper, resulting in the collapse of economies due to inflation, have been repeated in history. Since silver is white and copper is brown, the appearance of gold currency elaborately adulterated with them is not immediately distinguishable. The best-known example is brass, an alloy of copper and zinc, and naive people may be deceived easily without noticing the fraud. For dental use, 14 K gold is often used. I hear that there are heaps of gold ingots sleeping in the Swiss Bank. Isn't it a shame? I wish more gold was used for dental treatment.

In the past, gold foil was packed in tooth holes (cavities) to treat caries or as a lining for ceramics, because it is very pliable and has a sealing effect. At any rate, gold alloys have chemical stability, mechanical strength, and excellent durability, and they can be used with a sense of security for the treatment of at least molars if they suit you aesthetically (Fig. 26).

Fig. 26 Prosthesis (By courtesy of The Gold Information Center, NY).

Platinum is a precious metal and is chemically very stable and has been valued from old times as a material for ornaments similarly to gold. Recently, it has been used widely as a precious material for many electronic devices. The dental material prepared by adding a relatively high percentage of platinum to gold alloy to increase the strength is called platinum gold alloy (PGA). PGA is used primarily as metal dentures and clasps, which are hooks for fixing dentures to natural teeth.

## Story of plastics

Formally, plastic is a macromolecular resin and is a category of polymers (substances with a molecular weight of 10,000 or higher) along with fibers and rubber. Thus, polymers can be classified into plastic (often called resin in dentistry), fiber, and rubber (Fig. 27).

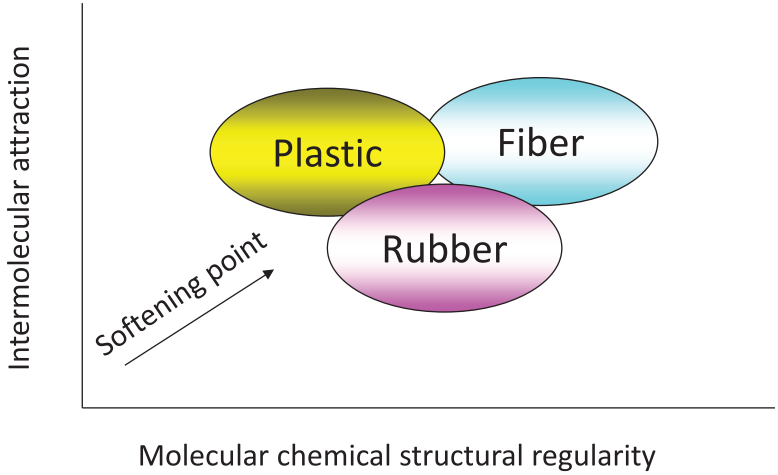


Fig. 27 Classification of polymers.

The best-known plastic is polyethylene (PE), which is made by polymerizing ethylene ($CH_2{=}CH_2$). Ethylene made by purifying crude oil by a technique called fracking is the origin of a wide variety of engineering/plastics and is a material indispensable for the manufacturing of plastics. The rapid economic growth of Japan is said to have been owed to PE plants. This is why oil shocks in the past have caused such a commotion.

Various plastics are derived from PE. For example, polyvinyl chloride (PVC) used as water and gas pipes is prepared by polymerizing vinyl chloride ($CH_2$=CHCl), in which one hydrogen atom (H) in PE is substituted with a chlorine atom (Cl). PVC is well-known as a material of synthetic leather, but it is said that, early after its development, it became sticky in summer and was hardened and soon broke into pieces in winter. I suppose there was indescribable effort before it was made tough and pliable all year round.

Teflon (PTFE) is also well-known as a material that prevents burning and sticking, and the surface of many products including frying pans is given PTFE treatment. PTFE is obtained by polymerizing tetrafluoroethylene ($CF_2$=$CF_2$), in which all 4 hydrogen atoms (H) in ethylene are replaced with F.

PMMA, which is derived from ethylene, is well-known as a dental material, particularly, denture material. PMMA is reported to have been developed in Germany during World War II as a result of the pursuit for unbreakable glass. In those days, the windshields of fighter planes were made of inorganic glass and were frequently broken by machine gun shots, etc., but PMMA developed as an organic glass (Plexiglas®) performed outstandingly. A denture material was made by adding a red dye to PMMA. Thereafter, various plastics have been tentatively used as dental materials, but none were superior to PMMA on overall assessments, and it is still used as a denture material.

MMA ($CH_2=(CH_3)(COOCH_3)$), the monomer of PMMA, is prepared by substituting one H of ethylene with ($-CH_3$), substituting the other H with ($-COOH$), then attaching ($CH_3COOH$) by ester binding ($-COOCH_3$) (Fig. 28).

Generally, products with a wide variety of shapes are produced industrially by softening polymerized PMMA by heating and infusing it into molds with pressure. This method is reasonable for manufacturing a product with the same shape in large numbers, because the same mold can be used many times even if large equipment is necessary. However, this method is inappropriate to prepare objects each of which has a unique shape such as a denture in a dental technique department. So, a very wise method was devised in dentistry. That is: dough is prepared by kneading already polymerized powder of PMMA and unpolymerized monomeric MMA, only wax is removed from the plaster cast prepared in advance, the resultant space is filled with the dough, and the cast is heated to induce polymerization as the initiator (benzoyl peroxide: BPO) mixed in PMMA in advance is decomposed and generates active radicals.

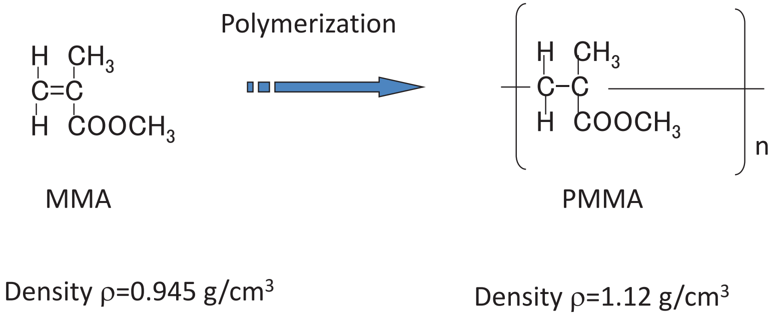


Fig. 28 Polymerization of MMA monomer.

Incidentally, do you know that the adhesive called bone cement used for fixing artificial hips in orthopedics is made of PMMA as are dental materials? Since bone cement does not conduct heat, MMA prepared in advance by adding a catalyst (such as DMPT and DHPT) is mixed with PMMA, and BPO is stimulated to initiate the reaction. However, as heat is generated by the reaction, and intact bone nearby is occasionally damaged, research to solve this problem is in progress. Also, surgery may be performed without using bone cement (cementless surgery).

In fact, catalyst type PMMA similar to bone cement was once used also for dental treatment, but, because it considerably contracts in polymerization, has no adhesiveness to the tooth substance, and is unsatisfactory in strength, it ceased to be used as a filling material. It is still used for the repair of fractured or chipped dentures. Then, appeared Bis-glycidyl methacrylate (Bis-GMA), a monomer that does not markedly contract in polymerization and is relatively hard after polymerization. The composite resin mentioned above is prepared by adding an inorganic filling material called filler to this monomer. However, it was found that a compound suspected to be an environmental hormone (endocrine disrupter) called Bis-phenol A was used in the manufacturing process of Bis-GMA, and it has recently been avoided and replaced with urethane di-methacrylate (UDMA). Since MMA is attached to both ends of these monomeric molecules, they are considered basically to be successors of MMA. Since they react at room temperature, the filling can be made readily and simply by mixing two pastes. Moreover, recently, a single-paste light-cured composite resin has become the mainstay. It can be used by dentists and dental hygienists with a sense of security and no worries about failure. However, since light is difficult to reach deep cavities, polymerization tends to be insufficient, so modifications, such as split filling, have been devised. Light-cured composite resins contain a photosensitizer called camphor quinone, which strongly reacts to light with a wavelength of about 470 nm, instead of the catalyst DMPT, and when it is exposed to light, it is decomposed and yield active radicals, which stimulate the initiator BPO to advance the polymerization reaction. Formerly, ultraviolet rays with a shorter wavelength were considered as a candidate, but as there is the risk of damaging the patients' eyes and skin, visual rays (wavelength: about 380-770 nm), which are safer, are used. Regarding the light source, halogen lamps used to be used, but, they have presently been substituted with light emitting diodes (LED).

## Story of ceramics

The word ceramics immediately reminds us of rice bowls, teacups, and clay figures and pots excavated from ancient remains. Commonly, ceramics, which are made by kneading, shaping, and firing clay, are called *yakimono* (fired-ware). However, their properties differ considerably depending on the components and temperature of firing. From ancient times, ceramics have been part of people's lives, serving as storages of food and art objects. A typical example would be celadon fired in official kilns during the Ming and Qing Dynasties of China. Under the protection of the dynasties, a large number of masterpieces were produced. Thin and almost translucent white porcelains are also famous. They are said to be made possible by special clay collected from Mt. Kaolin in the suburbs of Jingdezhen, Jiangxi Province, China (kaolin: $Al_2O_3$-$2SiO_2$-$2H_2O$). We are told that chinaware produced there was transported to the West across the Indian Ocean and

Mediterranean Sea and was highly valued by the noble class. The ocean route through which such articles were carried began to be called the Silk Road of the Sea as opposed to that of the land. In time, the technique was investigated in 18th century Meissen, Germany, and Sevres, France, where royal kilns were operated, leading to the dawn of the new era of romanticism.

In Japan, the technique transmitted from China and Korea was developed independently, and many potteries arose in various parts of the country and yielded splendid pieces of art. One of the earliest regions in which chinaware was commercially produced in large quantities is Seto in the present Aichi Prefecture, and as ambulant vendors travelled far and wide to sell it, “seto-mono (Seto ware) became a synonym for chinaware, sometimes causing misunderstandings.

However, since a large percentage of such traditional ceramics are composed of clay, their mechanical strength was inevitably low. In contrast, fine ceramics (new ceramics) have recently made their appearance to be used in electronic parts and laboratory instruments and begun to be used also in medicine and dentistry as materials of artificial bone and dental implants. These materials are very strong unlike conventional ceramics, and even ceramic knives are marketed.

On the other hand, dental ceramics contain little clay component, consisting of feldspar (80-90%), quartz (10-20%), and kaolin (0-5%) and have excellent strength. They are not as fragile as rice bowls or cups.

In making prosthetics to be filled in or mounted on teeth, porcelain powder is kneaded with water, the dough is put in a mold covered with gold foil, or put on a prosthetic called a crown, and heated after removing excess water. By repeating this procedure 2-3 times, the color of the prosthetics becomes close to that of natural teeth.

We often see celebrities smiling to exhibit beautiful teeth on TV, but some of such teeth are mounted with highly aesthetic prosthetics called metal bonds (Fig. 29). Metal bonds are metal crowns with only the visible front part covered by a ceramic material. Metal is exposed in part of the reverse side, but it cannot be seen unless the person opens the mouth wide or you look into the mouth. Presently, metal bonds are very favorably accepted as aesthetic prosthetics, but they are expensive, and the tooth must be ground by the thickness of both the metal and porcelain combined. In addition, the metal and porcelain are not bonded well, and caution is needed as the porcelain front surface is occasionally fractured if a strong force is applied. In such an event, there is no choice but to repair the fracture with resin. It is important to consider the durability without adhering to the appearance alone.

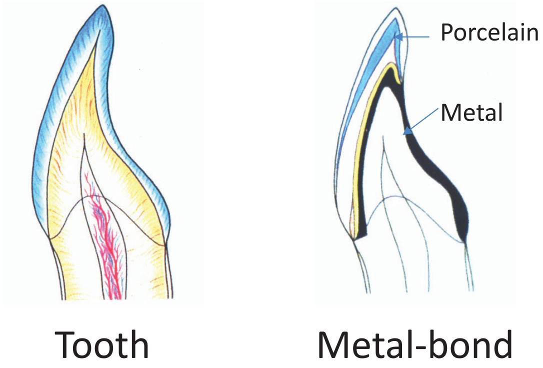


Fig. 29 Images of natural tooth and metal-bond.

In contrast to metal bonds and dentures, a completely novel treatment of planting a prosthetic into the jaw bone appeared in the 1960s. It is the implant. The material of the implant was pure Ti from the beginning, but alumina, which is a ceramic, was used for a period. Although alumina had sufficient mechanical strength, it was gradually disused, because it was less satisfactory in biological compatibility than was expected, and sintered hydroxyapatites came in to replace it. Sintered hydroxyapatites were satisfactory in biological compatibility but were, unfortunately, mechanically vulnerable and gradually became out of use. Today, primarily implants of pure Ti or Ti coated with hydroxyapatite are used.

## How about a cup of tea? This and that about tea

Raku ware is famous as the tea ceremony bowl, and there are expensive bowls called Red Raku and Black Raku. There are also tea containers rated as *meibutu* (renowned utensils). It is said that, in the period of warring states, territories to be given to war lords as rewards for deeds of arms became deficient, and, in trouble, the idea of outrageously pricing tea utensils and giving them as rewards as substitutes for territories was squeezed out. What an economically ingenious idea! However, I consider that some arts and crafts are priceless and are not exchangeable for whatever amount of money, but what do you think?

