CHAPTER -I

INTRODUCTION

1.1 INTRODUCTION OF BIOMATERIALS

The material used for developing body implants or interfaces are commonly called biomaterials. The common definition for biomaterials, "A biomaterial is any pharmacologically inert material, viable or non-viable, natural product or manmade, that is a part of or is capable of interacting in a beneficial way with a living organism" [1]. The field of biomaterials is a worldwide multi-billion dollar industry. Just under a decade ago, the lives of over 20 million patients were sustained, supported or significantly improved by biomaterials. It is estimated that this figure increases at 10 % per year. Worldwide, the market of initial and follow-up of organ replacement and prostheses exceeds $ 300 billion US dollars per year and represents between 7-8 % of total worldwide healthcare spending. In the United States alone, the costs of therapies enabled by organ replacement technology exceed 1 % of the gross national product [2]. These figures highlight the importance of biomaterial developments. Research in this area is constantly on going to enhances current and concept materials for future generations, improving the longevity of implants and the quality of the patient's life. This area of materials science is characterized by medical needs, materials characterization and design, basic research, advanced technological development, patient expectation, ethical considerations, industrial involvement and federal regulation [3]. Such multidisciplinary research requires expertise and techniques from a wide variety of subjects such as materials science, chemistry, molecular and cell biology, mathematics, engineering, biomechanics,

computer modelling, manufacturing, medicine and genetics. Biomaterial devices are available for joint and limb replacements, artificial arteries and skin, contact lenses, and dentures which aim to replace damaged or diseases tissues. However, prostheses may also be used for enhancement of the body of which the most well-known is the breast implant. Although use of such materials dates back to more than 2000 years ago, when gold, wood and glass have widely been used in dentistry and other medical disciplines [4]. We have witnessed a major paradigm shift around the early 1990s, when the term "Tissue Engineering" was coined as "a scientific discipline dedicated to the generation of new tissue using the principles of engineering in combination with an understanding and application of the biologic sciences" [5].

1.2 HISTORICAL BACKGROUND OF BIOMATERIALS

Biomaterials have been in use for over 2000 years [3] and even before the pre-Christian era. Throughout history materials have been found to be used with the body to replace lost or damaged parts of the body. The Aztecs, Chinese and Romans commonly used gold in dentistry. Wooden teeth and glass eyes have also been discovered throughout the course of history. In 1860 Lister became a pioneer for aseptic surgery which significantly improved the survival rate of many patients but notably compound fracture victims. However, it was not until the 1900s when the use of biomaterials really expanded with the use of bone plates and biomaterials for joints. Even so, it was not until 1975 that the Society of Biomaterials was formed. The biomaterials world has historically concentrated on replacement and, in more recent times, enhancement, but as we begin the 21st century the discipline looks set to become a regenerative science [6].

1.3 CLASSIFICATION OF BIOMATERIALS

Biomaterials are those capable of being in contact with the body fluids and tissues for prolonged period of time without eliciting any adverse reactions. The increasing demands for safe and reliable implants have resulted in the evolution of variety of biomaterials. Biomaterials are broadly classified as

1.3.1 Polymers

A large number of polymeric materials that have been used as implants or part of implants systems. The polymeric systems include acrylics, polyamides, polyesters, polyethylene, poly(methylmethacrylate) (PMMA), polylactides (PLA), plyglycolide and polyhydroxybutyrate [7]. Some other typical biomedical polymeric materials applications include: artificial heart, kidney, liver, pancreas, bladder, bone cement, catheters, contact lenses, cornea and eye-lens replacements, external and internal ear repairs, heart valves, cardiac assist devices, implantable pumps, joint replacements, pacemaker, encapsulations soft-tissue replacement, artificial blood vessels, artificial skin, and sutures. As bioengineers search for designs of ever increasing capabilities to meet the needs of medical practice, polymeric materials alone and in combination with metals and ceramics are becoming increasingly incorporated into devices used in the body.

1.3.2 Metals

The metallic systems most frequently used in the body as implant metals are the 316L stainless steels, cobalt-chromium alloys, titanium and its alloys [8]. Improvement of implant integration in bone can either be accomplished by cement fixation or the use of a porous bead implant surface to allow bone ingrowth or the application of bioactive ceramic coatings [9].

1.3.3 Composite Materials

Composite materials have been extensively used in dentistry and prosthesis designers were now incorporating these materials into other applications. Typically, a matrix of ultrahigh-molecular-weight polyethylene (UHMWPE) is reinforced with carbon fiber. These carbon fibres are made by pyrolizing acrylic fiber to obtain oriented graphitic structure of high tensile strength and high modulus of elasticity. The carbon fibres were 6 – 15 mm in diameter, and they are randomly oriented in the matrix. In order for the high modulus property of the reinforcing fiber to strengthen the matrix, a sufficient interfacial bond between the fiber and matrix must be achieved during the manufacturing process. This fiber reinforced composite can then be used to make a variety of implants such as intra-medullary rods and artificial joint. Since the mechanical properties of these composites with the proportion of carbon fiber in the composites, it is possible to modify the material design flexibility to suit the ultimate design of prostheses.

Composites have unique properties and are usually stronger than any of the single material from which they are made. Workers in this field have taken advantages of this fact and applied it to some difficult problems where tissue in-growth is necessary.

Examples:

- Deposited Al_2O_3 onto carbon
- Carbon / PTFE
- Al_2O_3 / PTFE
- PLA-coated carbon fiber

1.3.4 Ceramics

The most frequently used ceramic implant materials include aluminium and magnesium oxides, calcium phosphates, apatites and graphite [10]. Glasses have also been developed for medical applications. The use of ceramics was motivated by:

- inertness in the body,
- formability into a variety of shapes and porosities,
- high compressive strength, and
- excellent wear characteristics.

Among the bioactive ceramics, synthetic hydroxyapatite with a chemical composition $Ca_{10}(PO_4)_6(OH)_2$ has been extensively studied as a bone replacement material [2]. Materials used for bone applications are categorised into four groups: autografts, allografts, xenografts and alloplastics.

A Autografts

It involves removal of tissue from one site and transferring it to another within an individual. A common example of this is skin grafts in which skin layers may be transferred to a site of trauma or disease to restore function to that area. For bone tissue autografts, common sites available for donation are the iliac crest, tibia and ribs. Bone autografts have the advantage of being osteogenic, osteoinductive and osteoconductive and do not illicit a host immune response. Drawbacks of using this type of graft are a second surgical procedure is required to prepare the graft with related donor site morbidity and only limited amounts of tissue may be removed. For the patient, this generally means increased time in surgery, increased morbidity, longer recovery time and increased scarring.

A Allografts

They are also known as homografts, are the transplantation of tissue from one individual, either alive or deceased, to another within a species. The donated tissue is then processed and prepared to remove for implantation and used within a finite time period. Allografts are an attractive option when compared to autografts as they only require a single surgical procedure per patient, reducing discomfort, recovery times and post-operative problems such as infection. Furthermore, increased availability allows room for error. Unfortunately significantly inactivating pathogens in the donated tissue and reducing foreign body response poses a great challenge for scientists developing these tissues.

