Chapter 6 Implants and biomaterials (hydroxyapatite)

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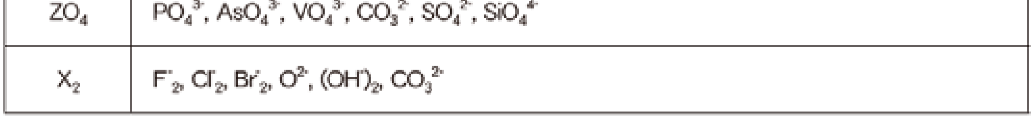
I. Hydroxyapatite[1) 2)]

The mineral apatite, can be represented by the formula, $M_{10}(ZO_4)_6X_2$ (or $M_5(ZO_4)_3X$), as a general term that can be applied to the crystal structures that will be mentioned later on in this chapter. The name "apatite" comes from the Greek spelling "apate" (απαταω) which means deceit. The reason for this was that the mineral appeared in different colors with a variety of crystal habits, and was thus often mistaken for precious minerals such as aquamarine or amethyst. This variation arises from the fact that the apatite structure has a particularly complex solid system. Each component (M, ZO4, and X) of the common equation $M_{10}(ZO_4)_6X_2$ can be replaced by a large number of different elements or solid states as listed in Table 3-6-1. M or X can also be absent. The most common form found in nature is calcium phosphate apatite, whereby the M and ZO_4 are Ca^{2+} and PO_4^{3-} groups. If the X is F, it is given the name fluorapatite, the main source of phosphorus, used as the standard mineral for hardness level 5 on the Mohs scale, making it an invaluable source for rock-forming minerals. When the X is OH^-, i.e. $Ca_{10}(PO_4)_6(OH)_2$, it is given the name hydroxyapatite, HA, which is the main subject of this report (Table 3-6-1 artificially synthesized hydroxyapatite crystal). HA is the key inorganic component of the hard tissues of vertebrae, and is an important substance in bioactive ceramic materials.

HA is one of many types of calcium phosphate (or calcium orthophosphate), but there are several others in this class. The main compounds of calcium phosphate are listed in Table 3-6-2. The simplest way to arrange these into order is by the calcium-phosphorus composition ratio. At physiological pH (7.2-7.6), HA is most stable. MCPM, DCPA, DCPD and TeCP are used as the ingredients of calcium phosphate cement. OCP is an intermediate that appears during extraction of the HA phase. Within TCP, α-TCP shows a stable phase between 1180 and 1430°C and transforms into its α'-phase at higher temperatures. β-TCP, a hypothermic phase material, is often used in the same way as HA, in bioactive ceramics. Its absorptivity when implanted into the body is significantly higher than that of HA. A material composed of a mixture of HA and β-TCP has also been developed, called biphasic calcium phosphate (BPC). A metastable amorphous phase material known as amorphous calcium phosphate (ACP) exhibits a significantly higher bioresorbable property than other crystalline calcium phosphate compounds.



M	Ca^{2+}, Mg^{2+}, Sr^{2+}, Ba^{2+}, Mn^{2+}, Fe^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Na^+, K^+, Al^{3+}
ZO_4	PO_4^{3-}, AsO_4^{3-}, VO_4^{3-}, CO_3^{2-}, SO_4^{2-}, SiO_4^{4-}
X_2	F^-_2, Cl^-_2, Br^-_2, O^{2-}, $(OH^-)_2$, CO_3^{2-}

Table 3-6-1 The main ions that can be consisted in the formula $M_{10}(ZO_4)_6X_2$

Fig. 3-6-1

Artificially synthesized hydroxyapatite crystals

II. The crystal structures of hydroxyapatite[2), 3)]

Fig. 3-6-2 shows a schematic diagram of the structure of HA. The structure shown here is the stoichiometric composition of HA whereby the unit cell is composed of $Ca_{20}(PO_4)_{12}(OH)_4$. The core framework of the structure is constituted mainly of the PO_4^{3-} tetrahedron, with two types of channel structures that lie parallel to the c-axis at the positions, $(x, y) = (0, 1/4)$ and $(x, y) = (1/3, 1/12)$. The OH^- ion ($z = 0 \pm 0.2, 1/2 \pm 0.2$) and Ca^{2+} ion ($z = 0, 1/2$) lie within each of these structures. Eight of the 20 Ca^{2+} in the unit cell are located at the Ca^{2+} site (Ca I), with the remaining 12 Ca^{2+} located on the second site (Ca II, $z = 1/4, 3/4$), which is also the peak of the equilateral triangle that is constructed from the OH^- ion channels. This Ca^{2+} is exposed on the crystal surface, thus playing a large role in the physical properties of HA, such as surface charge, and in interactions with organic compounds.