As to the history of tea, a book of medicine in China under the Han Dynasty (1st century BC) suggests that there was already a custom of drinking tea in those days. Tea is believed to have been introduced to Japan as monks who studied in China such as *Saicho*, *Kukai,* and *Eichu* brought tea seeds back in the Nara and Heian periods, when envoys were repeatedly dispatched to China.

Tea contains many ingredients good for the health including catechin (a kind of tannic acid with bitter taste: reduces cholesterol in blood), theanine (*umami* ingredient of tea: protects nerve cells), vitamins (protect the health of the skin and mucosa), fluoride (prevents caries), and various minerals (adjust physiologic functions). Recently, the term “anti-aging” has become popular, and an interest in healthy diets and rejuvenation techniques is growing. Tea is attracting attention as one of such items (Fig. 30).

Fig. 30 Japanese tea.

# I-4. CHANGES IN ARTIFICIAL BONE MATERIALS AND PROSPECTS FOR THE FUTURE

It was in the 20th century that metal biological materials began to be used in the field of medicine on a full scale, and stainless steel SUS316L (steel-special use stainless) was outstanding as an early material[19]. Stainless steel is a general term for special steel with excellent resistance to corrosion compared with usual carbon steel, and 18-8 stainless steel (Cr18%-Ni8%) is particularly well-known. In 1938, Vitalium® (Co-Cr-Mo alloy) was introduced by Strock (USA) and is still used widely as a material for various prostheses such as the artificial hip (Fig. 31). In addition, titanium, which is known as a biocompatible metal, is very popular, and since it was used by Linkow (USA) as artificial dental roots in 1967, it has been variously modified and improved by Bränemark and others to the present. Moreover, since Nitinol® (Ni-50at%Ti), which was developed as a shape memory alloy by Buehler in 1962, has superelasticity, it has also been applied to the medical field.

Concerning ceramics, alumina ($Al_2O_3$) attracted attention in the 1960s and was used as artificial bone and dental roots. However, it gradually faded away with the advent of biocompatible calcium phosphate ceramics, one of the first of which was Bioglass ($CaO$-$P_2O_5$-$SiO_2$). Although it had excellent biocompatibility, it was actually not used much because of its fragility. Thereafter, many sintered apatites appeared in the 1970-1980s, and various artificial bones ranging from the block type to granular type were produced.

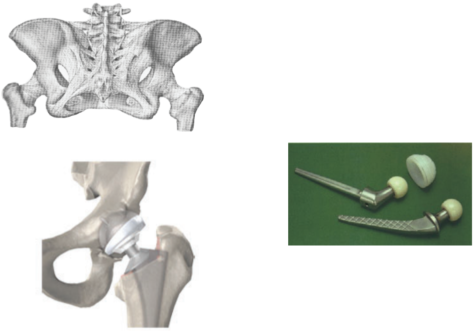


Fig. 31 Artificial hip prothesis (Photo: by courtesy of Kyocera Co. Ltd.).

However, the use of these sintered apatites was gradually reconsidered because of poor metabolic properties, and they were replaced with many resorbable calcium phosphates[20]. A variety of biomaterials with improved compatibility with bone were developed by using resorbable apatites and materials such as α-TCP, β-TCP, DCPD, OCP, and 4CP alone or by coating metal materials, such as titanium, with them.

Simultaneously, composites of these resorbable apaties and organic collagen, chitosan, or polylactic acid (PLA) have been tentatively developed. Furthermore, with the advent of tissue engineering, these materials were made porous to facilitate the infiltration of osteoblasts. We also developed a carbonate apatites/collagen scaffold with a chemical composition and metabolic properties close to those of natural bone[21-23] and confirmed that it is promising as a high-functioning bone regenerative biomaterial using animal experiments (Fig. 32) [23, 24]. A scaffold means a platform that provides support for the 3-dimensional proliferation of cells. Therefore, as regeneration advances, it is gradually metabolized and lost. Since carbonate apatites and collagen are originally biological materials, the components released after their degradation are reused for bone formation. No bad foreign bodies harmful to the body are left. Such metabolizable materials close to natural biomaterials are what we aim to develop and are ideal biomaterials “friendly to the body”. The carbonate apatites/collagen scaffold is expected to contribute to the regeneration of bone defects in the body and resorbed alveolar bone in the oral cavity and to be applied to the stabilization of dentures, maintenance of implants (artificial dental roots), and reconstruction of periodontal tissue. In addition, they have been demonstrated through animal experiments to exhibit dynamic osteogenic activities even in patients or sites of the body with a low regenerative ability by modifying bone morphogenetic proteins (BMP) and angiogenesis factors. However, they must be used in combination with conventional high-strength non-metabolizable ceramics and metals for large bone defects and at sites subjected to large forces such as joints.

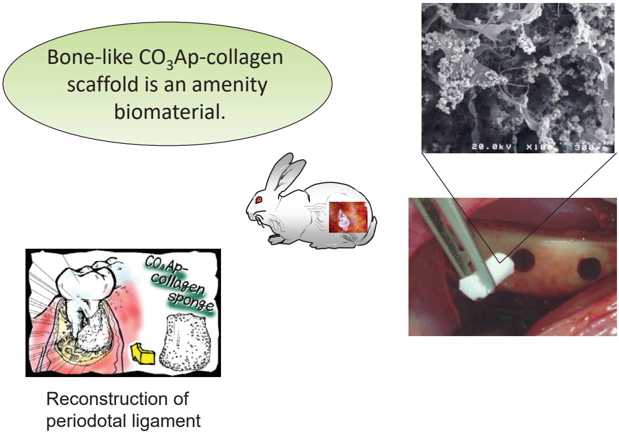


Fig. 32 Images of resorbable $CO_3$Ap-collagen scaffold.

Recently, patients who suffer fracture are continuing to increase year after year with the aging of the population. Many patients even in their 90s undergo hip replacement surgery. The need for biomaterials is expected to increase further. I hope that iPS cells will be put into practical use as soon as possible and that hard tissue regeneration is realized by a combination of biomaterials and tissue engineering.

Biomaterials are contributing as a bridge between the present and the future. I would like to propose them as a key to healthy aging thorough the understanding of biomaterials and their utilization according to individual needs.

# I-5. WHAT ARE MATERIALS FRIENDLY TO THE BODY?

Today, safety and security are demanded by both patients and medical workers in medical care circumstances. As medical care becomes more and more sophisticated, the operation of machines becomes more complicated, and new materials are introduced one after another. If the properties of materials are not fully understood, unexpected risks lie in wait.

As I have mentioned above, a large number of different materials are used in dentistry. Although they are used with approval by the Ministry of Health, Labour and Welfare after sufficient validation of safety, responses of the body are extremely complicated, and unpredicted adverse effects may occasionally appear. As indicated by the aphorism by Paracelsus (1493-1541), a renowned Swiss doctor, “All are poisons. Simply, their amount decides whether they act as poisons or not,” the utmost caution is needed in determining the amount of everything that is used. In addition, as the strength of response varies from one person to another, an assessment is made all the more difficult. Generally, the 50% lethal dose is used as an index in drug toxicity studies. The body has a threshold, under which the body patiently withholds responding. Therefore, responses of the body to a drug often show an S-shaped curve (Fig. 33). Since the slope of an S-shaped curve becomes gentle near 100%, it is difficult to determine the dose that provokes a 100% response. That is why the value near 50%, where the slope is relatively sharp, is used as an index. This value is technically called the

50% dose. Also, the dose is expressed as the amount per 1 kg of body weight, but some people are more sensitive than other people regardless of the body weight, and responses cannot be represented in terms of plain figures as in mathematics. This is how the body works.

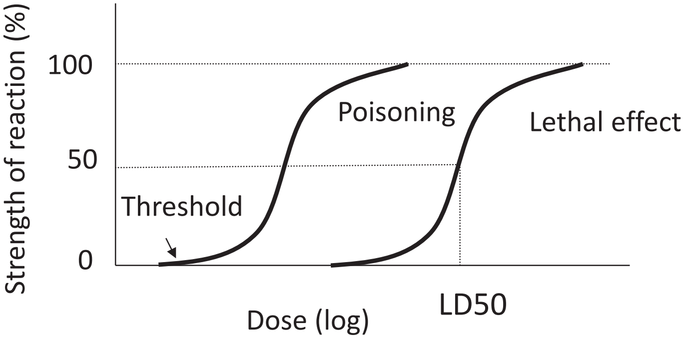


Fig. 33 Correlation between strength of reaction and lethal dose (LD50).

For example, fluoride, which has been used for the prevention of caries, is effective if used in a minute dose but may cause mottled teeth or osteosclerosis in a larger dose and even death in a very large dose.

Mercury used to be used widely as a dental filling material. Since it forms amalgams with silver alloys, the effect of mercury itself is not so great, and it is also used in a limited amount. However, mercury, even if it is inorganic and very small in amount, causes anxiety from the viewpoint of biosafety. Particularly, in Japan in the period of rapid economic growth from the 1960s to the 1970s, mercury contamination of seawater progressed due to waste discharge from factories and caused serious neuropathies such as Minamata disease. A large amount of mercury was discharged into the sea and was converted to organic mercury in the body of fish that ate it, and highly reactive poisons, such as methyl mercury, accumulated in fish bodies.

In humans who ate the fish without knowing it, mercury bound to nerve cells and caused extreme disabilities and suffering. Partly because of this experience, mercury was replaced with composite resin as a dental filling material earlier in Japan than in Western countries.

It is generally believed that immune responses to artificial materials can be ignored, but research of the compatibility of artificial materials to cells has just begun. It has been shown that, when cells are brought into contact with artificial materials, they seem to first secrete protein and observe its behavior before they make judgments about the compatibility of the materials. Ligands are materials that specifically bind to particular receptors such as integrin, which is known to be an adhesion molecule (Fig. 34)[16].

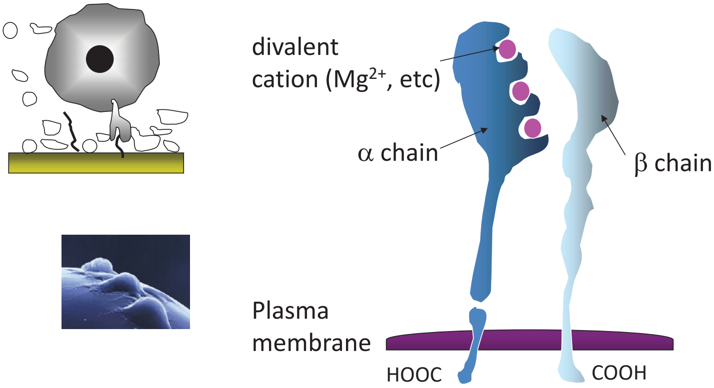


Fig. 34 Image of cell adhesion with adhesion molecule, integrin.

Ligands and receptors are like keys and keyholes. However, unknown factors still lurk in the subsequent step of recognition. Such is the situation with cells alone, and a far greater uncharted territory lies ahead in research of the whole body. It is impossible to judge biosafety from the results of cell experiments alone. In this sense, the term “biocompatibility” has not been fully accepted in science. Therefore, ethical brakes are needed in the applied research of regeneration medicine. However, life science has advanced remarkably, and secrets are unveiled little by little. The time when “biocompatibility” is firmly established as a scientific term will come sooner or later.

Since the living body is very delicate but, at the same time, very patient, sufficient caution is necessary in using artificial materials. Teeth are likely to be seen as simple objects, because they are exposed out of the body and show little metabolism, but they are innervated, and the gums and alveolar bone are metabolized daily. There is the possibility that materials incompatible to the living body may be eluted over a long period and do harm, resulting in alveolar pyorrhea and oral cancer. This is exactly why materials more friendly to the body are longed for now.

I have talked about various things, but what I really wanted to convey is the idea of “What are materials friendly to the body?”, which is the theme of this chapter. The lifespan of humans used to be around 50 years. Compared with those days, life is richer now, science and technology have advanced greatly, medical skills have been improved, and the average life expectancy has been considerably prolonged. This in turn has increased the opportunities for us to resort to biomaterials. Unlike trained Zen monks, it is usually difficult for us to maintain our mental stability if we have health problems, and we tend to lapse into a psychological condition opposite to a state of enlightenment. In an aging society, it is important for us to be more interested in medical artificial materials so we can lead at least reasonably healthy, if not comfortable, lives by keeping on good terms with biomaterials including dental materials. I recommend that my readers sufficiently learn about materials and have close consultations with experts in receiving treatments.

# PART II

# FURTHER DETAILED KNOWLEDGE OF APATITES

# II-1. MYSTERY OF APATITES

It is well known that coral is composed of inorganic calcium carbonate (Aragonite). Shells are also composed with calcium carbonate and keep a number of shapes and colors as well. On the other hand, we human beings are supported by bone and our daily metabolism. It is said that mammals selected phosphate instead of carbonate when they landed on the shore from the sea. However, fish still have skeletons, which are composed of calcium phosphate, hydroxyapatite. Fish scales are also composed of hydroxyapatite. Therefore, it appears that an additional secret is hidden in this phenomenon.

