HYDROXYAPATITE

by

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1. Introduction

Bioceramics are a class of biomaterials that have found a variety of role in the human body as surgical implants. Introduced in the 90s, initially as the retrograde filling materials, then as sealers for root canal, they have been used in a wide array of medical application such as imparting structural stability to the joints, tissue replacements, coatings for the biocompatibility of implants. Contrary to what is normally thought, Bioceramics are not porcelain type ceramics, but are biocompatible, non-toxic, non-expanding biomaterials. They are usually chemically stable within the vast range of temperatures.

One of the most important property of the bioceramics is their ability to form hydroxyapatite which is major component of the bone. About 50% by volume and 70% by weight, the human bone is consisted of Hydroxyapatite. Further, carbonated hydroxyapatite is the prime mineral in the human dental enamel and dentin.

1.1 Classification of Bioceramics

A synthetic material when inserted into the human body elicits a biological response. The composition of the material determining this response, depends on the mechanism of the tissue interaction on the response of the tissue with the implant. Bioceramics, thus are classified in accordance with their response to the tissue:

1. Bio-inert
2. Bio-active and
3. Bio-degradable

Bio-inert ceramics as the term suggests, refers to biomaterials that show minimal to no interaction with the surrounding tissues. The body responds to these by forming a non-adhering capsule of connective tissues around the bioinert materials. Examples of bioinert includes alumina, zirconia, carbon and titanium.

Bio-degradable ceramics refers to biomaterials that are either soluble or are dissolvable. The properties of solubility or dissolution depends on the composition of the bioceramic. Examples of biodegradable materials include calcium oxide and tri-calcium phosphate.

Bio-active ceramics are the materials that experience interfacial interactions with the tissues. They show the phenomenon of osteoconduction through the process of ion-exchange reaction resulting in the formation of biologically active bone-like apatite (such as Hydroxyapatite)

layer. (*1*) The adsorption of bone growth mediating proteins at the surface of bioinert surfaces triggers chemical bonding between the bone and the surface. Example of these include synthetic calcium phosphate salts like hydroxyapatite and glass ceramics. (*2*)

1.2 Calcium Phosphate Salts

Calcium Phosphate is one of the most important inorganic compounds in the biological formations. (*3*) Calcium Phosphate salts, by definition, consists of three major elements as part of the orthophosphate anions:

1. Oxygen, present in the reduction state of -2
2. Phosphorous, present in the oxidation state of +5
3. Calcium, present in the oxidation state of +2

Because of the abundance of these chemical elements, it is expected to find calcium phosphate throughout the planet in various forms. Thus, natural calcium phosphates are found in the different region in the igneous rocks as deposits of apatites, minerals fossil bones, microbial pseudomorphs. (*4*) In biological formations, calcium phosphates are formed by biomineralization which is a process where living organisms produce minerals which are required for their survival. Thus, major amount of calcium phosphate is present in the form of carbonated hydroxyapatite (HAP) in vertebrates (such as humans) which tend to present in the bone, teeth and tendons, giving them the stability and hardness. The main constituent of the human bone are calcium orthophosphates (~50-60 wt.%), collagen (~30-40 wt.%) and water (~10 wt.%). (*5*) In lower animals, calcium phosphate in present more in the amorphous mineral phase.

Hydroxyapatite (HAP) is the most stable form of calcium phosphate at normal temperatures and pH between 4 and 12. One of the factors separating HAP from other calcium phosphates is its higher in-vivo stability. Therefore, it has become of prime importance in the process of catalysis, fertilizer and pharmaceutical industries, water treatment processes, and mostly because it is the prime inorganic constituent of bones and tooth. (*5*) (*6*) Due to biomineralization, HAP is formed due to functional irregularities in arthritis, calcification of transplanted cardiac valves and formation of bladder, bile stones. This importance of HAP has led to extensive research in various areas of the mechanisms of the formation of HAP crystals to its applicability as implants in the biomedical field. (*7*) Thus, for biomineralization related phenomenon, HAP is considered as a model compound. (*8*)

Apart from the HAP, there are several other calcium phosphates that occur in the pathological mineralization. Due to the variety of calcium phosphate, abbreviations and parameters have been introduced to distinguish between different compounds. These include the molar Ca/P ratio and the solubility. The general trend of the parameter is that the lower the Ca/P ratio, the more acidic and soluble in water.(5) The figure below shows the various calcium phosphate salts.

Ca/P ratio	Compound	Formula	Solubility at 25 °C, $-\log(K_{sp})$	Solubility at 37 °C, $-\log(K_{sp})$
0.5	monocalcium phosphate monohydrate (MCPM)	$Ca(H_2PO_4)_2 \cdot H_2O$	1.14	no data
0.5	monocalcium phosphate anhydrate (MCPA)	$Ca(H_2PO_4)_2$	1.14	no data
1.0	dicalcium phosphate dihydrate (DCPD, "brushite")	$CaHPO_4 \cdot 2H_2O$	6.59	6.63
1.0	dicalcium phosphate anhydrate (DCPA, "monetite")	$CaHPO_4$	6.90	7.02
1.33	octacalcium phosphate (OCP)	$Ca_8(HPO_4)_2(PO_4)_4 \cdot 5H_2O$	96.6	95.9
1.5	α-tricalcium phosphate (α-TCP)	$\alpha\text{-}Ca_3(PO_4)_2$	25.5	25.5
1.5	β-tricalcium phosphate (β-TCP)	$\beta\text{-}Ca_3(PO_4)_2$	28.9	29.5
1.2–2.2	amorphous calcium phosphate (ACP)	$Ca_x(PO_4)_y \cdot nH_2O$	[c]	[c]
1.5–1.67	calcium-deficient hydroxyapatite (CDHA)	$Ca_{10-x}(HPO_4)_x(PO_4)_{6-x}(OH)_{2-x}$ $(0<x<1)$	≈85.1	≈85.1
1.67	hydroxyapatite (HA)	$Ca_{10}(PO_4)_6(OH)_2$	116.8	117.2
2.0	tetracalcium phosphate (TTCP)	$Ca_4(PO_4)_2O$	38–44	37–42

Table 1: Properties of commonly occurred calcium orthophosphates.