A Xenografts

They are the transferring of tissue from one species to another. Common examples of this include porcine and bovine heart value transplants into humans which have proved successful. The advantages are similar to allographs in that there is an increased donor number. However, issues related to pathogens associated with the implant and trans-species infection has reduced the potential offered by these types of biomaterials. Moreover, ethical issues and religious views can further limit xenograft use.

A Alloplastics

Due to restrictions of other donor types, researchers have begun to give increasing attention to alloplastic. These materials are synthetically produced devices which can be manufactured into many shapes and sizes out of an almost infinite number of material or material combinations. The advantage of this graft

type is that there is unlimited resource. In addition pathogen contamination does not pose a serious threat, as these materials should be designed to allow sterilisation. Disadvantages include unwanted foreign body immune response upon implantation. The human body is a complex system and often alloplastics are very simplified replications of single components of this system. This means the device may not always accurately mimic the mechanical properties, composition or role of the replaced tissue. This study in part aims to address some of these principal issues.

1.4 BIOCOMPATIBILITY

Biocompatibility is related to the behaviour of biomaterials in various environments under various chemical and physical conditions. The term may refer to specific properties of a material without specifying where or how the material is to be used. For example, a material may elicit little or no immune response in a given organism, and may or may not be able to integrate with a particular cell type or tissue. The ambiguity of the term reflects the on-going development of insights into how biomaterials interact with the human body and eventually how those interactions determine the clinical success of a medical device (such as pacemaker or hip replacement). Modern medical devices and prostheses are often made of more than one material so it might not always be sufficient to talk about the biocompatibility of a specific material [11].

Also, a material should not be toxic unless specifically engineered to be like ‘smart” drug delivery systems that target cancer cells and destroy them. Understanding of the anatomy and physiology of the action site is essential for a biomaterial to be effective. An additional factor is the dependence on specific anatomical sites of implantation. It is thus important, during design, to ensure that

the implement will fit complementarily and have a beneficial effect with the specific anatomical area of action.

1.5 APPLICATIONS OF BIOMATERIALS

Biomaterials are used in:

- Heart valves
- Skin repair devices (artificial tissue)
- Orthopaedics
- Dental implants for tooth fixation
- Tissue

The following are the details of the biomaterial applications.

1.5.1 Heart Valves

In the United States, 45% of the 250,000 valve replacement procedures performed annually involve a mechanical valve implant. The most widely used valve is a bileaflet disc heart valve, or St. Jude valve. The mechanics involve two semi-circular discs moving back and forth, with both allowing the flow of blood as well as the ability to form a seal against backflow. The valve is coated with pyrolytic carbon, and secured to the surrounding tissue with a mesh of woven fabric called Dacron (du Pont's trade name for polyethylene terephthalate). The mesh allows for the body's tissue to grow while incorporating the valve [12].