Bone is famous as a vital mineral safe. Phosphate is stored and used as a source of the life energy, ATP and a genetic component. A DNA chain is a long, unbranched polymer, composed of only four types of subunits[16]. The nucleotides are linked together by covalent phosphodiester bonds that join the 5'carbon of one deoxyribose group to the 3'carbon of the next.

Fundamentally, hydroxyapatite in the calcium phosphate group is the most stable in the solution. Phosphoric acid dissociates at each of the three steps in the solution. Therefore, hydroxyapatite with $PO_4^{3-}$ and $OH^-$ apt to precipitate there, differing from other calcium phosphates. At the alkali condition, $PO_4^{3-}$ ions mostly exist with excess OH- ions. When $CO_3^{2-}$ ions substitute into the $PO_4^{3-}$ positions, ionic balance is broken. This is well known as the susceptibility of caries in the dental field.

Hydroxyapatite (Fig. 17) has two columnar axes of Ca. These axes contribute to make hydroxyapatite crystals stable. On the contrary, since the axes are not single, the center of the crystal is sometimes blurred. We tend to make a crucial mistake in understanding the properties, because it is difficult to decide which of the defects of $Ca^{2+}$ ions in the left or right axis affects the occurring phenomena, when compared with a single axis. The shift of $OH^-$ ions from stable positions was established by Key et al.[9]. The shift of 0.03 nm is approximately 5 % of the c-axis dimension (0.688 nm) of hydroxyapatite. This value is enough to create an imperfection in the crystal, contrary to fluorapatite's.

We reported the dislocation of fluoridated hydroxyapatite synthesized using a multi-step fluoride supply system[25], as after-mentioned in PART II-5. High-resolution transmission electron microscopy showed a slender hexagonal shape similar to homogeneous hydroxyapatite in cross-sections perpendicular to the c-axis, but some lattice imperfections.

# II-2. SPECIFIC PHYSICOCHEMICAL PROPERTIES OF FLUORIDATED APATITES

Apatites are very interesting but strange substances. Almost all elements in periodic table could be substituted into the apatite crystals[5]. Most interesting element is fluorine, which can substitute stoichiometrically during apatite synthesis. Fluoride dramatically contributes to the preventive of dental caries[4], while inorganic substance of human hard tissues such as bone and teeth are composed with carbonate apatites[26, 27]. On the other hand, fluoride sometimes induces dental and skeletal fluorosis. We focused our research interest to the physicochemical properties of fluoridated hydroxyapatites.

Fluorine is a chemically active element, which contributes to the chemical reaction as a solvent of mineral stones and as a tool of glass processing. It also contributes to the caries prevention. Biological apatites such as inorganic substances of bone and teeth contain many trace elements in addition to the main components of calcium and phosphate ions. Most of them are hard to substitute stoichiometrically during the apatite synthesis. However, fluoride ions can easily substitute into the $OH^-$ position of hydroxyapatite stoichiometrically during synthesis. Maximum amount of fluorine substituting into the apatite crystal is 3.8 wt%.

It is generally considered that the solubility of apatites is affected by crystallinity in relation to crystal size and defect[28, 29]. The *a*-axis dimension of fluoridated hydroxyapatites decreased with the increase of the degree of fluoridation. The crystallinity of fluoridated hydroxyapatites increased initially, then decreased, and finally increased again with the degree of fluoridation, whereas the decrease in relative solubility of fluoridated hydroxyapatites was proportional to the logarithm of the fluoride content. Furthermore, the crystallinity of the apatites showed similar patterns of variation with the degree of fluoridation[30], irrespective of carbonate content. These patterns appear analogous to the complex pattern of the fluoridated hydroxyapatites.

# II-3. ROLES OF TRACE ELEMENTS IN APATITES

Biological apatites contain many trace elements, such as $Mg^{2+}$, $Zn^{2+}$, $Fe^{2+}$, $CO_3^{2-}$, $F^-$. These elements sometimes affect significantly the physicochemical properties of apatites (Table 3). Tooth enamel is well-crystallized, contrary to poorly-crystallized dentin and bone (Fig. 35)[1, 31]. It is well known that a number of elements can substitute into the apatite crystal structure[5]. The behaviors of many trace elements have been reported.

## Cationic ions

The interesting ion is $Mg^{2+}$ ion. Magnesium exists in human minerals, especially as a phosphorus complex in bone, and in chlorophyll in plants. It is known to be closely related to the metabolism and physiology of human body. Hexokinase, for example, which transfers a phosphoryl group from ATP to a variety of six-carbon sugars, requires $Mg^{2+}$ for activity in the human cell[15]. In that human hard tissues contain certain amounts of magnesium, it may play an important role in the initial formation of tooth apatites and have a significant effect on their physicochemical properties. However, since magnesium is a minor constituent of human enamel, dentin and bone[26], the nature of its association with the mineral phase, especially hydroxyapatite, and its contribution to the properties of biological apatites are still not clear. Since this ion has smaller ionic radius (0.064 nm) than that of $Ca^{2+}$ ion (0.099 nm), it was thought early time that $Mg^{2+}$ ion is hard to substitute into apatite crystal. Mostly, it was considered that it was

**Table 3. Effect of trace elements on crystallinity and solubility of hydroxyapatite.**

| | Crystallinity | Solubility |
|---|---|---|
| $Mg^{2+}$ | ↘ | ↗ |
| $Zn^{2+}$ | ↘ | ⇢ |
| $Fe^{2+}$ | ↘ | ↘ |
| $CO_3^{2-}$ | ↘ | ↗ |
| $F^-$ | ↗ | ↘ |

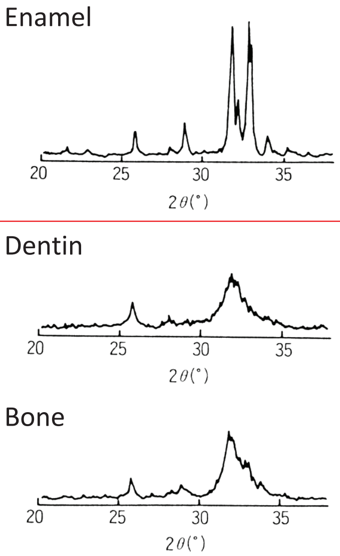


Fig. 35 X-ray diffraction patterns of enamel, dentin and bone.

absorbed at the surface of the crystal[32] or combined with organic compounds[33]. However, firstly LeGeros suggested that a part of $Mg^{2+}$ ions could substitute into the apatite crystal[34]. Okazaki et al.[35] also demonstrated this fact considering the change of c-axis dimension of Mg-containing apatites. It is estimated that the maximum Mg content keeping the apatite structure may be 1/5 of calcium ions. Above the Mg content, TCP with Mg is formed.

Neuman and Mulryan[32] reported that the presence of magnesium slowed the precipitation and crystallization processes. LeGeros reported that the combined presence of carbonate and magnesium or magnesium pyrophosphate is more effective in facilitating the formation of amorphous materials than when these ions present singly with the phosphate[36].

The Zn concentration is greatest in the top layer of the enamel and Zn probably accumulates in the enamel even before the tooth has erupted[37]. Zn ions are attracting attention as a promoter of bone formation. It was reported that Zn-doped TCP/HAp ceramics stimulated osteoblastic differentiation in cultured marrow stromal cells[38] and that in zinc-releasing calcium phosphate ceramics, the optimum zinc content for promoting bone formation was 0.316 wt%[39]. However, the crystallographic properties of Zn-containing hydroxyapatite are not well understood.

The crystallographic properties of synthesized Zn-containing hydroxyapatites were examined, and compared with those of Mg-containing hydroxyapatites. The crystallinity of hydroxyapatite, which was well-crystallized, dramatically decreased with the increase of zinc content, analogous to the tendency of Mg-containing hydroxyapatites[35]. The (002) reflections of Zn-containing hydroxyapatite shifted to the high angle direction[40]. This means that the $Zn^{2+}$ ions were taken up into the $Ca^{2+}$ positions of hydroxyapatite crystals, supporting the result of chemical analysis. The ionic radius of $Zn^{2+}$ ions (0.074 nm) is much smaller than that of $Ca^{2+}$ ions (0.099 nm) and larger than that of $Mg^{2+}$ ions (0.065 nm).

Iron, an indispensable element for human life, forming for example the core of hemoglobin molecules in red cells[26], is also a trace element in tooth mineral[4]. As mentioned before in PART I-1, in East Asia tooth black "Ohaguro" was traditionally applied, and the agent used contains iron, which could have contributed to caries prevention[41]. Iron accumulates in the outer surface of the enamel. Analyses of total enamel have shown considerable variation in the iron content of individual human teeth, probably due to differences in iron ingestion and age[26]. However, the form in which iron is deposited in the enamel and the mechanism of its acquisition are unknown. There is some evidence that iron is a component of the organic matrix of the enamel[42], and it has been postulated that it may serve as a bridge between the matrix and the mineral phase[43]. Such a mechanism would be of limited significance because these findings do not prove the accumulation of iron in the outer surface of the enamel.

Ferrous ions ($Fe^{2+}$) affect significantly the crystallinity and solubility of apatites as compared with ferric ions ($Fe^{3+}$)[44]. The decrease of the c-axis dimensions was not significant. The ferrous ion has the same charge as the calcium ion, but a smaller radius, 0.075 nm as compared to 0.099 nm. Therefore, it seems reasonable that the contraction in the lattice dimensions occurs when $Fe^{2+}$ ions substitute at $Ca^{2+}$ positions. $Fe^{3+}$ ions would be less likely to substitute at $Ca^{2+}$ positions than $Fe^{2+}$ ions because of the loss of electron neutrality and their smaller ion radius (0.064 nm). The crystallization of apatites was inhibited in the presence of iron.

## Anionic ions

It is well known that an increase in the carbonate content in enamel is correlated with increased caries susceptibility[45]. In addition, the wide variations in the reported values for the carbonate content in enamel may reflect actual changes in the carbonate distribution during and after mineralization[46]. These variations must be closely connected with caries susceptibility.

Biological apatites contain several %(w/w) $CO_3^{2-}$ ions. Long time ago, it was speculated that bone mineral might be composed with calcium phosphate and calcium carbonate $CaCO_3$. However, LeGeros clarified that the inorganic composition of hard tissues such as bone and teeth is composed of carbonate apatites[27]. In general, $CO_3^{2-}$ ions can substitute into both $PO_4^{3-}$ position and $OH^-$ position. It is said that the apatite synthesized in aqueous system contain $CO_3^{2-}$ ions in the $PO_4^{3-}$ position. When ions substitute into the positions, a-axis dimension decreases[47]. The crystallinity of $CO_3$apatites decreases with the increase of $CO_3^{2-}$ content and the solubility increases. i.e. the caries susceptibility and bone metabolism increase with the increase of $CO_3^{2-}$ content.

On the other hand, Kay et al.[9] reported form a crystallographic standpoint that the incorporation of fluoride into the crystalline lattice of apatitic tooth mineral brings about a greater stability. Driessens[48] and Moreno et al.[49] reported the same findings from a study of the solubility of fluoridated hydroxyapatites. A study of the physicochemical properties of synthetic fluoridated hydroxyapatites showed that there is no close relationship between their solubility and crystallinity at low fluoride content. Furthermore, the crystallinity of the apatites showed similar patterns of variation with the degree of fluoridation irrespective of carbonate content[50].

# II-4. WHAT ARE BIOLOGICAL $CO_3$APATITES

With constantly improving knowledge of structural detail now being provided by precision techniques in X-ray and neutron diffraction, the study of the structure-property relationships at the atomic level of organization seems to be especially promising. Atomic-scale mechanisms and the effects of substitution are particularly important in biological apatites which, apparently, always show considerable substitution and other crystalline defects[51].

LeGeros et al. [34] reported that the interference of carbonate with the crystallization of apatites and its weakening effect on the bonds in the structure increase the solubility, thereby contributing to the susceptibility to caries of dental apatites containing carbonate. Synthesized $CO_3$apatites changed dramatically their crystal features[47]. The apparent solubility of $CO_3$apatites over pH 4 - 8 at 37 °C was related approximately to their crystallinity. In particular, the degree of increase in the solubility of $CO_3$apatites having high crystallinity changed greatly in the region of 0 - 3 wt% of carbonate content, which approximates to that of enamel apatites.

Various kinds of materials: ceramics, metals, polymers and their composites were considered as bone substituting materials[52-54]. In particular, many different kinds of hydroxyapatites and other calcium phosphates such as tricalcium phosphate (TCP)[55], dicalcium phosphate dihydrate (DCPD)[56] octacalcium phosphate (OCP)[57], tetracalcium phosphate (4CP)[58] have been studied because they are composed mainly of calcium and phosphate, as are bone and teeth. Synthetic hydroxyapatites have been eagerly investigated and applied as hard tissue biomaterials for many years, because of their good biocompatibility[59, 60]. Mostly, sintered hydroxyapatites were developed as representatives of bone and tooth roots. They showed a relatively good bonding to natural bone[61]. Unfortunately, however, these hydroxyapatites were permanent non-metabolized materials, and those porous and non-porous block-type hard materials had a brittle nature, even if laminated by metals[62], and they had the possibility of cracking or exerting a long-term influence on the surrounding tissues since they remained in the body without metabolizing. Therefore, the recent focus has been on biodegradable materials[63-65].