2. Synthesis of Hydroxyapatite (HAP)

Crystallization in a controlled environment like a laboratory is carried out in a fluid medium, at a particular temperature, contained in a crystallizer. The final crystalline product that has to be obtained can be controlled by managing the composition and other parameters of the medium. On the other hand, the parameters defining crystallization occurring biological systems, such as in humans, plants, pearls, mollusc shells are controlled by nature. This not only makes it difficult to study the biologically occurring crystals but also understanding the composition of these crystals. As is the case with any type of crystallization process, he nucleation and crystal growth in a system of calcium phosphate is a function of supersaturation.

2.1 In-vivo methods

Biomineralization is the process via which various minerals are formed at a certain condition of temperature, pressure and pH in the biological systems. These are composites of proteins, polymeric carbohydrate molecules and crystals of many inorganic compound.

Biomineralization processes are differentiated in to two types:

1. Cell-mediated mineralization
2. Matrix-mediated mineralization

Cell-mediated mineralization appears within cellular structure called vesicles and matrix-mediated mineralization occurs outside of the cells in microcellular bodies of proteins and polysaccharides. (*9*) (*10*) Because biomineralization involves diffusion through the means matrix medium, it is composed of two parts:

1. Precipitating crystalline phase
2. Organic supporting structure called matrix

In humans, the matrix is composed of Proteoglycans and collagen fibres in cartilage and bones and Glycoproteins and polysaccharides in teeth. The biominerals involved in the matrix is *calcium oxalate, calcium phosphate, calcium carbonate*.

For the synthesis of HAP via the process of crystallization in the biological systems, some degree of supersaturation of the precipitating phase has to occur. Some of the factors that lead to this mineralization are the concentration of the ions, solution pH, inhibitors and the structure of the supporting organic matrix. It is because of the organic matrix that heterogenous nucleation of the phase is induced. (*10*) The exact mechanism of the aforementioned factors to induce the nucleation is not known well. (*11*) The maturation pathway of the nanocrystalline apatites, however has been recognized depending on the composition of the medium, though more work needs to be done in this regard. Figure 1 shows the main route for this progression.

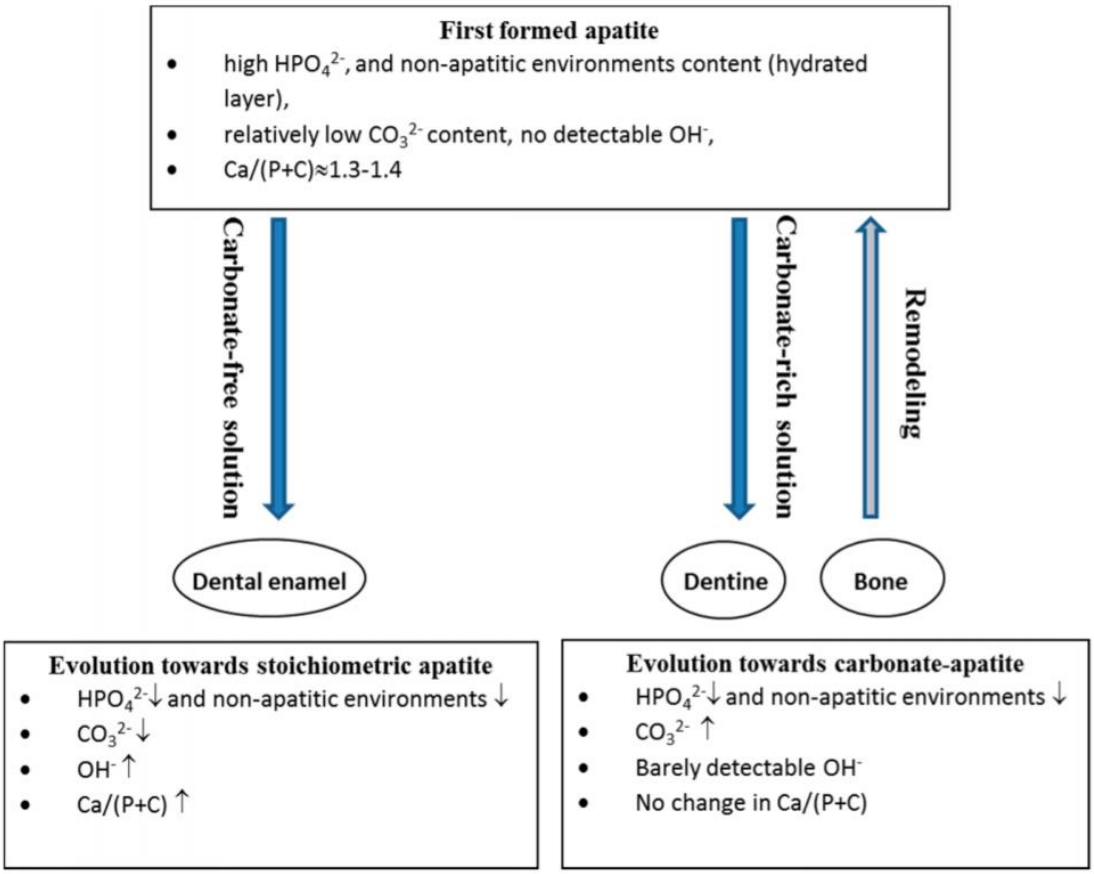

Figure 1: Maturation pathways of apatites in dental enamel, dentin and bone. (*12*)