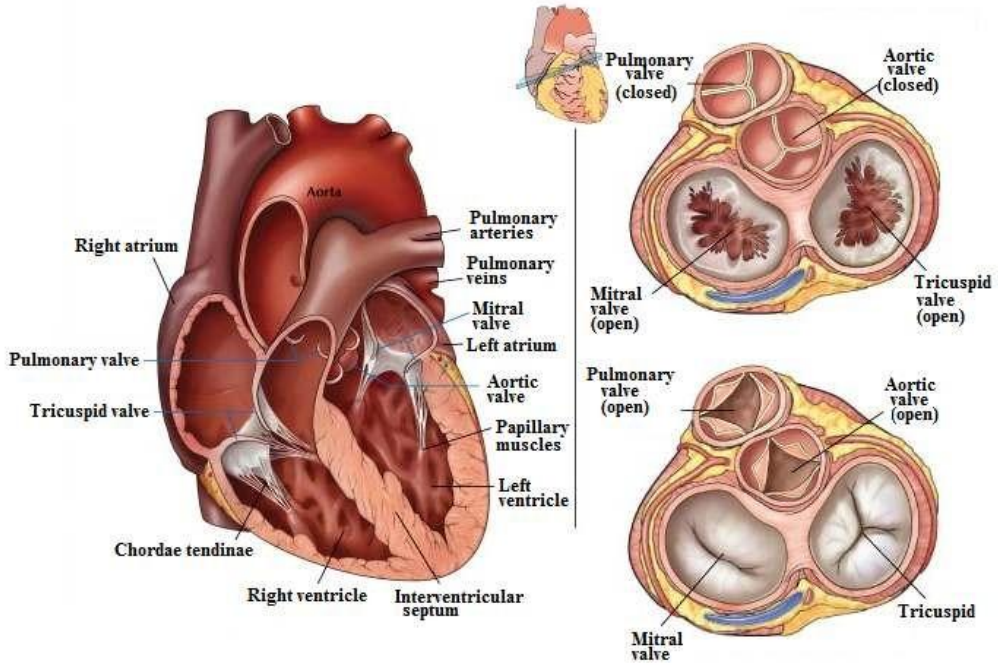


Fig. 1.1 The picture of heart valve anatomy

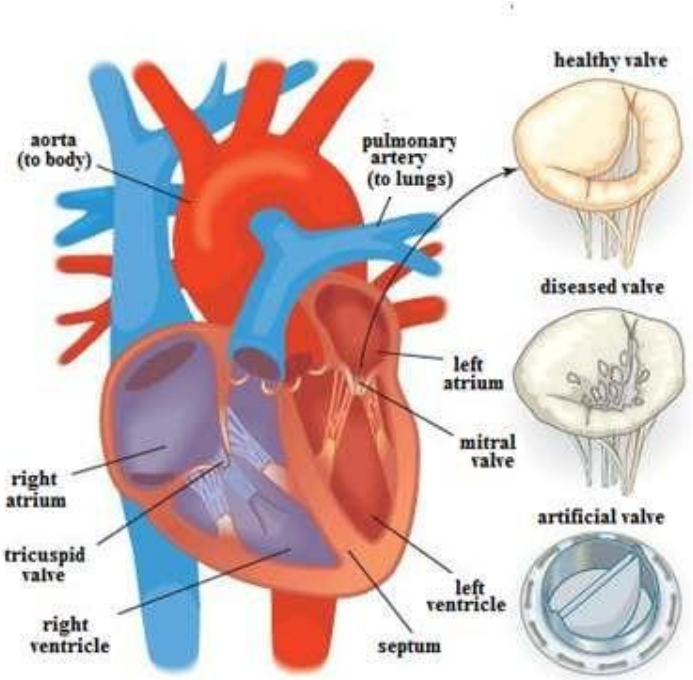


Fig. 1.2 Heart valve implants

1.5.2 Skin Repair

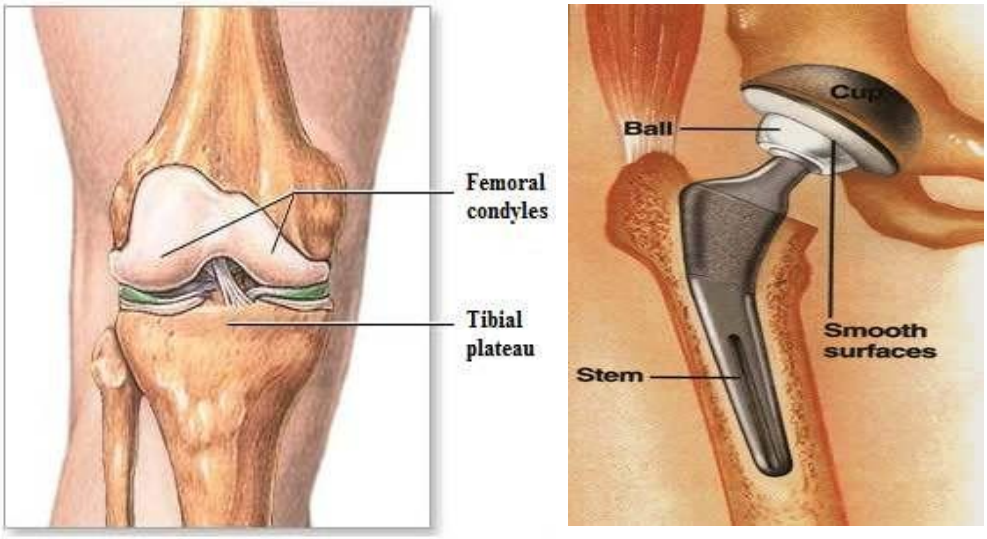
Most of the time "artificial" tissue is grown from the patient own cells. However, when the damage is so extreme that it is impossible to use the patient's own cells, artificial tissue cells are grown. The difficulty is in finding a scaffold that the cells can grow and organize on. The characteristics of the scaffold must be that it is biocompatible; cells can adhere to the scaffold, mechanically strong and biodegradable. One successful scaffold is a copolymer of lactic acid and glycolic acid [12].

Fig. 1.3 Skin graft repair in thigh

1.5.3 Orthopaedics

Metallic, ceramic and polymeric biomaterials are used in orthopaedic applications. Metallic biomaterials are normally used for load bearing members such as pins and plates and femoral stems etc. ceramics such as alumina and zirconia are

used for wear applications in joint replacements, while hydroxyapatite is used for bone bonding applications to assist implant integration.



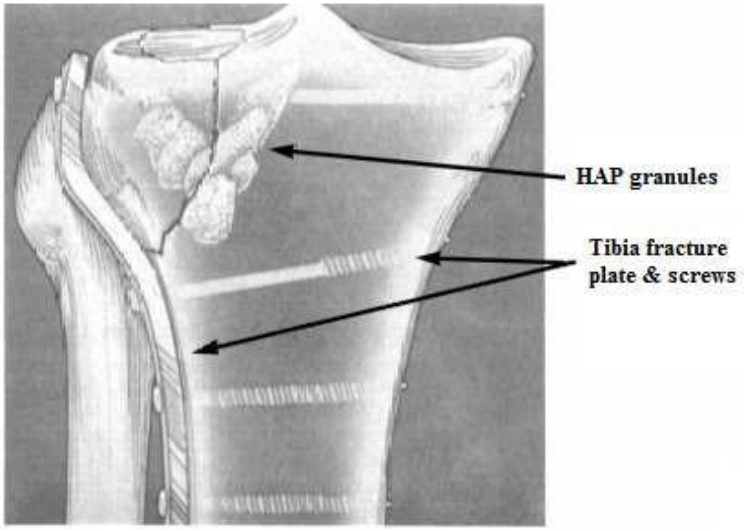


Fig. 1.4 Figures showing the joints in legs and the orthopaedic implants

1.5.4 Dental Applications

Ceramics have found uses as tooth implants including alumina and dental porcelains. Hydroxyapatite has been used for coating on metallic pins and to fill large bone voids resulting from disease or trauma. Polymers are also used as orthodontic devices like plates and dentures.

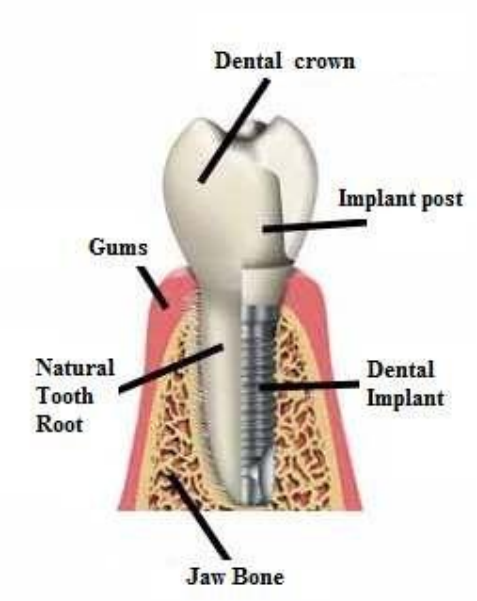


Fig. 1.5 Figures showing natural tooth and dental implants

1.6 CERAMICS

Ceramics used for orthopaedic implant materials include hydroxyapatite, tricalcium phosphate, alumina, Bio glass and other bioactive glasses. Conventional engineering ceramics such as alumina were initially attractive biomaterial options due to their excellent properties of high strength, good biocompatibility and stability in physiological environments. Due to lack of chemical bonding between sintered alumina and bone tissue, its applications are limited. However, alumina can be polished to a high surface finish and possesses excellent wear resistance and therefore is often used for wear surfaces in joint replacement prostheses. Femoral heads for hip replacements and wear plates in knee athroplastic have been fabricated using alumina. In the last 30 years, a move towards ceramic material which bone tissue can achieve a direct bond with, such as hydroxyapatite and Biogases have attracted the attention of biomaterial researchers.

1.7 HYDROXYAPATITE

Hydroxyapatite is a member of the apatite group of ceramics. The term "apatite" is derived from the Greek apatê, which means deceit or deception. It was called such for its diversity of form and colour [13]. It has been tested many times as artificial bone since it is similar to the natural bone though devoid of such organic constituents as collagen and polysaccharides [14]. HAP is the major component, and an essential ingredient, of normal bone and teeth. It makes up bone mineral and the matrix of teeth. Hydroxyapatite, also called hydroxyl apatite, is a naturally occurring mineral in the form of calcium apatite with the formula $Ca_5(PO_4)_3(OH)$, but is usually written as $Ca_{10}(PO_4)_6(OH)_2$ to denote that the crystal unit cell comprises two entities. Hydroxyapatite is the hydroxyl end member of the complex apatite

group. The OH^- ion can be replaced by fluoride, chloride or carbonate, producing fluorapatite or chlorapatite. It crystallizes in the hexagonal crystal system. Pure hydroxyapatite powder is white in colour. Naturally occurring apatite can, however, also have brown, yellow, or green colorations, comparable to the discolorations of dental fluorosis. Up to 50% of bone by weight is a modified form of hydroxyapatite (known as bone mineral) [15]. Carbonated calcium-deficient hydroxyapatite is the main mineral of which dental enamel and dentin are comprised. Hydroxyapatite crystals are also found in the small calcifications (within the pinegland and other structures) known as corporaenacea or 'brain sand'.