To obtain better handling properties, apatite-collagen/gelatin composites have been developed. We have synthesized $CO_3$apatite with chemical composition and crystallinity similar to those of bone at pH 7.4 and 60 °C (Fig. 36)[1, 21, 31, 63]. The apatite powder was mixed with collagen solution, whose antigenicity had been removed by enzymatic treatment, and formed into apatite-collagen pellets. After insolubilization by UV-irradiation, the composites showed remarkably reduced disintegration and maintained their shape. They showed good biocompatibility when implanted beneath the periosteum cranii of rats. The UV-irradiated sample kept its feature well and was packed with newly created materials.

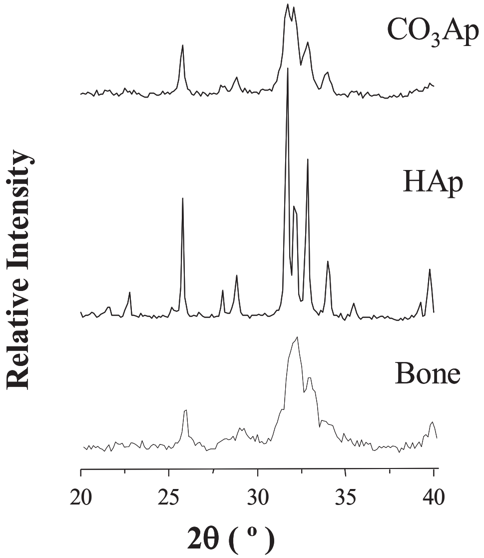


Fig. 36 X-ray diffraction pattern of $CO_3$apatite together with those of hydroxyapatite and human bone.

# II-5. SYNTHESIS OF HETEROGENEOUS FLUORIDATED APATITES

Biological apatites contain many trace elements[4], and sometimes form heterogeneously. To investigate such complicated phenomena in biological systems, we studied heterogeneous apatite formation. Since it is well known that fluoride contributes to caries prevention[66-69], including biomaterials applications[70, 71] and affects significantly on the enamel maturation, we focused on the fluoridated apatites. When a two-step fluoride system was adopted to simplify the phenomena, two different types of heterogeneous fluoridated apatites were formed[72, 73], hydroxyapatite covered with fluorapatite (H-FAp type apatite), and fluorapatite covered with hydroxyapatite (F-HAp type apatite). Although high-resolution transmission electron microscopy (HR-TEM) showed homogeneous hydroxyapatite was slender hexagonal and fluorapatite was typical hexagonal shape (Fig. 37)[73], these two crystals showed different physicochemical properties; H-FAp type apatite was comprised of slender hexagonal crystals, while F-HAp type apatite was typically hexagonal. H-FAp type apatite was less soluble than F-HAp.

When we synthesized the fluoridated hydroxyapatite using a multi-step fluoride supply system[25], high-resolution transmission electron microscopy showed a slender hexagonal shape similar to homogeneous hydroxyapatite in cross-sections perpendicular to the c-axis and the structural damage in the core of the crystal, although no boundary of step-like layers was observed. Some lattice imperfections and line dislocation (Fig. 38) were found in the core composed of many white spots, while crystal lattices in the outer layer were arranged almost perfectly.

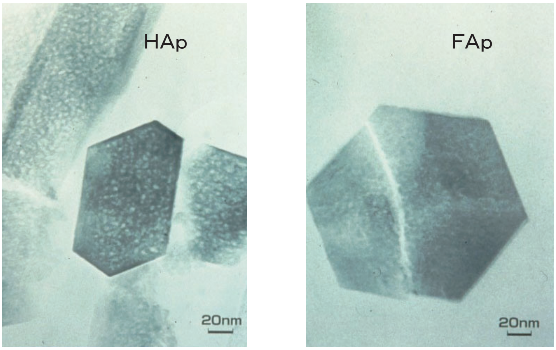
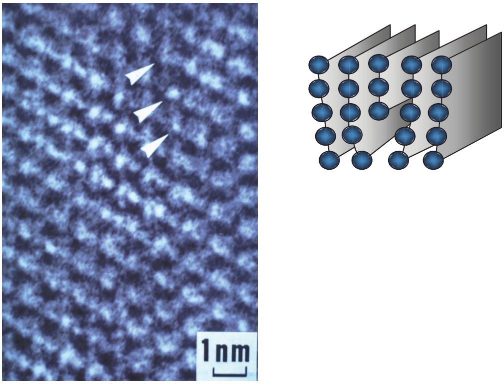


Fig. 37 HR-TEM photos of hydroxyapatite (HAp) and fluorapatite (FAp).



Crystal dislocation

Fig. 38 High-resolution transmission electron microscopy (HR-TEM) of sectional heterogeneous fluoridated hydroxyapatite crystal synthesized with a multi-step system and its dislocation.

# II-6. FUNCTIONALLY GRADED FLUORIDATED HYDROXYAPATITE (FGFAP)

Functionally graded (gradient) materials, FGM, have been developed for use in engineering fields[74]. These materials are utilized as adhesive agents, or for their thermal or electronic conductivity. The graded method is very useful because phenomena can be examined more smoothly. In biological systems, bamboo has a typical functionally graded structure: a negative density gradient of fibers in the thickness direction. Bone also has a functionally graded structure from the surface cortical compact toward the inner cancellous material. In human teeth, a negative gradient of fluoride concentration from the surface of tooth enamel toward the dentin-enamel junction[4] can be formed. We speculate that even each crystal composing enamel layer may have a fluoride distribution in the crystal structure. Therefore, we designed a continuous gradient fluoride supply system by developing the previous step-like fluoride supply system.

The X-ray diffraction pattern of the precipitate synthesized heterogeneously at 80°C and pH 7.4, FGFAp, was typically apatitic, although its crystallinity was lower than those of homogeneous hydroxyapatite, HAp and fluorapatite, FAp[75]. Calcium and phosphate contents were not significantly different from homogeneous HAp and FAp. Fluoride content of FGFAp was half of that of homogeneous FAp. High-resolution transmission electron microscopy (HR-TEM) of sectional FGFAp crystals revealed the typical hexagonal shape similar to that of homogeneous fluorapatite. Structural damage was observed in the inner core, the boundary of which was not clear, although a clear boundary for two-layer H-FAp apatite was observed previously[73]. ESCA analysis clearly showed the negative gradient distribution in F1s intensity (atomic concentration) of fluoride from the crystal surface toward the inner core. The effects of fluoride on tooth enamel have been described in mature enamel[76, 77] and in dental fluorosis[78]. Although fluoride profiles in the enamel layers are known, a fluoride gradient in a single apatite crystal has not been demonstrated. Our findings concerning the gradient of fluoride concentration in the apatite crystal is not entirely satisfactory.

# II-7. FUNCTIONALLY GRADED MG-CONTAINING $CO_3$APATITE (FGMGCO$_3$AP)

Recently, one of the main focuses of biomaterials research has been biological adhesion. It has been reported that cationic ions appear to be related to the activity of adhesion molecules such as those of the integrin family[16]. We can speculate that $Mg^{2+}$ ions also play some role in cell adhesion. To investigate the effect of the magnesium ions contained in the apatite crystals on cell adhesion, we considered how to prepare apatite that contained magnesium. It is easy to synthesize $CO_3$apatite with a certain magnesium content[79]. However, this apatite was poorly crystallized and too soluble. We now endeavored to synthesize functionally graded $CO_3$apatite containing Mg, producing a negative gradient of magnesium concentration from the surface toward the core.

Interface affinity (wettability) is affected by morphology, surface mobility, surface composition, electrical charge *etc*.[80]. Since hydroxyapatite is an ionic crystal, the interface affinity is strongly related to the surface composition and electrical charge. Adhesion molecules such as those of the integrin family were examined in terms of cell structure and function. It was reported that the cationic ions are related to the adhesion behavior of integrin. Especially divalent cations such as $Mg^{2+}$ ions play some role in cell adhesion.

The adhesive mechanism of cell adhesion molecules in living systems has been thoroughly investigated[81-86]. The first to be well characterized was fibronectin, a large glycoprotein found in all vertebrates. Fibronectin is a dimer composed of two very large subunits. Each chain is approximately 2,500 amino acid residues long. The Arg-Gly-Asp (RGD) sequence is part of the major cell-binding site. This tripeptide is solvent exposed and lies on a conformational mobile loop, consistent with its cell adhesion function[82]. All known RGD receptors are members of the integrin family of cell adhesion molecules. However, the mechanism and specificity of integrin binding to RGD-containing ligands remains unclarified.

Recently, adhesion molecules such as those of the integrin family were examined concerning cell structure and function[16]. It was reported that the cationic ions are related to the adhesion behavior of integrin (Fig. 34). The binding of integrin to specific ligands is dependent on the presence of divalent cations, with most but not all cells preferring $Mg^{2+}$ to $Ca^{2+}$ ions[87]. We can speculate that $Mg^{2+}$ ions also play some role in cell adhesion. Thus, magnesium seems to be an important factor even in controlling *in vivo* bone metabolism since it plays a part in both bone formation and resorption[88]. Mg accumulates in bone as substitution ions in hydroxyapatite crystal, adsorbing ions at the crystal surface, and binding with organic substances[89, 90]. In addition, magnesium plays many important roles in the human body, such as in reducing the chances of cardiovascular disease[91], promoting catalytic reactions[15], and controlling biological functions[31]. We can speculate that $Mg^{2+}$ ions also play some role in cell adhesion. Thus, magnesium seems to be an important factor even in controlling *in vivo* bone metabolism since it plays a part in both bone formation and resorption[88]. $Mg^{2+}$ ions may contribute to the bone metabolism of osteoclast and osteoblast action with the integrins at their cell surfaces.

At first, carbonate apatite containing Mg, FGMg$CO_3$Ap, was synthesized at 60 ±1°C and pH 7.4±0.2 using a gradient magnesium supply system (Fig. 39)[23, 92]. The X-ray diffraction analysis of synthesized FGMg$CO_3$Ap showed a poorly crystallized apatitic pattern similar to that of human bone and Mg-free $CO_3$Ap when compared with well-crystallized hydroxyapatite synthesized at 60°C without a carbonate source. SEM observations showed that the FGMg$CO_3$Ap crystals were smaller and coagulated, while the hydroxyapatite crystals[30] exhibited typically hexagonal plate-like features. ESCA analysis clearly showed a negative gradient distribution of Mg1s intensity (atomic concentration) of magnesium from the crystal surface toward the inner core.

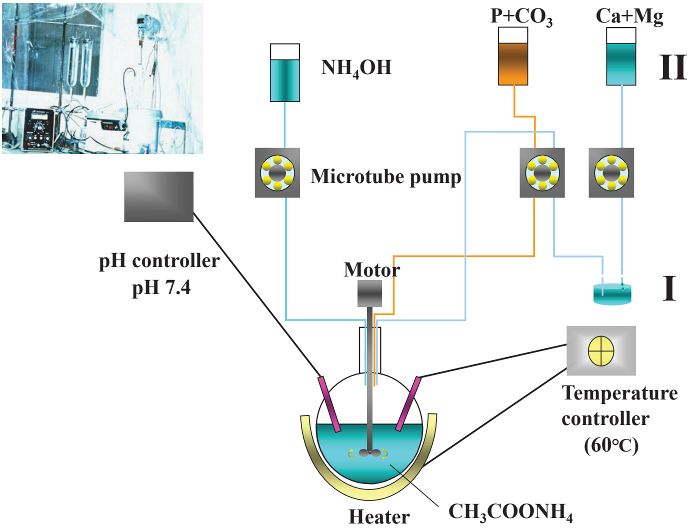


Fig. 39 Apparatus for synthesis of FGMg$CO_3$Ap crystal.

Then, FGMg$CO_3$Ap-collagen composite was prepared for osteoblast adhesion and animal experiments. After 4 wks of incubation, many more osteoblasts adhered to the FGMg$CO_3$Ap-collagen composite than to the $CO_3$Ap-collagen composite and the layer they formed was thicker. Alizarin red staining of the FGMg$CO_3$Ap-collagen composite showed a thicker layer of extracellular matrix (ECM). The underpart of the layer was redder than that of the $CO_3$Ap-collagen composite. This suggests that the osteoblast cells were activated and that mineralization had developed.

After 4 weeks of implantation in the rabbit femur, both the FGMg$CO_3$Ap-collagen composite and the $CO_3$Ap-collagen composite showed a clear bone formation, although the control hole with no implantation appeared to also have been repaired with a thinner layer of new bone (Fig. 40)[23]. The bone density of the FGMg$CO_3$Ap-collagen composite was higher than that of the $CO_3$Ap-collagen composite.