The initial precipitate at only contains carbonate ions and a large amount of HPO_4^{2-} ions. As nucleation progresses, the amount of carbonate ions increases with the simultaneous decrease of HPO_4^{2-}. Thus, Carbonate deficient solution gradually helps in the formation of stoichiometric apatite and carbonate rich solutions produce carbonated hydroxyapatite. When initial carbonate ions are not present, the hydrate layer which is composed of mainly bivalent anions and cations, grows progressively into apatite with the increase in Ca/P ratio. (*12*)

2.2 In-vitro methods

Several methods have been devised to prepare HAP crystals throughout the literature. These include, layer hydrolysis, sol-gel crystallization, solid state reactions, plasma techniques, wet chemical routes that renders precipitations at low temperature. (*13*) (*6*) Although these synthetic procedures are advantageous due to low cost but leads to the formation of non-stoichiometric products. (*14*) These deviations are mainly due to inconsistencies in the crystal structure such as lattice vacancies and substitution of ions.

The formation of synthetic HAP crystals, from any method, proceeds via the formation of intermediate precursors phases (tricalcium phosphate and octacalcium phosphate) from the saturated solutions.

Nancollas and Mohan, in 1970, proposed a method for the growth of HAP crystal which has been followed extensively throughout the literature. They seeded the stable supersaturated calcium phosphate solutions with crystals. The HAP crystals were prepared by the addition of 05M $CaCl_2$ solution to 0.3M Na_2HPO_4 at 70^oC and a pH in between 8.5-10. (*15*) This process was further modified by addition 0.1M KOH instead of disodium hydrogen phosphate with the addition of 0.3M KH_2PO_4 over a time period of 30 minutes. The pH was adjusted at 9.5 and temperature at 70^oC. This was then precipitated to get a solid which is washed with distilled water and refluxed for 24 hours. This product was aged for 4 months at 37^oC and the pH being readjusted to 7. (*6*) The product thus obtained has a Ca/P ratio of 1.67 which is the same of the respective ion in the molecular formula of HAP crystals.

3. Crystal Structure of Hydroxyapatite

The structure of HAP is important to understand the properties of the compound because it is the main component in bone and teeth.

Apatite describes a class of compounds having a hexagonal crystal system and space group of P63/m (Hexagonal bipyramidal). (*16*) Apart from HAP, fluorapatite is one another apatite that has been researched well primarily because it helps in preventing tooth decay or dental caries. The chemical formula of HAP is $\mathbf{Ca_{10}(PO_4)_6(OH)_2}$ and the lattice parameters of unit crystals are a = b = 9.42Å and c = 6.87Å. (*16*) The unit cell of the HAP has two equal edges inclined at 120^o to each other and possesses vertical symmetry-axes of three kind.

3.1 Structure of the unit cell

The unit cell of HAP has 10 Ca^{2+} atoms. Six of these atoms lie completely within the unit cell thus forming a triangular group. Because the allocation of Ca^{2+} atoms is four, eight Ca^{2+} atoms lie at the periphery of the Ca(I) position which are shared by adjacent unit cells. Similarly, eight OH^- ions are shared by the adjacent unit cell which lie at the edge of the unit cell. The 10 $(PO_4)^{3-}$ groups lie completely within the unit cell. (*17*)

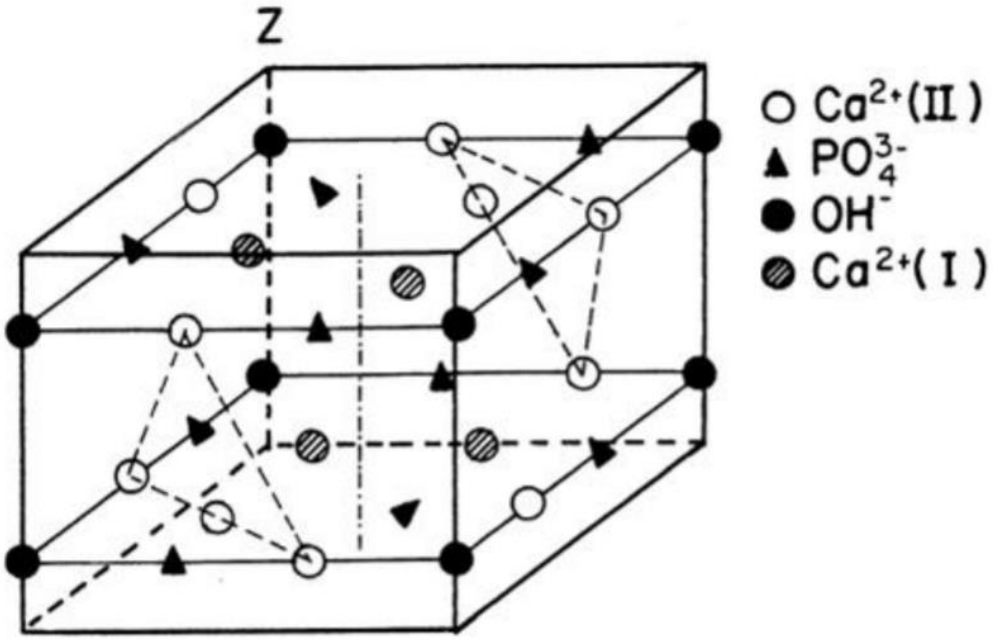

Figure 2: Unit Structure of HAP. (*17*)

The arrangement of the atoms around these symmetrical elements was by Beevers et. al. in 1945. Along the three-fold axis, there is a chain of calcium atoms one half of the c-axis apart. (*18*) These Ca atoms are bonded to three oxygen atoms above and below the neighbouring Ca atoms, thus forming a continuous chain, the figure of which is represented below.