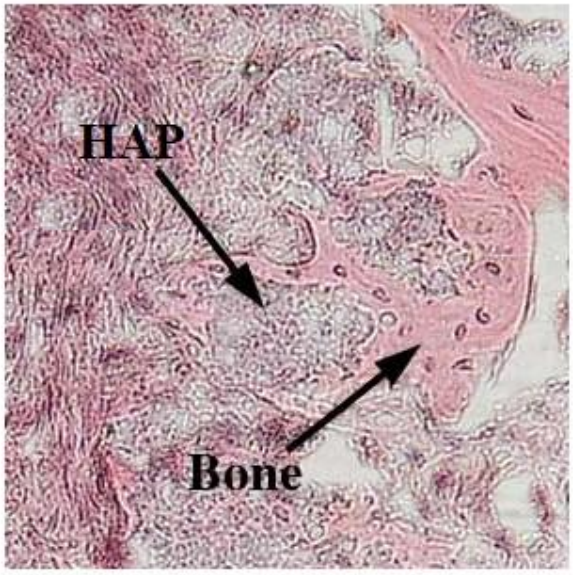


Fig. 1.6 Shows nature of bone and HAP

1.8 BONE ANATOMYAND HISTOLOGY

1.8.1 Bone Composition and Microstructure

Bone is a complex material with many levels of organisation. The composition of bone differs depending on species, age, dietary history [16] and the presence of disease [17], however, general values can be given. The main

constituents are collagen (20 wt. %), calcium phosphate (69 wt. %) and water (9 wt. %) [18]. Other organic materials exist in small quantities (1-2 wt. %). These organic components are made up of 58 wt.% proteins, 40 wt.% lipids and trace amounts of sugars, citrate and lactate ions [19].

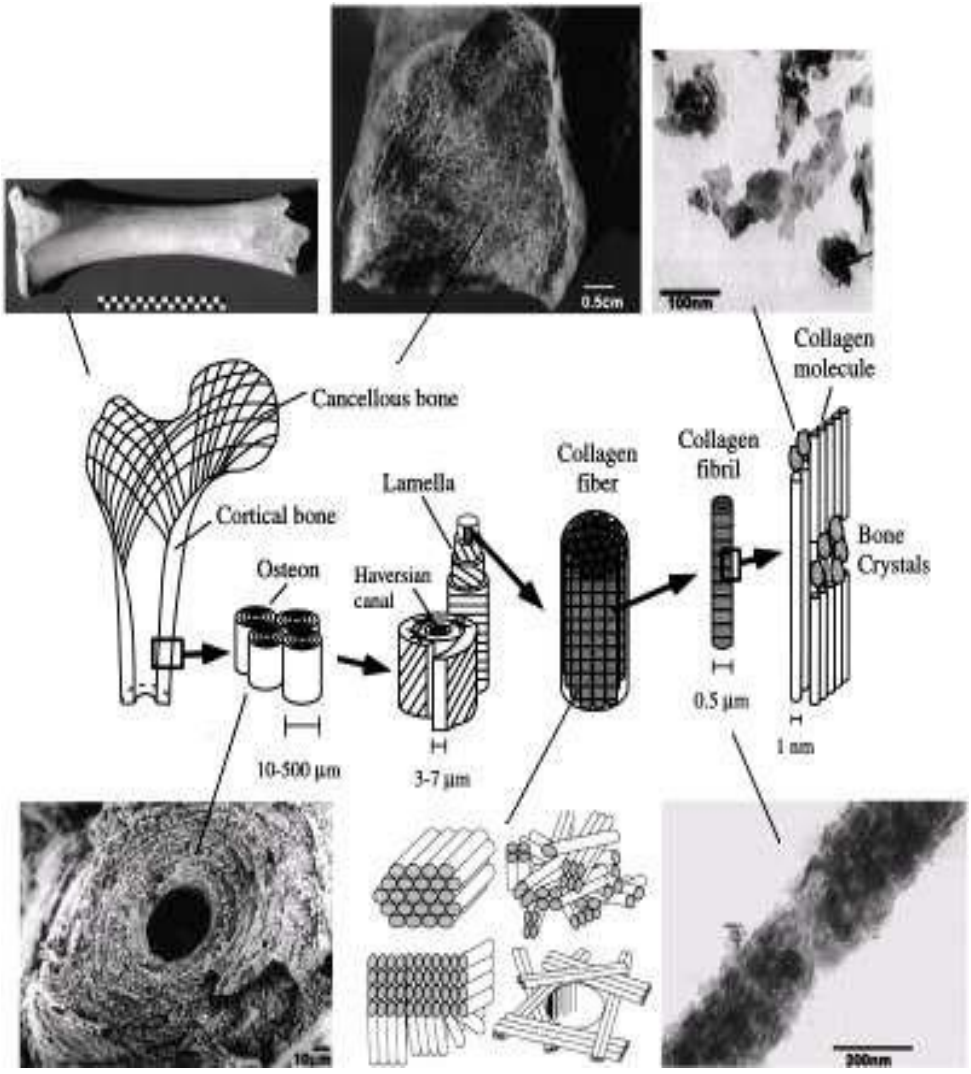


Fig. 1.7 Shows the hierarchical structure of bone down from the macrostructure down to the apatite and collagen nano and sub-nano structures

Collagen fibres, are considered to be the matrix and may vary from 100 to 2000 nm in diameter. Stiffness is supplied by bone mineral crystals, which were previously thought to be pure hydroxyapatite but are now referred to as apatite due to unique acid phosphate groups [20]. These mineral crystals, which are approximately 20-40 nm long and 1.5-5 nm thick [21], are deposited parallel to the collagen fibres. Figure 1.1 shows the hierarchical structure of bone from the macrostructure down to the apatite and collagen nano and sub-nano structures [22,23].

1.8.2 Bone Type

Two mature types of bone exist; compact and cancellous. Compact bone, sometimes known as cortical bone is largely found in the shafts of long bones. Cancellous bone, also known as trabecular or spongy bone, is found in the vertebrae and at the ends of long bones.) This variety of bone contains numerous pores which are filled with either red or fatty marrow [21], which are responsible for the synthesis of red and white blood cells. Both of these types of bone can be made up from a second sub-class termed woven (primary) or (secondary) lamellar bone. Woven bone is considered to be immature and unstructured, which can be found in the prenatal and neonatal stages, in newly formed bone and in the metaphysial region of growing bones in healthy individuals. More structured lamellar bone begins to form about 1 month after birth and actively replaces woven bone [17].

1.8.3 The structure of Long Bones

For the purpose of this thesis only the structure of long bones is considered, however detail of short, flat and irregular bone types can be found elsewhere [24,25].