The stoichiometric crystal structure of HA has a monoclinic system with $P2_1/b$ as its space group, and lattice constants of $a = 0.942$ nm, b □ $2a$, c = 0.688, $r = 120°$ However, if this unit cell is divided into two at the plane $y = 1/2$, the two halves become equivalent, so that this structure essentially has the symmetry of a hexagonal crystalline structure. The factor that defines the symmetry of a unit cell is the arrangement of OH^- ions. The OH^- ions are aligned on the $y = 1/4$ plane facing the same direction with regards to the c axis, but the OH^- ions on the $y = 3/4$ plane all face in the opposite direction, thus resulting in repetition in the b-axis direction that is double that of a. However, at high temperatures (>211°C), or if the structure is non-stoichiometric, with inclusion of impurities or holes, this neat arrangement becomes disordered, and the space groups become hexagonal $P6_3/m$. The structure of HA is broadly determined by the positions of Ca (as M) and PO_4 (as ZO_4) creating space group of $P6_3/m$, and is often represented by the chemical formula $M_{10}(ZO_4)_6X_2$, in accordance with $b = a$ as the unit cell.

Biological apatite is a non-stoichiometric form of HA, characterized by Ca^{2+} deficiency but containing trace elements. The trace elements include positively charged ions, Mg^{2+}, Na^+, K^+; and negatively charged ions, Cl^- and F^-. The most common replacement is with the CO_3^{2-} ion which constitutes 5-8% of apatite in bone, by weight. The CO_3^{2-} ion can replace all of the OH^- and certain PO_4^{3-} within the HA structure, termed A-type and B-type replacement, respectively. B-type replacement is common in bones, and is an essential factor in altering the melting point of the biological apatite. Increases in positive charge with the replacement of PO_4^{3-} by CO_3^{2-} are balanced out either by the loss of Ca^{2+} sites or by the introduction of Na^+ ions.

	組成	Ca/P
monocalcium phosphate anhydrous (MCPA)	$Ca(HPO_4)_2$	0.50
monocalcium phosphate monohydrate (MCPM)	$Ca(HPO_4)_2 \cdot H_2O$	0.50
dicalcium phosphate anhydrous (DCPA·monetite)	$CaHPO_4$	1.00
dicalcium phosphate dihydrate (DCPD·brushite)	$CaHPO_4 \cdot 2H_2O$	1.00
octacalcium phosphate (OCP)	$Ca_8H_2(PO_4)_6 \cdot 5H_2O$	1.33
alpha tricalcium phosphate (α -TCP)	$Ca_3(PO_4)_2$	1.50
beta tricalcium phosphate (β -TCP)	$Ca_3(PO_4)_2$	1.50
hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	1.67
tetracalcium phosphate (TeCP)	$Ca_4(PO_4)_2O$	2.00