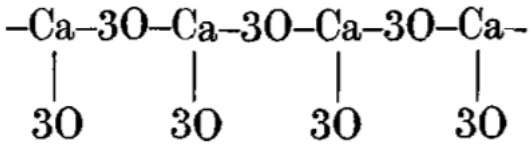

Figure 3: Arrangement of Ca atoms around Oxygen (*18*)

Thus, each Ca atoms is surrounded by nine oxygen atoms. The oxygen atoms that are linked to only one Ca are alternately raised and lowered parallel to the c-axis. This makes the oxygen atom to be at the same time members of the PO_4 group. Furthermore, the oxygen atom projecting outwards from the PO_4 groups are shared by neighbouring Ca-O columns. Thus, the adjacent columns linked by two bonds per c-axis giving the hexagonal network throughout the structure. (*18*) (*19*) This is represented below in the figure.

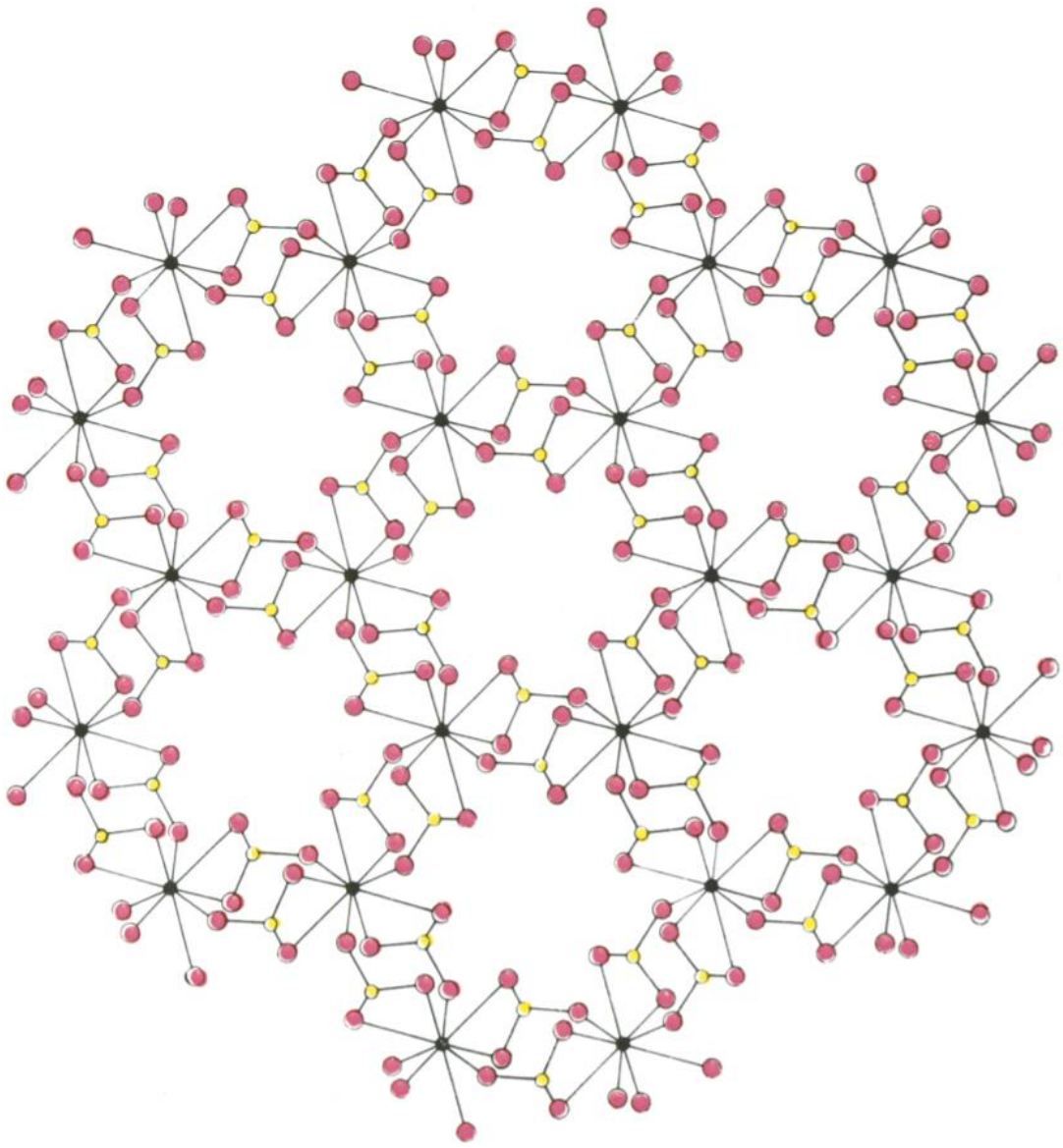

Figure 4: Arrangement of the PO_4, O_2 atoms around Ca atoms (*18*) (**Legend**: Pink: Oxygen atom, Black: Calcium atoms, Yellow: Phosphate atoms)

4. Differences in the Structure of Biological Occurring and Synthetic HAP

Stoichiometrically, biological HAP and synthetic HAP have minor differences which corresponds to major functional differences between the two. These minor differences, which arises due to the way in which they are form, involves imperfections in the crystal structure in the biologically occurring HAP. This entails detentions of different ions in the three sublattices. Specifically, the site of Ca^{2+}, could be occupied by monovalent or bivalent ions such as $Mg^{2+,}$ Na^+, K^+, Cl^-, F^- and PO_4 tetrahedral sites could be substituted by CO_3^{2-}, As, S and OH^- ion by CO_3^{2-}, Cl^-, F^-. (*20*) (*21*) Mostly, the PO_4 tetrahedral sites and the hydroxyl sites are replaced by

carbonate ions. Due to which, the biologically occurring HAP ions is considered as carbonated apatite.