Finally, modification of gradational $Mg^{2+}$ ions on the apatite crystals was successfully completed. The apatite crystals showed no special function on the surface but did promote cell adhesion. Furthermore, as a scaffold material, the FGMg$CO_3$Ap-collagen composite could contribute to bone formation. Since this composite is easy to process into any form, it is extremely useful in the reconstruction of bone defects. It also offers the possibility of being used as a scaffold in the regeneration of hard tissue by combining with tissue engineering[94] together with cytokines, mesenchymal stem cells (MSCs), and/or embryonic stem (ES) cells.

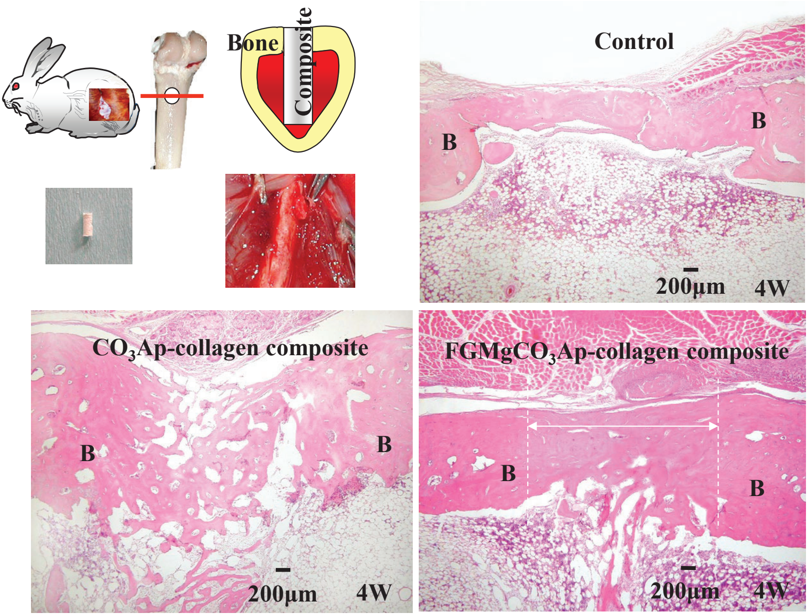


Fig. 40 Bone formation ability of FGMgCO$_3$Ap-collagen composite compared with CO$_3$Ap-collagen composite and control after 4 wks implantation.

# II-8. BIOCOMPATIBILITY OF $CO_3$AP-COLLAGEN SPONGE SCAFFOLDS

## Biomimetic $CO_3$Ap-collagen sponge scaffolds with optimal carbonate content

To examine the interrelation of $CO_3^{2-}$ contents in apatite crystals and biocompatibility, hydroxyapatite and $CO_3$apatites with different carbonate contents were synthesized, mixed with atelocollagen, and made into sponge scaffolds[95].

A recent focus has been how cells can invade scaffold materials and a 3D cell culture can be established. Ohgushi *et al.*[96] reported that a porous hydroxyapatite with a several hundred μm pore size is open to osteoblast invasion. We also successfully created a 70 wt% $CO_3$Ap-collagen sponge with 50 – 300 μm pore sizes[97]. This sponge has a chemical composition and crystallinity similar to bone. X-ray high-resolution microtomography revealed a clear image of the 3D structure of the sponges. The porosity of 70 wt% $CO_3$Ap-collagen sponges was $72.6 \pm 2.4\%$ and appeared to be the most favorable biomaterial from the viewpoint of natural bone properties. Osteoblasts could invade through the sponge bottom; however, the sponge material appeared to shrink during culture or animal experiments. First, we considered ensuring enough space in which new bone could easily form, utilizing the concept of guided bone regeneration (GBR)[98], and we newly created a sponge reinforced with a porous HAp-frame[99]. Our concept is that sponge as cancellous bone and the frame as trabecular bone are hybridized similar to natural bone. Bone formation was carried out on frame-reinforced $CO_3Ap$-collagen sponge scaffolds, after which they were implanted beneath the periostum cranii of rats. Unfortunately, bone formation was not sufficient.

Many researchers now believe that bone repair is entering a new era[94, 100]. Tissue engineering has started by erecting scaffolds toward rapid bone regeneration, especially for older patients and areas with lower regeneration ability. In general, for the therapeutic use of hard tissue biomaterials, a number of biomaterials have been investigated[101-104]. In particular, titanium and sintered hydroxyapatite have been used from the viewpoint of biocompatibility[53, 59, 102]. To obtain better handling properties, apatite-collagen/gelatin composites[21, 22, 23, 64, 105, 106] in addition to various kinds of calcium phosphate cements[57, 107-112] have been developed. The concept of biomaterials has appeared to shift to biodegradable scaffold biomaterials (Fig. 32, Fig. 41).

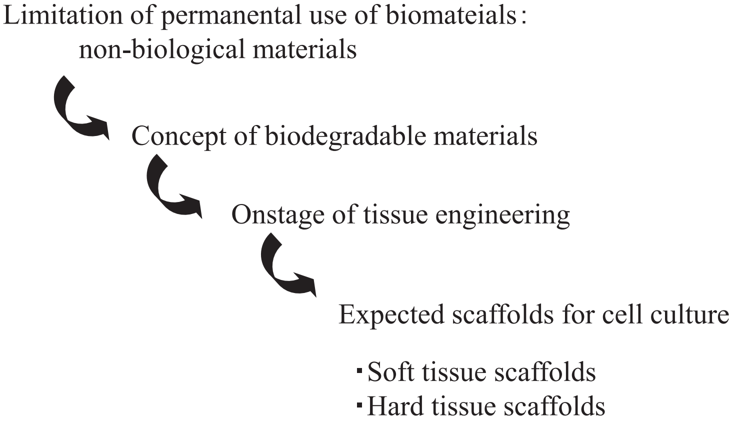


Fig. 41 Onstage of scaffolds in tissue engineering field.

On the other hand, biological apatites contain several percentages (4~6 wt%) of carbonate in apatite crystals[4, 113]. Carbonate contents significantly affect apatite crystallinity and solubility[47], and presumably dental caries susceptibility[45]. During the bone metabolism process, osteoclasts dissolve bone apatites with hydrogen ions in the closed environment by themselves, like dental caries phenomena[114]. We speculate that the carbonate contents of apatite crystals also affect bone formation and optimum carbonate content in apatite crystals may exist. Therefore, we examined the effect of *in vivo* bone formation using $CO_3$Ap-collagen sponge scaffolds with different carbonate contents.

Five types of apatites synthesized in the previous study[95] were used. Synthesis procedures were as follows. Hydroxyapatite (HA) and $CO_3$apatites ($CO_3$Ap or CA) with four different carbonate contents (0.01CA, 0.03CA, 0.06CA and 0.3CA) were synthesized at $60\pm1$°C and pH $7.4\pm0.2$.

X-ray diffraction patterns of $CO_3$Ap-collagen scaffolds showed apatitic patterns and were almost identical to $CO_3$apatite crystals. Crystallinity decreased with increasing carbonate contents and $CO_3$apatite with 4.8 wt% (= 0.80 mmol/g) carbonate content (0.06CA) appeared to have crystallinity similar to that of human bone.

In long-term implantation for 24 weeks, 0.03CA-CS, 0.06CA-CS, 0.3CA-CS showed uniform surrounding bone and could not be distinguished, although hydroxyapatite and $CO_3$apatite with low carbonate content 0.01CA-CS were slightly opaque on X-ray radiography. The area implanted using 0.06CA-CS with carbonate content similar to that of human bone was almost perfectly regenerated.

$CO_3^{2-}$ ion is an essential element in biological apatites and in the human body. Carbonate ions are related significantly to human metabolism. Bicarbonates in blood and body fluid contribute to stabilize the buffer action. We can speculate that cells may adapt to the surrounding environment, such as carbonate concentration in the solution through daily bone metabolism (Fig. 42). Osteoblasts and osteoclasts appear to adapt to bone properties such as the crystallinity and chemical compositions of bone apatites, including carbonate contents. Therefore, our concept of hard tissue biomaterials is that bone-like CO$_3$apatite is suitable and biocompatible. The carbonate content of synthetic CO$_3$apatite, which is similar to that of bone, 4-6 wt%[113], probably creates a suitable metabolic condition for bone in relation to apatite crystallinity and solubility.

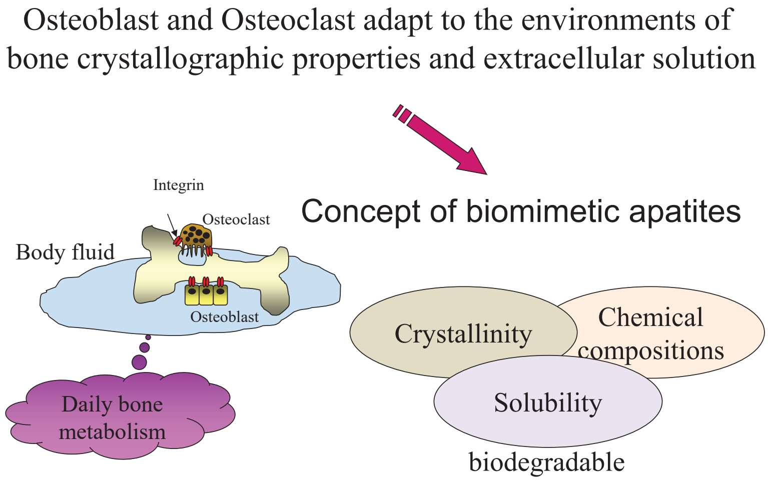


Fig. 42 Suitable bone regeneration.

Brown et al. reported an important solubility isotherm, from which we can speculate apatite metabolism in living tissue[115, 116]. From the viewpoint of solubility isotherm, other calcium phosphates such as TCP, DCPD, OCP, 4CP et al. seem to have quite different solubility from that of hydroxyapatite.

After long-term observation of 24-wk implantation, 0.06CA-CS was still significantly higher compared with 0.3CA-CS. It is considered that, in 0.06CA-CS, this is attributed to the space maintenance ability, which is a requirement for the bone graft material, and that material is absorbed and decomposed, and new bone forms in the space[117]. Thus, 0.06CA-CS has similar carbonate content to bone apatite of a living body, and it can work as biomimetic material.

## $CO_3Ap$-collagen sponge scaffolds with cytokines

Cytokines such as BMP2[100] and BMP7 have been successfully used to create and promote new bone growth using fixing biomaterials[94]. We investigated the acceleration of bone formation with rh-BMP2 using frame-reinforced $CO_3Ap$-collagen sponge scaffolds. To develop a new biodegradable scaffold biomaterial reinforced with a frame, synthesized $CO_3Ap$ was mixed with neutralized collagen gel, and the $CO_3Ap$-collagen mixtures were lyophilized into sponges in a porous HAp-frame ring. X-ray diffraction and FT-IR analyses together with chemical analysis indicated that synthesized $CO_3Ap$ had crystallinity and a chemical composition similar to bone. SEM observation showed that the $CO_3Ap$-collagen sponge had a suitable pore size for cell invasion. In proliferation and differentiation experiments with osteoblasts, ALP and OPN activity were clearly detected. When these sponge-frame complexes with rh-BMP2 were implanted beneath the periosteum cranii of rats, sufficient new bone was created at the surface of the periosteum cranii 4 weeks after implantation[118]. These reinforced $CO_3Ap$-collagen sponges with rh-BMP2 are expected to be used as hard tissue scaffold biomaterials for therapeutic purposes to aid rapid cure.

## $CO_3$Ap-collagen scaffolds with angiogenesis factor

The process of angiogenesis starts from digestion of the basement membranes of blood vessels by endothelial cells[119]. Cells subsequently migrate, proliferate and form tube-like structures. Numerous researchers have reported that these cellular responses are carefully regulated by signals from various growth factors and cytokines, including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and interleukin 8[120-124].

Osteopontin (OPN), one of the extracellular matrix proteins, is a phosphoric acid protein containing a large quantity of sialic acid. This protein is widely distributed in areas such as bone tissue, kidney, brain and skin. Osteopontin participates in bone metabolism and mediates inflammatory responses and angiogenesis[125].

Recently, the novel binding sequence Ser-Val-Val-Tyr-Gly-Leu-Arg (SVVYGLR) has been identified as an amino acid sequence in OPN involved in angiogenesis[126, 127]. This motif might be important in pathological conditions, as SVVYGLR is adjacent to the RGD sequence in osteopontin and is exposed by thrombin cleavage. Synthesizing a sequence of SVVYGLR as a neovascularization growth factor has been done artificially[128].

When $CO_3$Ap-collagen sponges with the synthetic motif SVVYGLR peptide were implanted beneath the back skin of the rat, new blood tubes were dramatically induced in 1 week[129], while no blood tubes were observed with the control sponge without SVVYGLR. SMA staining also indicated smooth muscular actin of blood tubes was stained red for the sponge with

SVVYGLR. These results suggest that $CO_3$Ap-collagen sponges combined with SVVYGLR form a useful high-quality scaffold biomaterial contributing to the angiogenesis necessary for bone formation. They will be useful as scaffolds for bone construction and regeneration during artificial tooth implantation and setting dentures on weak alveolar bone.

$CO_3$Ap-collagen sponges are useful by themselves for therapeutic applications in normal cases of regeneration without any modifications. Furthermore, they can be utilized as a rapid cure biomaterial, by emphasizing the adhesion motif and cytokines such as growth factor BMP or angiogenesis factor SVVYGLR in poor regeneration cases (Fig. 43).