Table 3-6-2 The major calcium phosphates

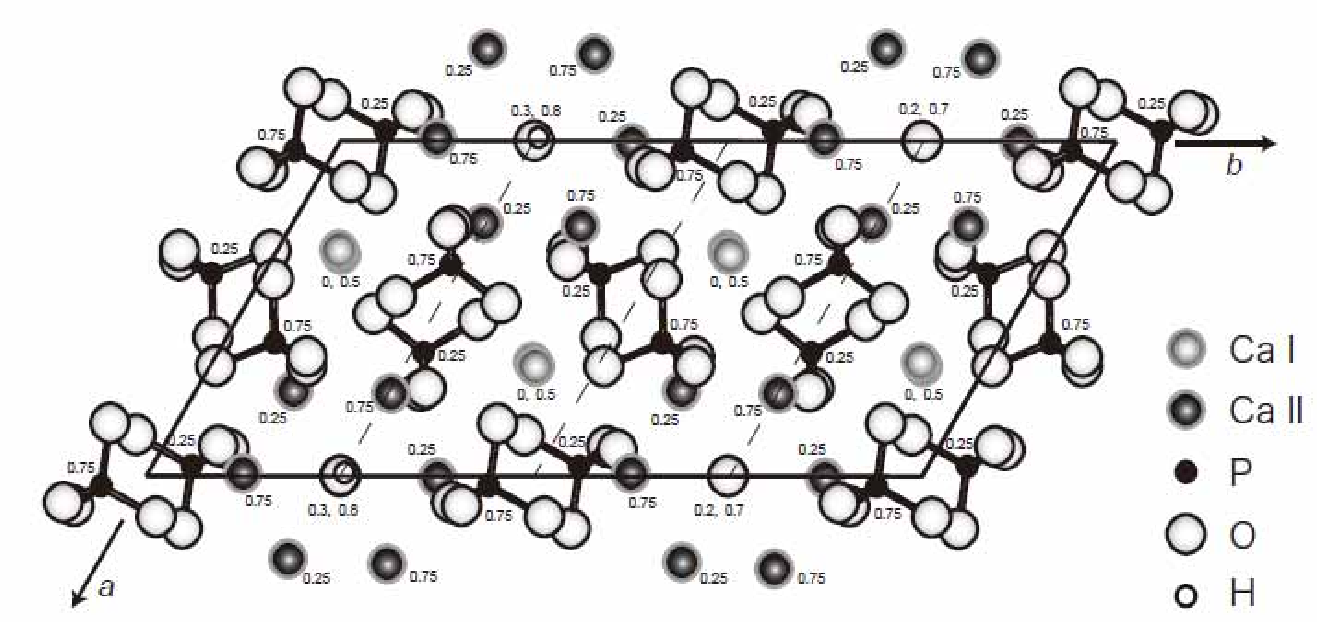


Fig. 3-6-2 The crystal structure of hydroxyapatite

The *c*-axis is perpendicular to the plane of the paper. The *x, y, z* that are mentioned in the text correspond to the direction of *a*, *b*, *c* in the diagram with defining the repetitive cycle as 1. The numbers in this diagram are the coordinates of the *z*-axis. The hydrogen atom of OH^- ions on the $y = 3/4$ plane are hidden behind the oxygen atom

III. Synthesis and applications of hydroxyapatite ceramics[4),5)]

The raw material powder used in simple HA ceramics can be synthesized using the wet method. Wet methods can largely be divided into neutralization reactions and reactions between calcium and phosphorous salts. In neutralization reactions, phosphoric acid solution is steadily added into a solution where calcium hydroxide is suspended in water. The chemical equation for this is shown below,

$$10Ca(OH)_2 + 6H_3PO_4 + H_2O \rightarrow Ca_2(PO_4)_6(OH)_2 + 18H_2O$$

Meanwhile, an example of the reaction between the calcium salt and the phosphorous salt is given as,

$$6Ca(NO_3)_2 + 6(NH_4)_3PO_4 + H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18NH_3 + 20HNO_3$$

There are other methods of synthesis, such as the dry method whereby calcium and phosphorous compounds are directly reacted and heated to complete the reaction, or by the removal of organic matter from bones extracted from fish or cows. As an example of the dry method,

$$6CaHPO_4 \cdot 2H_2O + 4CaCO_3 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 14H_2O + 4CO_2$$

The above reaction is conducted at temperatures above 1000°C. In the extraction from bone, the crushed bone undergoes enzymatic treatment to break down the proteins, and is rinsed before heat treatment at 800°C. The product of this method contains trace elements such as Mg.

Sintered bodies are generally formed by the adding organic polymer binding agents to the raw powdered materials, which are then compacted and heated under atmospheric pressure to temperatures of roughly 1200°C. Sintering aids are not usually required under normal circumstances.