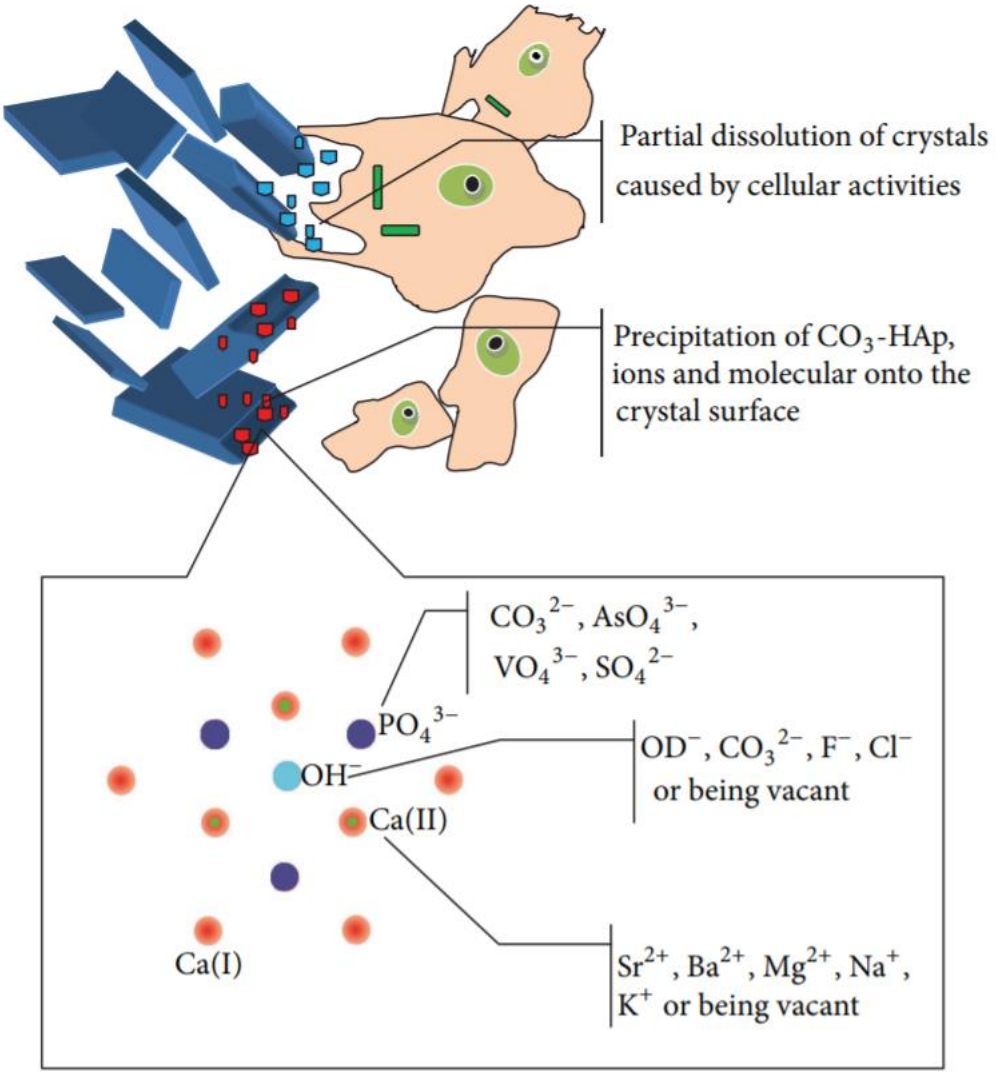

Figure 5: Partial dissolution of biological apatite in vivo in the crystal of HAP (*20*)

Thus, the apatite hosts carbonate in two positions:

1. Hydroxyl sublattice producing type A carbonate apatite
2. Phosphate sublattice forming type B

$$\text{Type A}: \; Ca_{10-x}\cdot(PO_4)_{6-x}\cdot(HPO_4)_x\cdot(OH)_{2-x} \text{ with } 0 \leqslant x \leqslant 2$$
$$\text{Type B}: \; Ca_{10-x}\cdot(PO_4)_{6-x}\cdot(CO_3)_x\cdot(OH)_{2-x} \text{ with } 0 \leqslant x \leqslant 2$$

The nature of this substitution is such that the electrical neutrality is replaced in the crystal structure by adjusting number of positive and negative ions. (*22*) (*23*)

The crystal structure and morphology constitute other differences between biologically occurring and synthetic HAP. There have been various figures of the reported cell parameters due to the variation in biological HAP. The parameters of biological HAP prepared from bovine bone by sintering to 700^{o}C were $a = b = 9.429$ Å, $c = 6.885$ Å. (*24*) On the other hand, the cell parameters of enamel in human and shark teeth were stated as $a = b = 9.445$ Å, $c = 6.833$ Å and $a = b = 9.377$ Å, c = 6.881 Å, respectively. (*20*) (*21*) Furthermore, the HAP crystals in bone and teeth are very highly ordered and oriented.