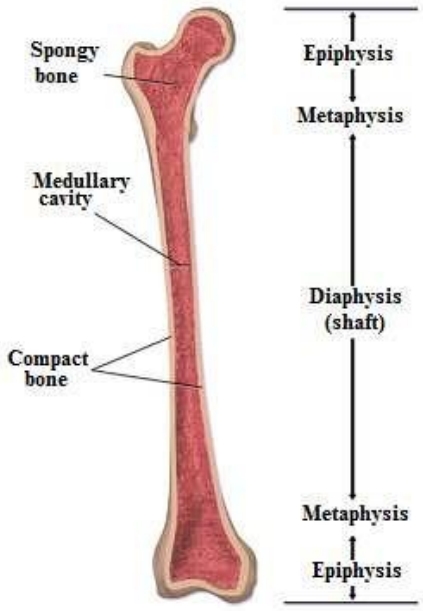


Fig. 1.8 Schematic of a long bone showing the ephiphysis and diaphysis regions

Long bones are present in the arms and thigh and are made up of two distinct the diaphysis and the epiphysis (Figure 1.8.). Sections which is made up of a hollow section of cortical bone. This central areas cavity contains the medullary arterial supply and is occupied chiefly by fatty marrow. The epiphysis is the end section on the bone which is comprised of trabecular or spongy bone. The epiphysis is made up of hyaline cartilage which is important for bone lengthening during childhood, then upon bone maturation progressing into adulthood. The entire bone, except the joint ends where articular cartilage is present, is surrounded by the periosteum. The periosteum consists of an inner osteogenic layer (cambium), which provides appositional growth before maturity, and an outer fibrous layer which is purely supportive. The presence of the active cambium, with longitudinal arterioles, makes the periosteum thick. However, within the mature long-bone the cambium is thin and tenuous.

Deorganized bone and some sea corals (porites) are used to make implants [26-29,20]. The X-ray diffraction, dentine, and bone patterns for enamel are depicted in Figure 1.9.

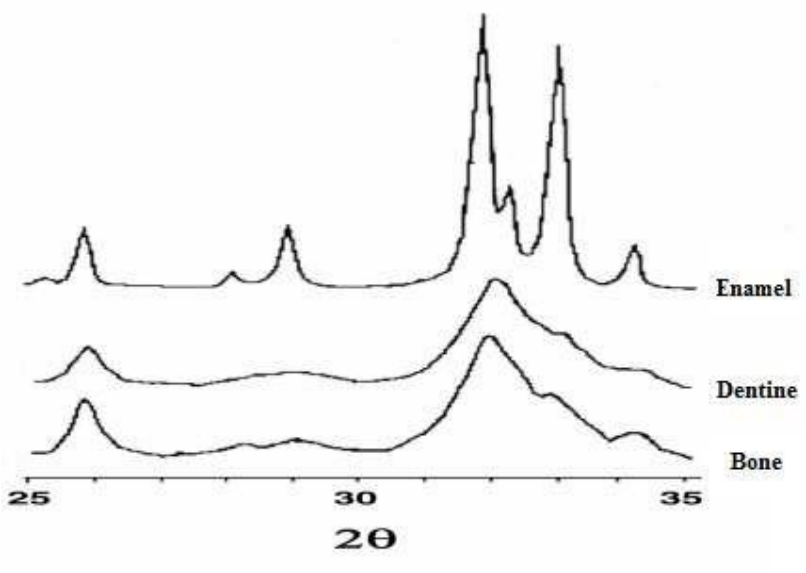


Fig. 1.9. X-ray diffraction patterns of enamel, dentine, and bone.

As can be seen enamel has a sharp and well-defined pattern, as opposed to dentine and bone, which is due to the larger crystallites and higher crystallization of the mineral phase as shown in the Fig. 1.9.

1.9 COMPOSITION AND STRUCTURE OF HAP

There are two sources of apatite: one biological and the other from mineral deposits, such as phosphate rock or phosphorite, a sedimentary rock the essential mineral components of which is carbonate fluoroapatite [13]. Bone and teeth contain a HAP-like mineral component that supports the majority of load in vivo. The chemical composition, crystal structure, and other properties of enamel, dentine, and bone are summarized in Table 1.1.

Table 1.1 Composition and physical properties of apatites in adult human enamel, dentine and bone

Composition	Enamel	Dentine	Bone
Calcium, Ca^{2+}	36.50	35.10	34.10
Phosphorus, as p	17.70	06.90	15.20
(Ca/P) molar	01.63	01.61	01.71
Sodium, Na^{+}	00.50	00.60	00.90
Magnesium, Mg^{2+}	00.44	01.23	00.72
Potassium, K^{+}	00.01	00.05	00.03
Carbonate, as CO_3^{2-}	03.50	05.60	07.40
Fluoride, F^{-}	00.01	00.06	00.03
Chloride, Cl^{-}	00.30	00.01	00.13
Pyrophosphate, $P_3O_7^{4-}$	00.02	00.10	00.07
Total inorganic (mineral)	97.00	70.00	65.00
Total organic	01.50	20.00	25.00
Absorbed H_2O	01.50	10.00	10.00

Trace elements: Sr^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, etc.

Crystallographic properties: Lattice parameters (+0.0003 nm)			
a-axis	0.944100	0.942100	0.9410
c-axis	0.610100	0.610107	0.6109
Crystallinity index”	70~75	33~37	33~37
Crystallite size (nm)	0.13 x 0.03	0.020 x 0.004	0.025 x 0.003
Ignition products (1000 °C)	β-TCMP*+HA	β-TCMP*+HA	HA + CaO

Weight analyses based on ashed samples except for CO_3, which was determined on an unashed samples using an IR method. "Crystallinity index" is determined from the ratio of coherent to incoherent scattering in mineral OH^- apatite taken as 100 [30,31]. *β-TCMP = Mg-substituted β-tricalcium phosphate, or whitlockite in biological systems, β-TCP is always Mg substituted, $(Mg,Ca)_3(PO_4)_2$ [32].

Table 1.2 presents the many techniques for making HAP, of which the hydrothermal method is the most widely studied and commercially used. Calcium hydroxyapatite [33] ceramic is usually prepared from apatites obtained by precipitation or hydrolysis under nonacidic conditions and subsequently sintered at temperatures between 950 and 1300 °C. HAP has been synthesized and used to manufacture various forms of implants (solid and porous) and as a coating on other implants Precipitation can be obtained by the following reactions [34,35].

$$10Ca(NO_3)_2.4H_2O + 6(NH_4)_2HPO_4 + 8NH_4OH \xrightarrow{1100\,^\circ C} Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 46H_2O \quad (1.1)$$

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \xrightarrow{1100\,^\circ C} Ca_{10}(PO_4)_6(OH)_2 + 18H_2O + 12NH_3 \quad (1.2)$$

or by drop wise addition of phosphoric acid to a saturated solution of calcium hydroxide, $Ca(OH)_2$:

$$10Ca(OH)_2 + 6H_3PO_4 \xrightarrow{1100\,^\circ C} Ca_{10}(PO_4)_6(OH)_2 + 18H_2O \quad (1.3)$$