High pressure techniques can be applied for the preparation of compact bodies. By using techniques such as hot-press methods where a piston is used to press from above and below uniaxially, or the hot isostatic press (HIP) method where an inert gas acts as the pressure catalyst, generation of a transparent sintered body with a relative density of 100% is possible. The mechanical properties of a sintered compact of HA and that of cortical bone are shown in Table 3-6-3. The manufacture of porous material usually requires mixing of beads that decompose on heating or foaming of a slurry that consists of raw materials dispersed throughout a liquid. The porous material created with this foaming method is unstable and is therefore commonly stabilized by cross-linking with the polymer that was initially added to the slurry. Other methods include hydrothermal treatment of skeletal structures of natural coral (calcium carbonate as the main ingredient) in a phosphoric acid-containing solution 7); there are also examples of manufacture of a porous body with unidirectional pores oriented by growth of thin ice columns within an HA slurry 8), 9).

HA ceramics are remarkable for their marked ability to absorb certain organic compounds, and therefore have been used widely as adsorption separation agents for proteins and nucleic acids, in chromatography column resins, toothpaste additives and gas sensor element components. Most significant of all is their biocompatibility when used in osteoimplants. HA ceramics not only have low biological effects, but when inserted closely to bone, promote regenerative growth and facilitate direct chemical bonding with bone, without any need for the presence of other connective tissue. HA has become referred to as a “bioactive ceramic” because of its ability to actively and physiologically stimulate the surrounding cells. Osseointegration is a term commonly used in this field to describe an interaction where there is absence of connective tissue on the interface between material (typically titanium) and bone. However, as this does not have a strict definition with regards to connectivity, it can only be used as a general term. The interaction between bioactive ceramics and bone can be described as biointegration to differentiate this interaction from osseointegration, an umbrella term.

HA ceramics have already been used as filling agents in bone defects in the form of a sintered body, a porous body, granules (agglomerates), and cement. Improvements to these materials are being made with regards to their mechanical properties and bioactivity by compounding with organic polymers. A number of promising materials are currently under development for use as regenerative medicines, (e.g. cell scaffolds), or as vehicles for sustained release of drugs.

Typical HA ceramics in current use have a coating material over titanium-based implants, as this has been discovered to be invaluable due to its mechanical strength characteristics.

The introduction of HA coatings will be explored later in this manuscript.

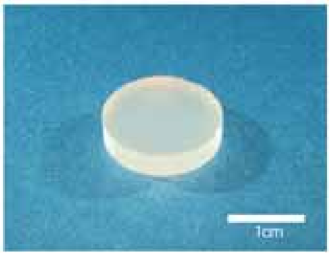
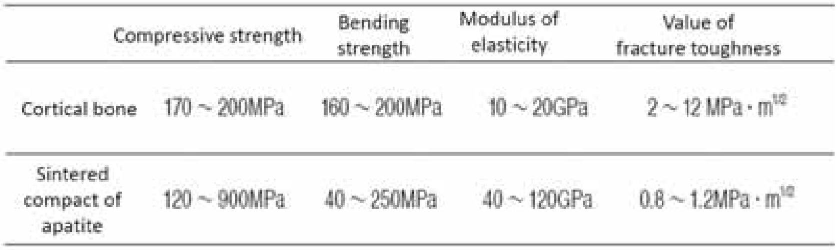


Fig. 3-6-3
Transparent sintering body of hydroxyapatite



	Compressive strength	Bending strength	Modulus of elasticity	Value of fracture toughness
Cortical bone	170 ~ 200MPa	160 ~ 200MPa	10 ~ 20GPa	2 ~ 12 MPa · $m^{1/2}$
Sintered compact of apatite	120 ~ 900MPa	40 ~ 250MPa	40 ~ 120GPa	0.8 ~ 1.2MPa · $m^{1/2}$

Table 3-6-3
The comparison of the mechanical properties of the sintered compact of hydroxyapatite and cortical bone

IV. Hydroxyapatite coating of titanium10)-12)

HA-coated implants constructed of pure titanium or coated titanium alloy are rapidly able to bond with bone that is very rigid, without the need for bone cement. For this reason, stability can be expected to be maintained for a long period of time. In this chapter, the course of development of HA coating methods and those in current practice are described, under the following headings: the wet method, the electro-chemical method, the vapor growth method and the thermal spraying method.