When it come to the dissolution behaviour, a recent study reported that solubility of biologically occurring HAP from the bovine bone is significantly higher than that of the synthetic HAP. This may be due to the fact that synthetic HAP is more crystalline than biologically occurring HAP. Additionally, the presence of Mg^{2+} may also contribute to the increased solubility of the biologically occurring HAP. (*25*)

The final key distinction in that biologically occurring HAP is always in a dynamic state and is removed and redeposited by cellular activity, whereas synthetic HAP can only be modified by physiochemical processes.

5. Interest in Bones and Teeth

Bones and teeth are heterogenous, hierarchical, composite materials. To elucidate this, figure 7 shows the hierarchical nature of bones and teeth using mouse tissues. It should be noted that all mammalian tooth and bones have similar structure at the microscopic level. (*26*)

Mineralization makes it possible for living tissues like bones and teeth to be replaced and replenished actively during life: bone and dentin are capable of regeneration while the enamel is acellular, so it is non-living tissue. But what sets the enamel apart, is its porosity. This allows for diffusion and chemical reactions to occur within the structure. This involves acidic dissolution and remineralization from saliva.

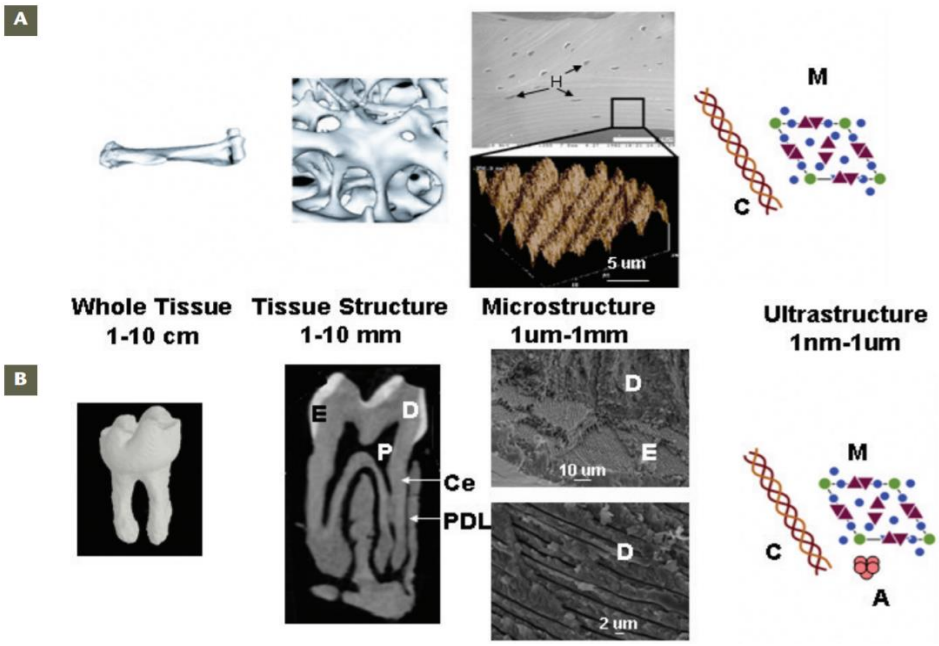

Figure 6: The hierarchical nature of the bones (A) and teeth (B), using mouse tissues. In (A), the microstructure shows peaks and troughs of the bone lamellae and the cartoon of the ultrastructure illustrates the major components, triple-helical collagen (structure C) and HAP (M): Ca^{2+} are shown in blue circles, OH^- are shown in green circles and PO_4^{3-} are shown in red triangles. In (B), the tissue structure shows cross section, with regions of enamel (E), dentin (D), pulp (P), cementum (Ce) and periodontal ligament (PDL). The cartoon of the ultrastructure includes amelogenin nanospheres (A, pink clusters) from enamel apart from the apatite and collagen. (*26*)

5.1 Hydroxyapatite Content in the Bones and Teeth

The composition of bones, enamel and dentin is different. Although the three contains the same elements for or less, it is the stoichiometry of each that makes them differ in the chemical and physical characteristics. Due to the presence of minor and trace elements no exact chemical formula for biological apatites are available, but the average composition has been derived based on the simplified model which do not account for the presence of type A carbonate replacing OH^- ions and the sodium content. (*27*) The composition are mentioned below: (*27*) (*28*)

Human bone apatite: $Ca_{8.1}\ Mg_{0.2}\ (PO_4)_{4.3}\ (HPO_4)_{0.5}\ (CO_3)_{1.2}\ (OH)_{0.3}$

Human dentine apatite: $Ca_{8.0}\ Mg_{0.4}\ (PO_4)_{4.4}\ (HPO_4)_{0.7}\ (CO_3)_{0.9}\ (OH)_{0.4}$

Human enamel apatite: $Ca_{8.8}\ Mg_{0.1}\ (PO_4)_{4.9}\ (HPO_4)_{0.6}\ (CO_3)_{0.5}\ (OH)_{0.9}$

HAP comprising the dentin has the dimension 50 × 30 nm which is much smaller than that of enamel. Further, the crystallinity and alignment of the crystals in dentin is poorer in dentin as compared to enamel. Therefore, dentin is much softer than the enamel and decays much more rapidly. (*10*) The crystallinity of the crystals of enamel and its composition makes the enamel

the hardest substance in the human body. Bone exhibits elongated plate-like nanocrystals (~50 ×25 ×10 nm)

The table below encapsulates the structural differences between the bone, enamel and dentin:

S. No	Diff	Bone and Dentine	Enamel
1.	**Carbon Content**	High carbonate content and higher number of inorganic substitutions	Low carbonate content and lower number of substitutions.
2.	**Organic Content**	Cells, Organic tissues, non-collagenous proteins are present	Total organic content <3%
3.	**Structure**	Plate like nanocrystals	Needle like nanocrystals
4.	**Dimension**	50 nm x 25 nm x 10 nm	Dimensions vary with species
5.	**Packing**	Agglomerated as parallel platelet crystals	Organized as rods of densely packed structures
6.	**Regeneration**	Can be regenerated over time	Very low regenerative power
7.	**Hardness**	1-2 GPa	3.5-5.0 GPa
8.	**Elastic modulus**	17-23 GPa	80-100 GPa

Table 2: Difference between Bone, Dentine and Enamel

6. Demineralization in Teeth

Demineralization is a process which reduces the number of mineral ions from HAP crystals of in the hard tissues, for example, in enamel, dentin, and bone. It leads to a substantial lose of mineral ions from the surface of the tissue thus destroying its integrity and making the hard tissues highly sensitive to pressure and temperature. In teeth, it leads to the cavities and tooth decay whereas in the bone, it leads to osteoporosis. Demineralization of teeth is a reversible process, allowing growth of demineralized HAP crystals to grow to their original size. (*29*)

Figure 7: Example of tooth decay on the left and a healthy tooth on the right (from http://giggag.com/post/36353)

6.1 Causes of Demineralization

The main cause of demineralization is caused by the:

1. Dietary acid consumed via food or drinks. This results in the direct contact between tooth surface and acids. The critical threshold pH that has been known to cause demineralization is 5.5. (*30*) The source of intrinsic acids in the oral cavity is from the backflow of gastric contents.
2. Microbial attack from bacteria present in the mouth. (*31*) *Streptococcus mutans*, Streptococcus sobrinus and *Lactobacillus* are carcinogenic pathogens which can withstand very low levels of pH. (*29*) They tend to metabolise fermentable carbohydrates like sucrose, fructose, glucose and cooked starch present in the surface of the enamel and release formic, acetic, propionic and lactic acids. The acid softens the tissues by solubilizing the calcium and the phosphates.

Carbonated drinks	pH	Juice drinks	pH	Other drinks	pH
Coke	2.7	Orange juice	3.4	Iced tea	3.0
Pepsi	2.7	Grapefruit juice	3.2	Fanta orange	2.9
7-Up	3.2-3.5	Cranberry juice	2.3-2.5	Red Bull	3.4
Sprite	2.6	Apple juice	3.4	Gatorade	3.3
Mountain Dew	3.2	Pineapple juice	3.4	Isostar	2.4-3.8
Dr. Pepper	2.9	Kiwi juice	3.6	Coffee	2.4-3.3
Lemon Nestea	3.0	Grape juice	3.4	Tea (black)	4.2
Root beer	3.0-4.0	Carrot juice	4.2	Beer	4.0-5.0
Ginger ale	2.0-4.0	Beetroot juice	4.2	Wine	2.3-3.8

Figure 8: pH of common available drinks that can potentially cause tooth erosion. (*32*)

6.2 Acid Dissolution

Acid erosion can take place via two mechanisms:

1. Direct Pathway: Hydronium Ions are released from the acids in the solution which binds with the phosphate and the carbonate in HAP of the enamel, releasing anions as result causing etching. The pH of the acid determines the extent of demineralization. Acids with pH<1 can cause surface etching in a very short time interval. When the pH is in between 2-4, nanoscale surface softening occurs. When the pH>4, subsurface dissolution occurs which can result in the formation of carious lesions. Common acids causing dissolution are acetic acid, lactic acid and phosphoric acid. (*29*)
2. Chelation: The COOH group of an acid like citric acid or phosphate acid, is dissociated to form H^+ and H_3O^+. This results in a COO^- anion to cause calcium chelation. This happens via the formation of soluble chelate complex with three calcium ions and is

strictly dependent on the strength of the anion. Because the chelate is soluble, it is carried from the enamel surface, resulting in the loss of HAP from the surface of the teeth. Common acids that cause acid erosion of teeth via chelation are citric acid, carbonic acid and phosphoric acid. (*29*)

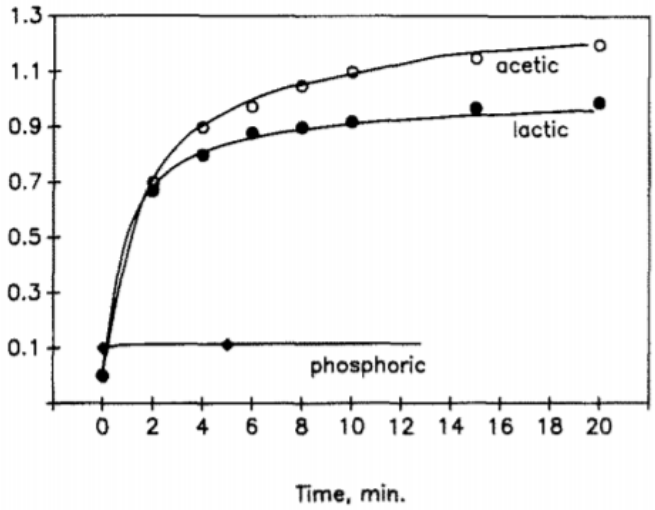

Figure 9: Change in calcium concentration as a function of time from observed pH changes of three acid solutions. In general, more dissolution occurs in acetic acid solution. (*33*)

7. Remineralization of Teeth

As the name suggests, the process of arresting and reversing the demineralization is called remineralization. Demineralization can be arrested particularly in the early stages of tooth decay. Some of the techniques involves fluoride therapy, diet control, probiotic bacteria and the use of dental fillers containing HAP or other calcium phosphate compounds.