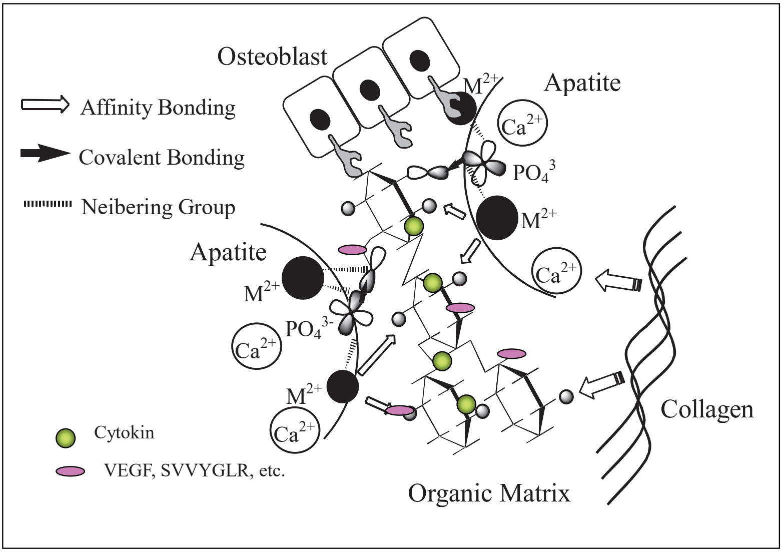


Fig. 43 Modification for bone regeneration.

# II-9. COMPUTER GRAPHICS OF APATITES

## Definition of crystals

A crystal may be defined as a solid composed of atoms arranged in a pattern periodic in three dimensions. In dividing space by three sets of planes, we can produce unit cells of various shapes. Oppositely, we call materials such as glass amorphous. The hydroxyapatite formed at the early nucleation stages in our body is thought to be an amorphous calcium phosphate. The French crystallographer Bravais worked on this problem and in 1848 demonstrated that there are fourteen possible point lattices and point lattice as synonymous. The systems are divided into cubic, tetragonal, orthorhombic, rhombohedral, hexagonal, monoclinic and triclinic, and each system is distinguished between simple cells (lattice symbol (P) or primitive (R)) and non-primitive cells (any other symbols: body-centers (I), face-centered (F), base-centered (C) )[11].

Gold (Au) and silver (Ag) often used as dental materials are cubic, and hydroxyapatite composed of teeth and bone is hexagonal. In detail, pure hydroxyapatite is monoclinic, but mineral hydroxyapatite is hexagonal[9] because many trace elements substitute into the crystal.

Since the interplanar spacing $d$ of hexagonal crystal[1, 9] is

$$1/d = (4/3)( h^2 + hk + k^2)/a^2 + l^2/c^2$$

We can calculate lattice constant such as $a$ (= $b$) and $c$ from the following equation using the Braggs law, $\lambda = 2d\sin\theta$,

$$\sin^2\theta = (\lambda^2/3)( h^2 + hk + k^2)/a^2 + \lambda^2 l^2/4c^2$$

X-ray diffraction is a tool for the investigation of the fine structure of matter. This technique had its beginnings in von Laue's discovery in 1912, and contributed to the development of crystallography, together with the X-ray discovery by Röntogen and Bragg's law.

Apatites and related compounds are of fundamental importance in several areas, including the structural chemistry of hard tissue. Hydroxyapatite is the first compound to be examined in a program which calls, in part, for the detailed refinement of several of these materials[9]. The space group and unit cell dimensions are, respectively, $P6_3/m$ and $a=b= 9.432$, $c= 6.881$.

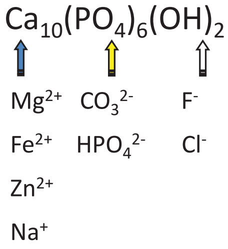
With constantly improving knowledge of structural detail now being provided by precision techniques in X-ray and neutron diffraction, the study of the structure-property relationships at the atomic level of organization seems to be especially promising. Atomic-scale mechanisms and the effects of substitution are particularly important in biological apatites which, apparently, always show considerable substitution and other crystalline defects[51].

Apatites are generally formulated as $A_{10}(BO_4)_6C_2$[5]. Hydroxyapatite is a kind of calcium phosphate and is the main inorganic component of hard tissues such as bone and teeth. Hydroxyapatite has a chemical formula of OH-containing apatites, and is an ionic compound in the hexagonal crystal group, theoretically expressed as $Ca_{10}(PO_4)_6(OH)_2$ (Fig. 15)[9].

## Display method of computer graphics

Information on the conformation of hydroxyapatite in relation to its crystallinity, solubility and/or mechanical strength is useful for understanding the mechanism and prevention of dental caries, the interaction between biomaterials and biological hard tissues. A ball-and-stick model of hydroxyapatite has been made using plastic spheres with a great amount of time and effort. Recently, computer graphics have developed remarkably. While a large computer was needed before, now this can be achieved easily with a personal computer and screen. Computer graphics allow one to view a model from any direction, and to change its elements and their sizes and position instantly. Therefore, hydroxyapatite substituted with trace element can be viewed at once (Fig. 44). Crystal models of fluoridated hydroxyapatite and fluorapatite, in which $F^-$ ions are substituted into some or all $OH^-$ positions, were also drawn. Data on the structural coordinates of the hydroxyapatite were put into a protein graphics program in Angstrom units[10].

Stoichiometric hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) is an ionic crystal with $Ca^{2+}$, $PO_4^{3-}$, and $OH^-$ as the main components, and the crystal system is hexagonal ($a=b=9.432$ Å, $c= 6.881$ Å, $\alpha=\beta=90^o$, $\gamma=120^o$) [9, 11]. It has been reported that almost all elements in the periodic table can adopt the positions of the main components above-mentioned[5]. Therefore, “apatites” means ‘thieves’ in the Greek language because they are very complicated substances. Thus, real apatites contain many trace elements.



$Ca_{10}(PO_4)_6(OH)_2$

$Mg^{2+}$ $CO_3^{2-}$ $F^-$

$Fe^{2+}$ $HPO_4^{2-}$ $Cl^-$

$Zn^{2+}$

$Na^+$

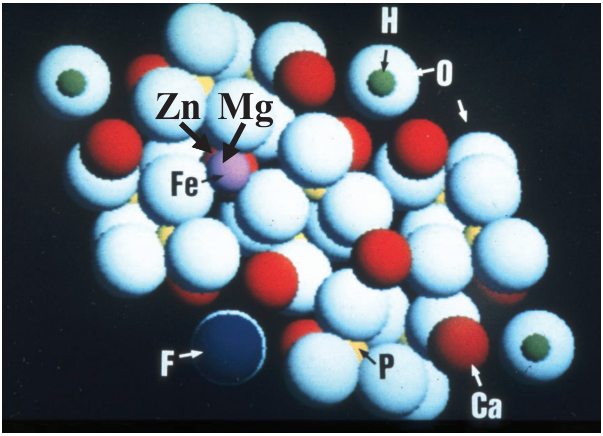


Fig. 44 Computer graphics model of hydroxyapatite with trace elements.

The analysis of hydroxyapatite was delayed until 1964[9], although the crystal structure of fluorapatite (FAp: $Ca_{10}(PO_4)_6F_2$) was initially analyzed in 1930 by Náray-Szabó[12]. Because $OH^-$ ions in hydroxyapatite shift just 0.3 Å from the stable position, it was difficult to determine the accurate crystal structure solely by X-ray diffraction analysis. Finally, it was determined by combining with neutron diffraction analysis[130]. Hydroxyapatite is sudo-hexagonal (rhombohedral), while FAp is a typical hexagonal crystal. Due to the development of computer science, Okazaki and Sato[10] could successfully construct a crystal model of HAp with a personal computer and the program[131] “PROTGRA”. In their paper, the computer graphics (CG) of HAp and FAP were shown accurately according to the radii of $Ca^{2+}$ (0.99 Å), $P^{5+}$ (0.33 Å ), $O^{2-}$ (1.40 Å ), $H^+$ (negligible), and $F^-$ (1.36 Å ) based on the data of Pauling[13]. $OH^-$ ions in hydroxyapatite shift just 0.3 Å from the stable position, while $F^-$ ions in fluorapatite are present at levels of just 1/4 and 3/4 of the height of the crystal unit cell. Lattice dimensions of a = 9.37 Å and c = 6.88 Å were adopted from the data reported by Náray-Szabó[12]. Compared with the structure of hydroxyapatite, that of fluorapatite can readily be seen to be crystallographically more stable. The computer graphics display demonstrated that fluoride ions serve to stabilize the hydroxyapatite crystals and prevent dental caries.

The PC software “PyMOL”, which can draw polymer CG on Windows, is recently often used worldwide. We considered the application of “PyMOL” to hydroxyapatite. At present, “PyMOL” is licensedby Schrödinger. We have already succeeded in the CG drawing of tetrafluoroethylene (PTFE), as aftermentioned in PART II-10. Using this software, we can freely view the stereo structure of any molecules. However, this software requires the three- dimensional coordinates of each molecule. Fortunately, we could use the data, which were obtained in our previous study[10].

The atomic radius of each element is set as the Von der Waals radius by default, and a ball and stick (BS) model is initially drawn. Therefore, each radius was changed into an ionic radius based on Pauling data[13], and then modified slightly to be able to visualize it clearly as follows:

$Ca^{2+}$ (0.99 Å), $P^{5+}$ (0.33 Å → 0.70 Å), $O^{2-}$ (1.40 Å → 1.20 Å), $H^+$ (negligible → 0.40 Å), $F^-$ (1.36 Å → 1.20 Å), and $Mg^{2+}$ (0.65 Å)

At first, we displayed both the original BS model of the hydroxyapatite with the Van der Waals radius and an ionic model. We can view the structure freely from any direction simply by operating a PC mouse.

We could display, as an example, the CG of fluoridated hydroxyapatite with the partial substitution of $OH^-$ by $F^-$, and Mg-containing hydroxyapatite with that of $Ca^{2+}$ by $Mg^{2+}$. Interestingly, some of the crystallographic properties of partially substituted fluoridated hydroxyapatites still remain unknown, although many studies have been conducted[8, 30, 68, 73].

A number of crystallographic studies on $CO_3$Ap have been reported[7, 20, 23, 27, 47]. Enamel apatite contains 1~3 wt% of $CO_3^{2-}$ ions, and bone and dentin contain more $CO_3^{2-}$ ions[7]. It is known that $CO_3^{2-}$ ions of biological apatites, which are created in aqueous solution, adopt mainly $PO_4^{3-}$ positions, contrary to $CO_3$Ap synthesized under dry conditions at a high temperature, in which $CO_3^{2-}$ ions adopt $OH^-$ positions. Interestingly, since the molecular weight of hydroxyapatite is 1,000 and that of $CO_3^{2-}$ ions is 60 (6 wt%), in the case of bone apatite, approximately one $CO_3^{2-}$ ion can replace six $PO_4^{3-}$ ions in a unit cell of hydroxyapatite.

We can also draw the CG of $CO_3$Ap. However, the accurate direction of $CO_3^{2-}$ is still unknown, because the content of $CO_3^{2-}$ ions in $CO_3$Ap is small, and the crystallinity of $CO_3$Ap decreases with an increase in the $CO_3^{2-}$ ion content. At present, we consider the possibility of $CO_3^{2-}$ existing parallel to the c-axis being strong from the viewpoint of the shortening of the a-axis observed on X-ray diffraction analysis.

# II-10. HEXAGONAL CRYSTAL STRUCTURES OF FAP AND PTFE

Fluorapatite (FAp) and polytetrafluoroethylene (PTFE) as biomedical materials, both of which contain fluorine, casually belong to the hexagonal crystal system. Especially, the hexagonal close-packed (HCP) structure is common to many metals. The HCP structure is so-called because it is one of the two ways in which spheres can be packed together in space with the greatest possible density and still have a periodic arrangement. This is generally true of all crystals[11], and probably of FAp and PTFE. However, it is interesting that FAp is an inorganic ionic crystal, while PTFE is an organic covalent-bond crystal. Generally, fluorine contributes to the physicochemical stability and in some cases to the biocompatibility, due to having the strongest electronegativity, i.e., chemical bonding, when compared with other atoms.

Since the discovery of X-rays in 1895 by Röntgen and establishment of Bragg's law in 1912 by the father and son, a number of studies on the crystallography of inorganic and organic compounds have been continued[11, 133, 134]. X-ray diffraction is a tool for the investigation of the fine structure of matter. This technique had its beginnings in von Laue's discovery in 1912 that crystals diffract X-rays, with the manner of diffraction revealing the structure of the crystals[11]. In the 1950s, crystallographic analyses of materials, especially polymers, were very popular using X-ray diffraction combined with neutron diffraction and electron diffraction methods. We researchers often have similar experiences at the same time, and probably the most popular subjects are focused on by many researchers worldwide. In particular, the crystal structure of DNA was successfully analyzed in 1953 by Watson and Crick[135, 136]. This double helical structure gave many researchers a special impression. One year later, polytetrafluoroethylene (PTFE) crystal analysis was reported in 1954 by Bunn and Howells[137]. PTFE delicately changes its phase near room temperature, as described later, although at a glance it appears to have a simple form compared with DNA.