A. Wet methods

In neutral or alkaline solution, HA is the most stable of the calcium phosphate compounds. By immersing a titanium plate into a solution that contains calcium and phosphate, it is possible to precipitate HA onto its surface. In many research examples, pseudo-body fluids have been used as the immersion liquid; this is also referred to as the biomimetic method. These pseudo-body fluids such as Kokubo solutions and Hanks solutions that contain concentrations of inorganic ions, and pH and temperature similar to those of natural body fluids, are supersaturated with HA in its ionic concentrations. Once titanium is placed in the solution, the surface becomes covered with amorphous ACP, which gradually converts to HA, becoming increasingly crystalline. In order to boost the rate of formation of the HA membrane, several methods may be used, such as increasing the ionic concentrations of pseudo-body fluids, treating the titanium surface with acid or alkali, or by injecting ions using the “dry process”.

In acid treatment, the titanium plate acts as the anode in electrolysis in solution, and its surface becomes coated with HA 13). Alkaline treatment is also effective whereby, after immersing the titanium plate in sodium hydroxide solution, an amorphous layer of sodium titanate is formed by heat treatment. If this product is soaked in the pseudo-body fluid, the Na^+ ions are removed from the same layer, increasing the pH adjacent to the titanium surface. This effectively increases HA saturation levels, forming Ti-OH groups on the plate surface, and inducing the nucleation of HA 14), 15).

An example of ion injection is where, by driving Ca^{2+} into the titanium plate, a calcium titanate layer is formed 16), and HA layer formation occurs rapidly in the pseudo-body fluid. The coating layer formed by the pseudo-body fluid is fairly even and has the advantage that its composition is close to that of biological HA. However, problems arise such as difficulty in controlling the shape, amount and composition of the layer of HA that is deposited, and the fact that that this layer can come off easily.

In the wet method, other fluids may be used apart from pseudo-body fluid, including fluid that contains colloidal HA, used in a direct coating method that is also known as the sol-gel process. Dip-coating is the most common method whereby once immersed in the solution, the layer is fixed further by heat treatment. Calcium nitrate and dibasic ammonium phosphate 17), calcium nitrate and triethyl phosphate18), or commercially available HA powder are commonly used as sources of calcium phosphate. Typically, the calcium titanate phase or titanium oxide phase are coated on the interface of titanium and HA.

B. Electro-chemical methods

It is also classed under the wet method since the film is formed in solution. The distinction lies in that the electrical energy is utilized as a more efficient means of membrane formation, and a method with improved adhesive ability.

Here, the titanium plate is very often used as the cathode for deposition. Using the titanium plate, electric current is passed through the acidic calcium phosphate solution, increasing the pH of the solution surrounding the plate, and forming an HA layer20). Depending on the reaction conditions, $H_2PO_4^{2-}$ ions or HPO_4^{2-} ions are reduced in the vicinity of the cathode releasing hydrogen and becoming PO_4^{3-} ions, and subsequently reacting with Ca^{2+} to finally yield HA21). There are reports of methods to control the particles to nanodimensions by application of ultrasound22).

The solubility of HA above room temperature declines as the temperature increases; and a thermal substrate method has been developed to utilize this property. Here, the temperature in the vicinity of the titanium plate is increased by electrical heating, passing an alternating current through the plate in a phosphate- and calcium-containing solution23), 24).

There are also reports of experiments that used electrophoresis25). Electrophoresis is the dispersing movement of charged particles in fluid under the influence of a spatially uniform electric field, created by application of a voltage. The charged particles then accumulate at the electrode. The two factors that must be considered are the size of the particles, which must be small enough to avoid precipitating out during electrophoresis, and the choice of the dispersion medium, with suitable stability and conductivity.

Even though this electrochemical method is simple and does not require complex equipment, the rate of layer formation is much faster than with the dip-coating method, and this approach has the added advantage that the coating can be applied to complex shaped objects. Further, it permits control of factors such as temperature, ionic concentration of the solution and current density, enabling control of the thickness and structural properties of the layer 26), 27). There are cases where heat treatment is still necessary as the final step.

C. Vapor growth method

There are two types of vapor growth method, physical vapor deposition (PVD) and chemical vapor deposition (CVD), but PVD has been the more popular of the two for application of HA coatings. PVD can be further divided into vacuum deposition, ion spraying and spattering.