Table 1.2 Preparation techniques for hydroxyapatite

Techniques	Starting materials	Synthetic conditions	Comments
Solid-state reaction	$Ca_3(PO_4)_2$ + $CaCO_3$ $Ca_2P_2O_7$ + $CaCO_3$	900~1,300 °C, usually with water vapour flowing	Ca/P = 1.67, large size, forms, inhomogeneous
Wet chemical Method	$Ca(NO_3)_2$ + $(NH_4)_2HPO_4$ $Ca(OH)_2$ + H_3PO4	R.T. ~ 100 °C pH: 7~1	Ca/P < 1.67, fine irregular crystals with low crystallinity, in homogeneous
Hydrothermal Method	Wet chemically prepared HAP, other calcium phosphates, seeding	100~200 °C (1~2 MPa) 300~600 °C (1~2 kbar)	Ca/P = 1.67, homogeneous, fine single crystals or large crystals
Gel growth Method	Gel + Ca_{2+} + PO_4^{3-}	R.T. ~ 60 °C pH: 7~10	Large monetite, brushite, OCP, but small Hap
Melt growth method	$Ca_3(PO_4)^{2-}$ PO_4^{3-} CaF_2, $CaCl_2$	1650 °C	Large crystals with lattice strain
Flux growth Method	CaF_2, $CaCl_2$ as flux $Ca(OH)_2$ as flux	1325 °C (FAp, ClAp) HAP	Large crystals with little lattice strain

The apatite family of minerals, $A_{10}(BO_4)_6X_2$, crystallizes into a hexagonal rhombic prism. HA has unit cell dimensions of a = 0.9432 nm and c = 0.6101 nm [36]. The atomic structure projected along the c-axis on the basal plane is given in Figure 1.10. Figure1.11 illustrates a three-dimensional view of the crystal structure of hydroxyapatite. The ideal Ca/P ratio of HA is 10/6, and the calculated density is 3.219 g/ml [37]. Hydroxyapatite structure projected down the c-axis on the basal plane. Three-dimensional view of the structure of hydroxyapatite crystal [38,39].

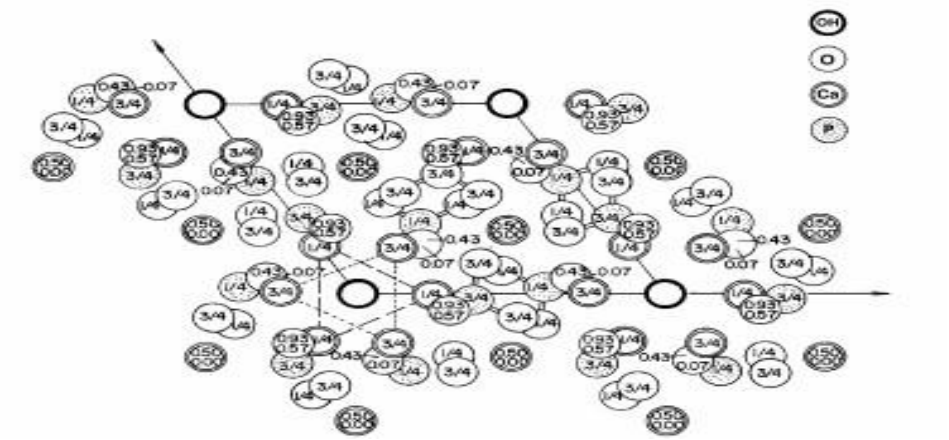


Fig. 1.10. Hydroxyapatite structure projected down the c-axis on the basal plane

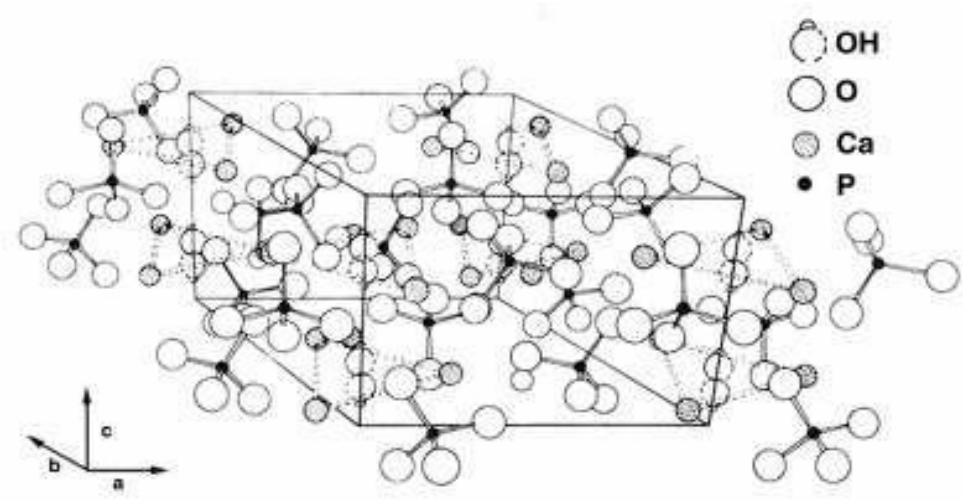


Fig. 1.11. Three-dimensional view of the structure of hydroxyapatite crystal.

1.10 PROPERTIES OF HYDROXYAPATITE

The (bio) chemical and mechanical properties of HAP are similar to those of bone and teeth. Their molecular structures are also similar, although the exact nature of the composite, the minerals and proteins, and their interactions are not fully understood.

1.10.1 Mechanical Properties

There is a wide variation in the reported mechanical properties of HAP Jarcho et al., [35] reported that fully densified polycrystalline specimens of HAP synthesized by them had average compressive and tensile strengths of 917 and 196 MPa, respectively. Kato et al., [40] noted a compressive strength of 3000 kg/cm^2 (294 MPa), a bending strength of 1500 kg/cm^2 (147 MPa), and a Vickers hardness of 350 kg/mm^2 (3.43 GPa).

Table 1.3 Elastic modulus of hydroxyapatite and mineralized tissues

Test method	Material	Elastic modulus (GPa)
Ultrasonic interference technique	Hydroxyapatite (mineral)	144.0 [19]
	Hydroxyapatite (synthetic)	117.0 [19]
	Dentine	021.0 [18]
	Enamel	074.0 [18]
Destructive technique	Human cortical bone	24.6–35.0 [14]
Resonance frequency	Hydroxyapatite (synthetic)	39.4–63.0 [59]
Technique	Canine cortical bone	12.0–14.6 [59]

The elastic modulus of HAP measured by ultrasonic interference and resonance frequency techniques is given in Table 1.3. Although there are some variations in values, depending on measurement technique, it is clear that HAP has a higher elastic modulus than mineralized tissues. Along this line of thought, it is interesting to note that the relatively smaller amount of organic material (mainly collagen) exists in enamel, which has a higher elastic modulus than bone and dentine. This fact is indirect evidence that the mineral portion of the hard tissue is

made of HAP. Poisson's ratio for the mineral or synthetic HAP is about 0.27, which is somewhat close to that of bone (0.3) according to Grenoble [41].