## Fluorapatite (FAp)

A number of fluoride studies in the medical field have been reported. Most of them have involved caries prevention or dental fluorosis (mottled teeth) in dentistry and osteofluorosis in medicine. Since human teeth and bone are composed of inorganic hydroxyapatite and organic collagen, active investigations concerning hydroxyapatite crystals have been continued[4, 6, 7]. Especially, crystallographic research on fluoridated hydroxyapatites has been continued since the 1970s[8, 30, 68, 73].

As mentioned in the previous section, the crystal structure of fluorapatite (FAp: $Ca_{10}(PO_4)_6F_2$) was initially analyzed in 1930 by Náray-Szabó[12], although the analysis of hydroxyapatite (HAp: $Ca_{10}(PO_4)_6(OH)_2$) was markedly delayed until 1964[9]. Hydroxyapatite is sudo-hexagonal (rhombohedral), while FAp is a typical hexagonal crystal. TEM photos of synthetic hydroxyapatite and fluorapatite also show a slender and typical hexagonal sectional morphology (Fig. 37)[73]. $F^-$ ions are present at levels of just 1/4 and 3/4 of the height of the crystal unit cell. Lattice dimensions of a = 0.937 nm and c = 0.688 nm were adopted from the data reported by

Náray-Szabó[12]. Compared with the structure of hydroxyapatite, that of fluorapatite can readily be seen to be crystallographically more stable.

## Polytetrafluoroethylene (PTFE)

Recently, fluoride compounds, mostly PTFE, are being applied as biomedical materials. PTFE is hydrophobic, biologically inert, non-biodegradable, and also has low friction characteristics and excellent slipperiness. The chemical inertness of PTFE is related to the strength of the fluorine-carbon bond. Therefore, PTFE has wide biomedical uses such as in artificial blood tubes, artificial lung membranes, catheters, sutures, and uses in reconstructive and cosmetic facial surgery as well as guided tissue regeneration (GTR) scaffolds[19, 138].

The crystal conformation of polytetrafluoroethylene (PTFE) has been extensively reported in the literature for many years since Bunn and Howells[137] first reported it in 1954. The PTFE temperature-pressure phase diagram accepted for over 60 years is very complicated and insufficient. The crystal structure of polytetrafluoroethylene (PTFE: $-(CF_2)_n-$ ) is unusual in having a number of crystal forms and also showing marked molecular motions within the crystal well below the melting point[139].

At first, Bunn and Howells[137] indicated that PTFE appears to be a chain polymer of very high molecular weight materials, as produced in the polymerization reaction, and it is highly crystalline below about 293 ºK (20 ºC). The melting point – the first-order transition from a partly crystalline to a completely amorphous structure – is about 603 ºK (330 ºC), much higher than that of the corresponding hydrocarbon polymer, such as 405 ºK (132 ºC) of polyethylene. They also indicated that the room-temperature transition was a composite, although most of the change in density occurs at 293 ºK (20 ºC). Later, Cutler et al.[140] stated that there was widespread agreement that a transition occurs at 292 ºK (19 ºC) between a structure for phase $Ph_2$ with a 15/7 helix and for phase $Ph_1$ with a 13/6 helix. The crystal structure of phase $Ph_2$ is hexagonal, with a= 0.566 nm and c= 1.95 nm, but the phase is considered to be imperfectly developed, and the cell contains only one chain. The morphology of the low temperature phase $Ph_1$ is uncertain, since it has been considered to be both triclinic and monoclinic[141]. Bunn and Howells[137] favored a pseudo-hexagonal (probably triclinic) structure with a = 0.55 nm and c = 1.68 nm when they studied fibers. They concluded in this case that $\gamma = 119.5^o$. Clark[142] came to the same conclusion, with a = 0.56 nm, c = 1.69 nm, and $\gamma = 119.3^o$.

Clark et al. reported that the first-order transition at 292 ºK (19 ºC) between form (phase) II and IV[139, 143] was an untwisting in the helical conformation of the molecule from a 13/6 conformation to a 15/7 conformation. Furthermore, Brown et al.[144] showed that the PTFE temperature-pressure phase diagram that had been accepted for many years was insufficient, requiring the addition of deviatoric stress dependence. They showed the hexagonal crystal model for type IV PTFE. They reported that the room temperature crystalline structure of PTFE phase IV (15/7 helical PTFE chain, hexagonal lattice) only exists over a narrow range of temperatures at atmospheric pressure.

As above mentioned, we could also obtained the CG molecular model of the twisted zig-zag chain of PTFE using the computer software PyMOL(Fig. 45)[132], together with an illustration of the hexagonal crystal structure model of the PTFE carbon backbone modified the data of Brown et al. [144]

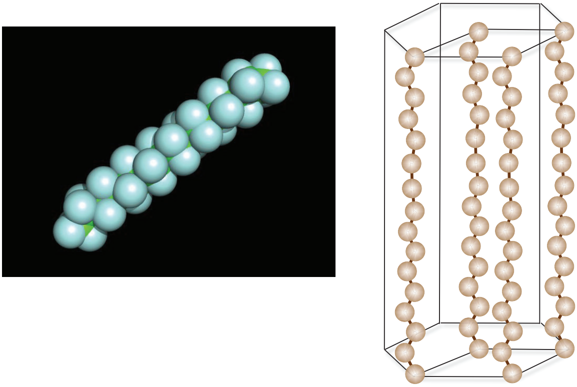


Fig. 45 Computer graphics model and illustration of PTFE crystal.

The ions or molecules of simple inorganic and organic compounds, when considered from the melt or solution, tend to arrange themselves in a regular manner in three dimensions, forming crystals. As Brown et al.[144] indicated that the phase diagram of PTFE is affected by the stress-strain. Sterilization by Co-60 γ-ray irradiation has recently become popular in the medical field[145, 146]. In this case, it has been reported that the mechanical strength of PTFE is significantly impaired[147]. Therefore, further PTFE research approaches based on crystallography are now expected.

Both FAp and PTFE, containing fluorine as a biomaterial casually maintain a hexagonal space lattice near room temperature. Their imperfect and/or heterogeneous crystallographic properties are still unknown, for example, in the case of partial fluorine substitution.

# II-11. A HYPOTHESIS FOR TOOTH ENAMEL FORMATION MYSTERY

## Tooth and magnesium

Tooth enamel is well crystallized, contrary to poorly crystallized dentin and bone. Magnesium is richer in dentin than in enamel, and especially significantly affects the physicochemical properties of teeth. In general, magnesium decreases the crystallinity of hydroxyapatite and promotes solubility. Magnesium also has important roles in the relationship to cells. Divalent ions such as magnesium promote cell adhesion and contribute to the metabolism of hard tissues. This may affect the formation of teeth and be related to regulation during maturation.

Teeth are hard tissues vital for human life because we maintain our health by chewing food. This biting action is also very important to maintain brain activity through the impulse of the biting force. These teeth are sometimes destroyed by caries or periodontal diseases. Teeth are structured with enamel, dentin and cementum supported by alveolar bone and periodontal membrane (ligament). Enamel is composed of almost 95wt% well-crystallized hydroxyapatite containing a few % of non-collagenous proteins and water[4]. Dentin is composed of approximately 60-70 wt% of poorly crystallized hydroxyapatite and 30-40 wt% of collagen.

Although the calcium and phosphate molar ratio approximately reflects their content in hydroxyapatite for enamel, it shows a higher value for dentin because several % of $CO_3^{2-}$ ions are substituted for $PO_4^{3-}$ positions in the apatite crystals of dentin[27]. The variety of these chemical compositions in hydroxyapatite crystals affects the physicochemical properties, especially the crystallographic and mechanical properties of teeth.

Furthermore, biological apatites contain many of trace elements such as magnesium[148], as described in PART II-3. The existence of magnesium in apatites is very limited unless carbonate or fluoride ions are simultaneously incorporated[34, 35]. The amount of magnesium incorporated in apatite is proportional to the magnesium concentration in the solution. The magnesium uptake is increased by the simultaneous incorporation of fluoride or carbonate. In particular, the incorporation of magnesium was accelerated with the increase of fluoride[149]. Furthermore, the magnesium content of the apatites increased slightly with the degree of fluoridation despite the constant magnesium concentration in the feed solution. A small amount of magnesium significantly affected the crystallinity of synthetic fluoridated apatites. The patterns of crystallinity with the increase of fluoride content were unique in each series with different magnesium contents[149]. The limited incorporation of magnesium is also observed in apatites prepared at temperatures above 900 °C by solid-state reaction[7].

The presence of Mg in apatites decreases the a-axis dimension, decreases crystallinity, increases $HPO_4^{2-}$ incorporation, and increases the extent of dissolution. Mg inhibits remineralization. In vitro, Mg suppresses the crystallization of apatite, stabilizes dicalcium phosphate dihydrate (DCPD: $CaHPO_4 \cdot 2H_2O$) and promotes its formation even in neutral pH, stabilizes amorphous calcium phosphate (ACP), and increases the solubility of synthetic apatites.

Some trace elements such as magnesium, sodium, zinc, fluoride and chloride have characteristic distribution patterns in human matured enamel and dentin[150]. For example, at the narrow surface layer of enamel, magnesium and sodium show a very steep decrease in concentration, whereas zinc, chloride and fluoride show a peak concentration much higher than in the rest of enamel. It seems likely that these specific patterns of distribution are related to the progressive mineralization pattern observed during the maturation stage, which is characteristic of the layers. Suga et al.[150] indicated by electron microprobe analysis that in the matured enamel of cow, dog, rat and guinea pig, magnesium, sodium and chloride have almost the same distribution patterns as in human matured enamel.

Magnesium is distributed in dentin at a much higher level than in enamel[90, 148]. Although the Mg distribution difference between dentin and enamel has not been clarified, it can be speculated that this phenomena may be related to the association of Mg with organic compounds such as collagen in dentin. Magnesium may also be related to caries susceptibility of teeth the same as carbonate[89]. Both magnesium and carbonate decrease crystallinity and increased the solubility of apatite crystals.

Although enamel develops from epithelial cells, dentin and bone develop from mesenchymal cells by epithelial-mesenchymal interaction[93]. The chemical composition and crystallinity of dentin and bone are relatively similar. Therefore, osteoblast behavior may be partially reflected in odontoblasts, which form dentin. Recently, adhesion molecules such as those of the integrin family were examined concerning cell structure and function. Divalent ions affect cell adhesion in relation to the integrin molecule as an adhesion molecule at the cell surface[16], and it has been especially, reported that $Mg^{2+}$ ions promote cell adhesion[92]. Integrins are crucially important receptor proteins because they are the main way in which cells both bind to and respond to the extracellular matrix, as mentioned before (PART II-7).

The latest researches have gradually clarified the unknown phenomena inside and outside cells with magnesium. $Mg^{2+}$ ions regulate numerous cellular functions, serving as a cofactor in many different enzymatic pathways. The cytoplasmic $Mg^{2+}$ ion, $[Mg^{2+}]_c$ is regulated by both passive (influx driven by the electrochemical gradient of this ion) and active (active efflux) transport[151]. It has been clarified that $[Mg^{2+}]_c$ is tightly regulated by $Mg^{2+}$ efflux depending on extracellular $[Na^+]$[152]. Therefore, we speculate that during the formation and maturation of teeth, $[Mg^{2+}]_c$ in ameloblasts and odontoblasts may have significant roles. In conclusion, it can be speculated that magnesium is strongly related to the maturation of teeth and their physicochemical properties.

## Tooth generation of fish as a reference of human tooth formation

Enamel formation is one of the most interesting and strange subject in dental field. Because human tooth enamel is well-crystallized[7] and has never been synthesized in vitro at the physiological temperature. Furthermore, human teeth do not molt again after permanent teeth have matured. There might be related to the existence of a control mechanism on tooth development. It is becoming clear that the driving force for precipitation of enamel crystals in early developing stages is regulated by cellular control of the transport of lattice ions, particularly calcium[153-156].

Recent development of tissue engineering is clarifying the mechanism of tooth formation and approaching to the regeneration of tooth. Tooth development is regulated by sequential and reciprocal interactions between oral epithelium and mesenchyme[157, 158]. Reciprocal signaling between the epithelial and mesenchymal tissues continues throughout tooth development, resulting in the formation of a tooth of specific size and shape, depending on its position within the jaw[159]. Young et al. presented histological, immunohistochemical, and molecular evidence demonstrating the successful bioengineering of complex tooth crowns closely resembling those of naturally developing teeth[160].

On the other hand, fish teeth can molt many times in a life. Fish, amphibian and reptile prepare tooth germs for several generations in addition to functional teeth. This is so-called polyphyodont. Mammal teeth are monophyodont or diphyodont. The enameloid of fish teeth has been thought to be analogous to the enamel of teeth of tetrapods and is similarly very highly mineralized, although its organic matrix is composed of ectodermal protein (enamelin) and mesodermal collagen fibers, whereas the enamel of tetrapods is composed only of ectodermal proteins (amelogenin and enamelin)[161, 162].