In the vacuum deposition method, the solid state is vaporized by heating under conditions of high vacuum. The vapor accumulates on the plate, is maintained at a certain temperature, and formation of a thin film occurs on the plate. Laser light is often used to evaporate the raw material to be deposited (laser abrasion) 28), 29). Laser abrasion uses laser light with a high energy density, and evaporates the material using its photochemical abilities. This method is also referred to as the pulse laser deposition (PLD) method. In a similar manner to vacuum deposition, the ion-plating method uses plasma to ionize or excite the evaporated particles, plating the material with highly energetic particles. This method is yet to be widely applied in HA coating.

Sputtering has been applied in various industrial fields as a technique for forming thin layers. Ion-guns or electric discharges are used to ionize inert gases such as argon. These inert gas ions are then accelerated to bombard the target raw materials to cause evaporation, and the target atom or clusters are forcefully ejected from the surface. In HA coating, radio-frequency magnetron-sputtering is often used 30), 31). When an insulator such as HA is used as the target of raw material to be evaporated, this method is an effective means for preventing the target from being charged by using high-frequency wave as the power to gain ions through discharge of electricity. Furthermore, when a magnetic field is applied to the area of electric discharge, the electrons emitted from the target surface rotate in response to the Lorenz force received, increasing the discharge current and stimulating ionization of the sputtered gas, thus accelerating the formation of the film coating.

The PVD method has the advantage of not only allowing the chemical composition of the coating layer to be controlled, but also regulating its crystalline and orientation properties through setting of the conditions for deposition32). The thickness of the coating formed by this method is less than a few micrometers and its most notable advantage is firm adhesion to the titanium plate.

D. Thermal spraying methods

The most popular method in current practice for coating titanium with HA is the plasma spray method, one of the thermal spraying methods. Here, the HA particles are melted with plasma flame at extremely high temperatures of over 10,000°C, and repeatedly sprayed onto the titanium plate at a speed of 100-200 m/s for layer formation. The plasma spraying method was one of the first HA film coating methods to be used, and therefore many industrial examples have been reported. However, the process of solidification requires rapid cooling from a critically high temperature, and it is not easy to control the thickness of the film layers. Generally, the HA coating formed by the plasma spray method is thick (tens of micrometers), and contain impurities such as calcium oxide and TeCP in an amorphous matrix, mixed in with highly crystalline HA, resulting in an inconsistently distributed structure33) with uneven density and uneven adhesive properties. The stability upon implantation becomes problematic. In order to overcome the issues, treatments are continuously renovated to increase the smoothness and to improve stability. Heat treatment in solutions such as pseudo-body fluid is the main method in current procatice, and the recrystallization of an HA coating mentioned in this text, falls under this heading.

V. Final word

In this report, use of HA as a coating material was summarized. HA ceramics exhibit prominent biological activity and show efficacy as artificial bone material, however, for these to replace physiological bone, obstacles still remain, in particular, with regards to mechanical strength. Brittleness has been noted to be a problem associated with the simplex form of the materials that fully utilize the excellent biological activity of HA ceramics. The most successful material so far to solve this problem has been HA-coated titanium. All of the current methods of coating have their advantages and disadvantages, from uneven layers that are close to the amorphous phase, to highly pure, highly crystalline layers. There have been reports of improvements in proliferation and differentiation of osteoblasts on highly crystalline HA layers of calcium phosphate from that observed on the surface of amorphous substrates34).

Conversely, in the biologically active process whereby the HA layer binds with normal bone, HA needs to solubilize partially, therefore a coating that is less crystalline and highly soluble is more advantageous when it comes to fusing with bone. Nevertheless, a highly soluble coating lacks stability and may be quickly eliminated after installation, which is inappropriate for the success of the implant.

As titanium itself does not have any physiological adhesive property, the HA coating is expected to act as a long-term mediator to stabilize and aid the function of the titanium implant.

In attempts to find an optimum coating method that is most practical, the most rational method currently available may be the application of after-treatment to convert, a calcium phosphate layer formed initially with an inexpensive method, to a highly pure, highly crystalline HA, to gain high adhesiveness.

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