1.10.2 Chemical Properties

Hydroxyapatite is considered as bioactive material, indicating that the ceramic may undergo ionization in vivo and that the rate of dissolution may depend on many factors including degree of crystallinity, crystallite size, processing condition (temperature, pressure, and partial water pressure), and porosity. Hydroxyapatite is soluble in an acidic solution while insoluble in alkaline and distilled water. Solubility in distilled water increases with addition of electrolytes. Moreover, the solubility of HAP changes in the presence of amino acids, proteins, enzymes, and other organic compounds. These solubility properties are closely related to the biocompatibility of HAP with tissues and its chemical reactions with other compounds. However, the solubility rate depends on differences in shape, porosity, crystal size, crystallinity, and crystallite size. The solubility of sintered HAP is very low. The rate of solubility is 0.1 mg/year in subcutaneous tissue [39]. Hydroxyapatite reacts actively with proteins, lipids, and other inorganic and organic species. The most interesting property of HAP is its excellent biocompatibility [42-44,31], the result of its suspected direct chemical bonding with hard tissues [40]. Hench et al. [45] reported epitaxial HAP crystal growth on the surface of Bioglass wafers (1.23 cm diam., 0.32 cm thick) after spreading a 0.254-mm thick layer of amorphous calcium orthophosphate precipitate on its surface. X-ray diffraction analysis of the crystallization of HAP showed an average crystal size of approximately 20 nm, which is in the same range as the observed size for in-vivo mineral crystals [46]. A scanning electron micrograph (Figure 1.6) of a fractured section shows dendritic growth of HAP crystals on a glass-ceramic surface [47].

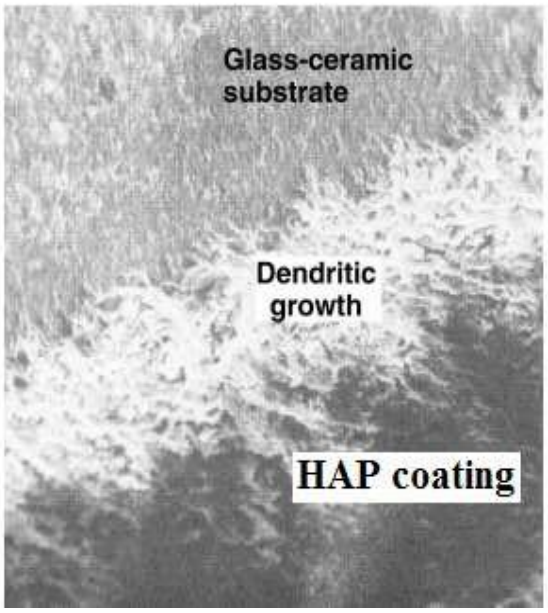


Fig. 1.12. Scanning electron micrograph depicting the morphology of hydroxyapatite crystallized on Bioglass substrate

1.11 DRAWBACKS OF USING CHEMICAL METHODS FOR THE SYNTHESIS OF HYDROXYAPATITE

Chemical method of HAP synthesis involves some toxic chemicals which are adsorbed on the surface that may have adverse effects in the medical applications. However, these methods employ organic solvents or organic templating agents which are potentially dangerous to the environment and biological systems [48].

1.12 ADVANTAGES OF USING GREEN METHOD FOR THE SYNTHESIS OF HYDROXYAPATITE

The synthesis protocols for HAP particles involving environmentally mediated materials like plant extract offers numerous benefits like eco-friendliness and compatibility for pharmaceutical as well as biomedical applications. Further, green template synthesis proves to be the best method rather than the chemical and

physical method, as it is reasonable, inexpensive, easily available and scaled up for large scale synthesis. There is no need to use high pressure, energy, temperature and toxic chemicals in the green synthesis of HAP. Therefore, in recent years many researchers have interested to synthesize nanoparticles with the naturally abundant green materials, since they are renewable, cost-effective and environmentally benign when compared to other synthetic organic templates [49-54]. The natural biological materials contain carbohydrates, proteins, fibres and polysaccharides in significant amounts. They are gifted, eco-friendly, biocompatible as well as biodegradable plant materials and remarkably used as antimicrobial, anticoagulant, anti-inflammatory agent and as antioxidants. They are also used as wound healing substance to tissue sites and as a composite material to alter the mechanical properties [55] and the degree of swelling [56,57]. In extracellular mineralization, such polysaccharides generally rich in carboxyl and hydroxyl groups can promote the binding of calcium ions (Ca^{2+}) from the solution to carboxylate ions. This initiates the crystal nucleation and growth [48] and hence helps in bone fixation thereby promoting bone regeneration [58-60]

1.13 NATURAL EXTRACTS AS TEMPLATE

Natural product extracts can be derived from many parts of green plants like root, stem, vegetable and fruits and they are discussed below one by one.

1.13.1 Root

A Carrot

The carrot (Daucus carota) is a root vegetable, usually orange in colour, though purple, red, white and yellow varieties exist. It has a crisp texture when fresh.

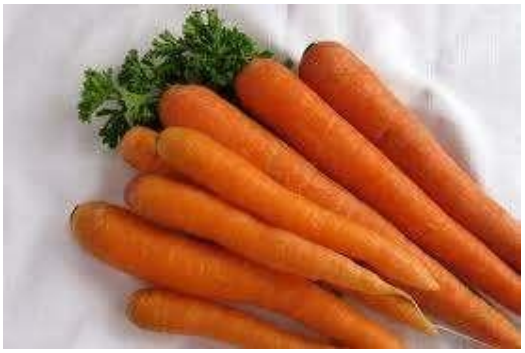


Fig. 1.13. Shows the picture of carrot

The most commonly eaten part of a carrot is a taproot, although the greens are sometimes eaten as well. Carrots are widely used in many cuisines, especially in the preparation of salads, and carrot salads are a tradition in many regional cuisines.

1.13.2 Stem

A Sugarcane

Sugarcane is any one of six to 37 species of tall perennial true grasses of the genus saccharum, tribe Andropogoneae, native to the warm template to tropical regions of south Asia sugarcane belongs to the grass family (Poaceae), an economically important seed plant family. The main product of sugarcane is sucrose, which accumulates in the stalk internodes. Sucrose, extracted and purified to specialized mill factories, is used as raw material in human food industries.

Fig. 1.14 Show the picture of sugarcane cultivation

1.13.3 Fruits

A Apple

The apple is the pomaceous fruit of the apple tree, species Malus domestica in the rose family Rosaceae. It is one of the most widely cultivated tree fruits, and the most widely known of the many members of genus Malus that are used by humans. Apples grow on small, deciduous trees. The tree originated in central Asia, where its wild ancestor, Malus sieversii, is still found today.

Fig. 1.15 Shows the picture of apple

A Tomato

The tomato is the edible red fruit the night shade solanum lycopersicum [61,62] commonly known as a tomato plant. The species originated in the South American Andes [62] and its use as a food originated in Mexico, and spread throughout the world following the Spanish colonization of the Americas. Its many varieties are now widely grown, sometimes in greenhouses in cooler climates. The tomato is consumed in diverse ways, including raw, as an ingredient in many dishes, sauces, salads, and drinks. While it is botanically a fruit, it is considered a vegetable for culinary purposes (as well as under U.S. customs regulations, see Nix v. Hedden), which has caused some confusion. The fruit is rich in lycopene, which may have beneficial health effects. The tomato belongs to the nightshade family, Solanaceae [61,63]. The plants

typically grow to 1-3 meters (3-10 ft) in height and have a weak stem that often sprawls over the ground and vines over other plants. It is a perennial in its native habitat, although often grown outdoors in temperate climates as an annual. An average common tomato weighs approximately 100 grams (4 oz) [64,65].