7.1 Remineralization from Fluoride Therapy

Fluorides are the most favoured remineralizing agent. (*34*) The method involves the use of topical fluoride, such as toothpaste and varnish. Fluorine displaces the calcium present in the biologically occurring HAP, forming fluorapatite (FAP). FAP has two main advantages over HAP:

1. It has much lower solubility than the original or demineralized FAP.
2. It forms solid state solution with HAP, by displacing OH^- ion. (*29*)

It is therefore, that fluorine is added to the drinking water. The Fluoride assists remineralization of enamel by acting as a catalyst with the phosphate ions dissolved in the saliva. (*35*) Further, the displacement of OH^- with fluoride counters the dissolution of HAP in lactic acid solution. (*36*) (*29*)

It is therefore for this effectiveness of fluoride ions, that fluorine therapy is preferred. But regardless of this, care should be taken not to exceed the overuse of fluoride. Exceeding the maximum recommended dose through fluorinated water, toothpaste or supplements may lead to dental and/or skeletal fluorosis. (*29*)

7.2 Remineralization from composites containing Hydroxyapatite

Skrtic et. al. came up with the idea of Amorphous Calcium Phosphates(ACP) as fillers for dental composites. They showed that ACP released significant amount of calcium and phosphate ions, thus arresting demineralization. Coupling agent such as silicon and zirconium enhanced the remineralization potential of ACP. There have been many studies regarding the coupling agent treatment of HAP with citric acid, acrylic acid, methacrylic acid improving the strength of the dental composites containing HAP. (*37*) (*38*)

HAP whiskers and nanofibers have been reported to further enhance flexural strength because of their excellent bioactivity and mechanical properties. (*39*) In attempts to improve the mechanical properties, various weight fractions of HAP fillers have been experimented with. Impregnation of the HAP nanofibers (2 wt.% or 3 wt.%) into the dental composites can substantially improve the strength of the resulting dental composites. Raising the wt. percent to 10% showed a decrease in strength due to the formation of HAP that served as defect site. (*40*) Below are studies by Swarup et. al. showing the effectiveness of the aforementioned remineralization techniques. The sample was demineralized in a demineralizing solution containing lactic acid and a pH of 4.5 and was later remineralized using nanohydroxyapatite powder and 2% neutral sodium fluoride solution.

Group	Baseline	After demineralization	After remineralization
Nanohydroxyapatite	1.57±0.10	1.28±0.09	1.52±0.10*,a
2% Sodium fluoride	1.56±0.10	1.28±0.10	1.40±0.10*,a

Figure 10: Surface zone mineral content of the enamel before and after remineralization (*34*)

Group	Baseline (μm)	After demineralization (μm)	After remineralization (μm)
Nanohydroxyapatite	1189.96±103.04	1163.20±103.55	1167.42±103.94a,b
2% Sodium fluoride	1188.22±106.09	1161.00±105.21	1162.93±105.60a,b

Figure 11: Enamel thickness before and after remineralization (*34*)

8. Demineralization–Remineralization in Bone

The mineralization process of the bone occurs via the process of bone remodelling wherein the mature tissues are replaced from skeleton by new bone tissue. This process occurs in two phases, the first being the deposition of osteoids by osteoblasts and the second being the actual formation of the bone. The mineralization rate of bones is 1 μm/day for humans. (*10*)

Demineralization results in the disruption in the physiochemical process of bone remodelling by change in the contribution of the osteons. Small decrease in mineralization results in large decrease in the strength of the bone resulting in the osteoporosis, osteogeneis imperfecta which is a genetic disorder associated with increased bone fragility, Paget's disease, Bruck's disease, renal osteodystrophy, etc.

Some drugs that reduce bone-resorption or promote bone formation were developed in the past but it has been found that they can have serious side effects, therefore bone cements/composites with remineralizing action have been developed and are being used widely in orthopaedic surgeries. (*10*) Poly(methylmethacrylate) (PMMA) is one such composite that is widely used as anchoring agent in hip and knee replacement. PMMA, however has poorer adhesion to bone therefore polymer composites reinforced calcium phosphates have been developed. Further, HAP is also used as filler with composites to form nondegradable cements. These HAP bone composites have been reported to exhibit bone-binding ability and enhanced cellular activity. As with the teeth, the concentration of HA has to monitored as increasing HA content leads to a decrease in the tensile strength. (*29*)

9. Conclusion & Perspective

Diversity and heterogeneity describe apatites the best. This can be attributed to their chemical richness with innumerable substitution within the crystal structure, defects that work just as fine, and strong surface reactivity. This has led to sophisticated compounds with large adaptability to many biological functions across animal species and other geological materials. The fact that chemical composition, non-stoichiometry, changes in the crystal size and orientation associated with the HAP and other calcium phosphate salts results in vastly varying physical properties and elaborates the complexity when dealing with such a system. From the perspective of material science, it is difficult is imitate such elaborate constructions through fabrication processes available. Although enormous progress has been made over the last few decades in understanding the mineralization process of HAP in the bone, dentin and enamel,

the understanding is far from complete. Beyond the fundamental understanding of how these complex chemical compounds associate to become a highly organized network of tissue matrices, there has to be work done in finding alternate calcium phosphates that have a biaxial strength of more than double of the present standards. Furthermore, remineralization dynamics needs to be work upon. Many other questions regarding the mineral depositions needs to be answered as well. While some of these questions may seem redundant, they set the stage for many future investigations.

10. References

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