It is very important to establish the enamel crystal formation. However, crystallographic approach to enamel formation has not been reported in detail because of difficulty as a monophyodont tooth. Therefore, it is interesting to examine the phenomena of molting teeth of fish. We selected the killifish, “medaka” (*Oryzias latipes*) as an object of our experiment because of short-term span of life.

Tooth enameloid of medaka as a representative of human tooth enamel was analyzed by HR-TEM, EPMA and micro-X-ray diffraction analyses to deepen the knowledge for the crystal formation of tooth enamel[3]. HR-TEM observation showed that enameloid crystal with incomplete slender hexagonal shape became sometimes fused each other when density of the crystals increased. EPMA analysis showed clear image maps of calcium, phosphate and iron. The element analysis indicated high concentration of calcium and phosphate. Micro-X-ray diffraction found that enameloid crystals are composed with relatively well-crystallized hydroxyapatite. Each (211), (112), (210) and (002) reflection was clearly detected. It is suggested that enameloid medaka is useful for research of developing teeth because of polyphyodont and short-term span of life.

In general, small materials such as medaka teeth are difficult for analysis using conventional X-ray diffraction equipment. In this study, we could successfully identify the crystallographic properties of medaka using micro-X-ray diffraction with the position sensitive proportional counter. Relatively clear separate peaks could be observed. This result indicates that medaka enameloid crystals are well-crystallized. If they were poorly-crystallized as dentin or bone, each peak would not clearly separate. The hydroxyapatites synthesized at 80 ºC and 40 ºC, which are control samples as models of enamel and bone, showed quite different X-ray diffraction patterns each other. The X-ray diffraction pattern of medaka enameloid was similar to that of simulated enamel-like hydroxyapatite.

We could observe enamel germ and developing tooth at the same time in Fig. 8. This observation clearly indicated that medaka teeth are polyphyodont[3]. EPMA element analysis for developing teeth showed high concentration of calcium and phosphate, and X-ray diffraction with PSPC revealed that medaka enameloid is composed with well-crystallized hydroxyapatite.

Fish enamel, called as enameloid or mesenchymal enamel, is formed in relation to the action of epithelium cell (mainly ameloblast) and mesenchymal cell (mainly odontoblast). That medaka tooth is composed with hydroxyapatite means that human enamel and medaka enameloid are made by the same mechanism in the living system, although human enamel molt just onetime through the life, so-called monophyodont in contrast to many times molten medaka teeth, so-called polyphyodont. It can be speculated that the gene difference between human enamel and medaka enameloid is not so great.

Why does medaka enameloid accumulate iron as a trace element? Water in the river, pond and supplied water contain a lot of minerals such as iron. Medakas drink their water. Medaka has bone, which produce red cells containing hemoglobin as an iron compound. Iron is stored in enameloid as a trace element of hydroxyapatite crystal or other iron compound. Since medaka enameloids easily molt and probably some of them are eaten to reuse, iron in enameloid appears to be an important source for red cell formation. Iron in hydroxyaptite composing enameloid can be dissolved easily by the strong acid in stomach of medaka. Although we could not detect fluoride ion in enameloid, it has been clarified by Suga[162] that under the coexistence with fluoride, the deposition of iron is accelerated.

It is interesting to note that, in some fishes, only the enameloid has a special ability to concentrate high fluoride and iron, whereas the immediately adjacent dentin does not have such ability. Suga et al.[162]

speculated that the high concentration of these elements conserved in the enameloid is due to some peculiar cytological functions of the ectodermal enameloid forming cells or due to peculiar chemical properties of the organic matrix, rather than the mechanisms controlling heavy mineralization.

## Tooth enamel formation

Enamel is composed with more than 95 wt% of hydroxyapatite and other non-collageneous protein and water. On the other hand, dentin and bone are composed with 70-60 wt% of hydroxyapatite and 30-40 wt% of type I collagen. Enamel hydroxyapatite is well-crystallized, while dentin and bone hydroxyapaties are poorly-crystallized. We could not have synthesized enamel-like crystals *in vitro* at 37ºC, biological temperature, although we can synthesize well-crystallized enamel-like crystal at 80ºC *in vitro* [30, 47].

It is thought to be two possibilities concerning enamel formation. First possibility is physicochemical phenomena in biological system, very mild longer-term enamel formation with regulated slow diffusion of ions occurs. Second possibility is the existence of a regulation system in enamel germ, which can synthesize well-crystallized hydroxyapatite, using a number of energy. In our daily life, the question occurs why the tooth enamel is extremely hard and strong, compared with dentin and bone. Relatively fast enamel formation occurs in killifish, medaka with polyphyodont enameloid[157, 162, 163]. This means that physicochemical hypothesis may be not suitable. Therefore, we propose a hypothesis that much energy is inevitable for enamel formation, which is regulated by biological system. ATP appears to have a very important key role.

## ATP synthesis in mitochondria

The generation of ATP by oxidative phosphorylation via the respiratory chain depends on a chemiosmotic process. A chemiosmotic process converts oxidation energy into ATP on the inner mitochondrial membrane[15].

Through the production of ATP, energy originally derived from the combustion of carbohydrates and fats is redistributed as a conveniently packaged form of chemical energy. Roughly $10^9$ molecules of ATP are in solution throughout the intracellular space in a typical cell, where their energetically favorable hydrolysis back to ADP and phosphate in coupled reactions provides the driving energy for a very large number of different coupled reactions that would otherwise not occur.

Mitochondria, which are present in virtually all eukaryotic cell, are membrane-bounded organelles that convert energy to forms that can be used to drive cellular reactions. Therefore, most energy necessary to enamel formation would come from ATP hydrolysis.

## Energy calculation

Although biological apatites are composed with carbonate-hydroxyapatite ($CO_3$apatites) and trace elements are partially substituted[4, 7], we here ignore the existence of carbonate ions and trace elements to simplify the estimation of free energy.

In general, free energy of hydroxyapatite can be written as

$\Delta G_f^o = -RT^o \ln K^o$ ------ (1)

using chemical equilibrium constant $K^o$ at 25ºC[163].

In the same way, for the hydroxyapatite synthesized at 80ºC,

$\Delta G_f^{80ºC} = -RT \ln K$ ------ (2)

We can calculate ln Ksp at 80ºC by outer estimation from the solubility isotherms equation of hydroxyapatite proposed by Patel and Brown[115, 116] under the assumption of monotonical decrease of Ksp above 37ºC, although the outer estimation appears to be risky. Then, ln Ksp at 80ºC can be estimated as -59.62, while ln Ksp at 25ºC is -58.52. Therefore, since the difference of both values are approximately 2 %, we can consider $\ln Ksp^{80ºC} \approx \ln Ksp^{o}$. Thus, It is known that the solubility product of hydroxyapatite does not change greatly at the certain temperature range.

The concept between solubility K and solubility product Ksp is quite same. Both are the ionic products.

Therefore, when assumed $\ln K \approx \ln K^{o}$ in equation (1) and (2),

$$\Delta G_f^{80ºC} = (G^{o} / T^{o})\ T \qquad \text{------ (3)}$$

Then, $\Delta G_f^{80ºC}$ can be calculated as -1770 kcal/mol using standard free energy of hydroxyapatite $G_f^{o}$ = -1494 kcal/mol[115]. Next, the difference of free energy between enamel-like hydroxyapatite formation and dentin bone-like hydroxyapatie formation can estimate as $\Delta G_f^{80ºC} - \Delta G_f^{37ºC} = (-1770 - (-1554)) = -216$ kcal/mol. This means that much energy supply from ATP is necessary for enamel-like hydroxyapatite formation at the biological temperature, 37ºC. The energy is equivalent to 29.6 mol of ATP for 1 mol of hydroxyapatite because the standard free-energy change $\Delta G_f^{o}$ for hydrolysis is – 7.3 kcal/mol[16].

## Existence of enamel gene

It is speculated that enamel gene appears to contribute to regulate the energy balance and morphology during enamel maturation. Amelogenesis can be divided into three major developmental phases: (1) synthesis of enamel proteins by ameloblast cells; (2) secretion of enamel proteins to the extracellular matrix; and (3) mineralization of the enamel extracellular matrix[163].

A terminology has been suggested, dividing enamel proteins into two major classes: enamelins (acidic glycoproteins with relative large molecular weight) and amelogenins (proline-rich, glycoproteins with relative lower molecular weight). Mouse, hamster, cow and human developing enamel matrices are highly enriched in amelogenins (65-70%) as compared to enamelins (10-15%). However, the proportion of amelogenins versus enamelins changes significantly during enamel maturation; the amelogenins slowly disappearing whereas the enamelins appear to be retained[161, 162].

## Regulation of enamel morphology

Tooth development is regulated by sequential and reciprocal interactions between oral epithelium and mesenchyme. At the late bud stage, a transient signaling center, the enamel knot, forms at the tip of the tooth bud and is fully developed during the cap stage, regulating the shape of the tooth crown[157, 158]. The enamel knots express more than 10 different signaling molecules, including sonic hedgehog (Shh), and several members of the fibroblast growth factor (FGF), bone morphogenetic protein (BMP), and wingless-type (Wnt) families. In these processes, ATP would be used to regulate the enamel morphology. During the enamel maturation of killifish, we observed ATPase activity, which suggests a proof of ATP accumulation near the developing enameloid[3]. This means a lot of energy was exhausted for apatite formation.

## Tooth regeneration

Therefore, we can consider the following possibility as tooth regeneration factors.

(1) Culture of enamel germ (ES cells, iPS cells, Reweaken gene, GDF (growth & differentiation factor).

(2) Chemical (artificial) regeneration, that is an elucidation of enamel formation mystery.

Recent research focus for enamel formation are (1) investigation of enamel apatite crystal growth, (2) investigation of concentration mechanism of cell membrane, (3) investigation of morphological enamel formation (Fig. 46). Especially, we need more knowledge on the embrology in addition to biomaterials and tissue engineering (Fig. 47). The design of morphology of hard tissues such as bone and teeth is a very interesting subject for the future, with biological materials expected to be man-made, not biological.

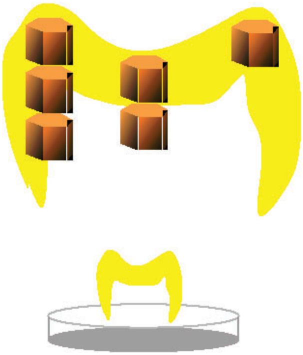


Fig. 46 Investigation schema of enamel crown formation.

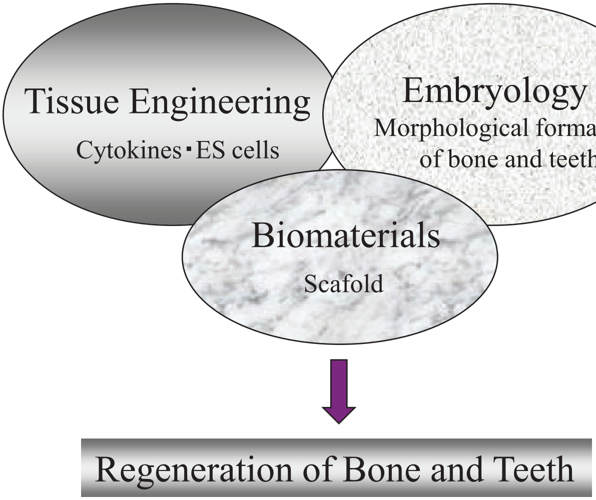


Fig. 47 Future Trends of Biomaterials.

# AFTERWORD

Seven years after the publication of the Japanese edition[2], and just when I was invited to publish an English translated edition, the new coronavirus began to run riot and shake the whole world. This coronavirus is very small (several tens to 100 nm) and has no proliferative ability but increases explosively by parasitizing living bodies. It may in time undergo a surprising evolution through mutations while playing a cat-and-mouse game with the development of drugs. In this respect, since artificial biomaterials show no antigen-antibody reaction, there is basically no concern about infection as long as the material is clean. Therefore, biomaterials may be regarded as safe materials. Life has evolved over a period of 3.5 billion years. I hope that life science someday makes viruses live on friendly terms with us humans without posing any threat.

# ACKNOWLEDGEMENT

This book is a translation with modification of the Japanese edition published by Gakken-shoin (PART I) [2], together with newly summarized knowledge of apatites mainly in our research results (PART II). I would like to express my sincere gratitude to the courtesies extended by Gakken-shoin. I also thank Ms. Helen Edwards, Cambridge Scholar Publishing and Ms. Mayumi Osaki, Gakken Shoin for their great help. Furthermore, I deeply appreciate great efforts and contribution of my many coauthors and colleagues.

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## Author Profile

**Masayuki OKAZAKI, PhD**

Graduation: Graduate School of Engineering, Kyoto University, Japan (1976).
Teaching: Osaka University and Hiroshima University, Japan (1976-2011).
Professor Emeritus, Hiroshima University in 2011.

AWARDS:
Scientific Award of The Japanese Society for Biomaterials (2007).
Scientific Award of The Japanese Society for Dental Materials and Devices (2010).

SPECIALITIES:
Apatite Chemistry
Biomaterials for Hard Tissue Regeneration