Fig. 1.16 Shows the pictures of tomato plant

A Pineapple

The pineapple (Ananas Comosus) is a tropical plant with edible multiple fruit consisting of coalesced berries [66] and belongs to the Bromeliaceae family [67]. Pineapple may be cultivated from a crown cutting of the fruit [68], possibly flowering in 20-24 months and fruiting in the following six months [68,69]. Pineapple are consumed fresh, cooked, juiced and preserved and are found in a wide array of cuisines.

Fig. 1.17. Shows the picture of pineapple and its slices

A Banana

A banana is an edible fruit produced by several kinds of large herbaceous flowering plants in the genus Musa [70]. The fruit is variable in size, colour and firmness, but is usually elongated and curved, with soft flesh rich in starch covered with a rind which may be green, yellow, red, purple or brown when ripe. The fruits grow in clusters hanging from the top of the plant. Almost all modern edible parthenocarpic (seedless) bananas come from two wild species - Musa acuminata and Musa balbisiana.

Fig. 1.18. Shows the pictures of banana tree and its fruit

A Grapes

A grape is a fruiting berry of the deciduous woody vines of the botanical genus Vitis.

Fig. 1.19. Shows the picture of hanging grapes

Grapes can be eaten raw or they can be used for making wine, jam, juice, jelly, grape seed extract, raisins, vinegar and grape seed oil. Grapes are a non-climateric type of fruit, generally occurring in clusters.

A Tamarind

Tamarind (Tamarindus indica) is a leguminous tree in the family Fabaceae indigenous to tropical Africa.

Fig. 1.20. Shows the picture of tamarind

The genus Tamarindus is a monotypic taxon, having only a single species. The tamarind trees produce edible, pod-like fruit which are used extensively in cuisines around the world.

1.14 APPLICATIONS OF HYDROXAPATITE

Hydroxyapatite can be found in teeth and bones within the human body. Thus, it is commonly used as a filler to replace amputated bone or as a coating to promote bone ingrowth into prosthetic implants. Although many other phases exist with similar or even identical chemical makeup, the body responds much differently to them. Coral skeletons can be transformed into hydroxyapatite by high temperatures; their porous structure allows relatively rapid ingrowth at the expense of initial mechanical strength. The high temperature also burns away any organic molecules such as proteins, preventing an immune response and rejection. Many modern implants, e.g. hip replacements and dental implants, are coated with hydroxyapatite. It has been suggested that this may promote [71] osseointegration. Porous hydroxyapatite implants are used for local drug delivery in bone [72,73].

1.14.1 Bone Fillers

Hydroxyapatite may be employed in forms such as powders, porous blocks or beads to fill bone defects or voids. These may arise when large sections of bone have had to be removed (e.g. bone cancers) or when bone augmentations are required (e.g maxillofacial reconstructions or dental applications). The bone filler will provide a scaffold and encourage the rapid filling of the void by naturally forming bone and provides an alternative to bone grafts. It will also become part of the bone structure and will reduce healing times compared to the situation, if no bone filler was used.

1.14.2 Bioceramic Coatings

Coatings of hydroxyapatite are often applied to metallic implants (most commonly titanium/titanium alloys and stainless steels) to alter the surface properties. In this manner the body sees hydroxyapatite-type material which it is happy to accept. Without the coating the body would see a foreign body and work in such a way as to isolate it from surrounding tissues. To date, the only commercially accepted method of applying hydroxyapatite coatings to metallic implants is plasma spraying.

1.14.3 Orthopaedic Applications

When the bone is damaged due to injury and illness, the defects are generally filled with natural bone because artificial bone materials have the problem of bioaffinity. However, the natural bone can induce infection problems and antigenic reaction [74], where the HAP has been used in bone-ingrowth, sintered dense hydroxyapatite for implants [75-77] and as a coating material for metallic implants because it is more soluble in physiological environment [78,79].

1.14.4 Cardiovascular Applications

In the cardiovascular, or circulatory system (the heart and blood vessels involved in circulating blood throughout the body), problems can arise with heart valves and arteries, both of which can be successfully treated with implants. The heart valves suffer from structural changes that prevent the valve from either fully opening or fully closing, and the diseased valve can be replaced with a variety of bioceramic substitute materials.

1.14.5 Ophthalmic Applications

The tissues of the eye can suffer from several diseases, leading to reduced vision and eventually, blindness. Cataracts, for example, cause cloudiness of the lens. This may be replaced with synthetic biomaterials (intraocular lens).

1.14.6 Dental Applications

Within the mouth, both the tooth and supporting gum tissues can be readily destroyed by bacterially controlled diseases. Dental caries (cavities), the demineralization and dissolution of teeth associated with the metabolic activity in plaque (a film of mucus that traps bacteria on the surface of the teeth), can cause extensive tooth loss. Teeth in their entirety and segments of teeth either can be replaced or restored by a variety of biomaterials like HAP, glass and other ceramic materials.

1.14.7 Drug Delivery Systems

One of the fastest growing areas for implant applications is controlled and targeted delivery of drugs. Many attempts have been made to incorporate drug reservoirs into implantable devices for a sustained and preferably controlled release. Biomaterial like HAP finds extensive application in drug delivery systems.

1.14.8 Other Applications of HAP

HAP also finds its applications in pathology, chromatography, archaeology and in animal structures

A Pathology

Hydroxyapatite deposits in tendons around joints in the medical condition calcific tendinitis.

A Chromatography

The mechanism of hydroxyapatite chromatography is complicated and has been described as "mixed-mode" ion exchange. It involves nonspecific interactions between positively charged calcium ions and negatively charged phosphate ions on the stationary phase HAP resin with protein negatively charged carboxyl groups and positively charged amino groups. It may be difficult to predict the effectiveness of HAP chromatography based on physical and chemical properties of the desired protein to be purified. For elution, a buffer with increasing phosphate concentration is typically used.

A Archaeology

In archaeology, hydroxyapatite from human and animal remains is analysed in order to reconstruct ancient diets. The mineral fractions of bone and teeth act as a reservoir of trace elements, including strontium. It has been established that the ratio of strontium to calcium in bone hydroxyapatite broadly reflects an animal's diet during the period before its death when the bone was being formed (5–10 years in the case of human remains), or in the case of dental mineral in childhood. In either case analysis of Sr/Ca ratios allows an individual's diet to be classified as carnivorous, herbivorous or omnivorous and either predominantly marine or terrestrially based. However the difficulty of compensating for post-mortem contamination of archaeological samples through interaction with groundwater continues to cast doubt on the reliability of the method. Stable isotope analysis is considered a more viable alternative, although strontium and other trace mineral analyses of dental samples are commonly used in situations where this is impossible because the collagen content of the bone has completely decayed (i.e. for Palaeolithic samples) [80,81].

A Animal structures

The clubbing appendages of the Peacock mantis shrimp are made of an extremely dense form of the mineral, which is being investigated for potential synthesis and engineering use [82,83].