CHAPTER 1

INTRODUCTION

1.1 BIOMATERIALS

In modern world, millions of people are suffering from different types of diseases or injuries such as heart attack, tissues damage, bone cancer, dental, hip, knee and eye infection, etc. The effective treatment of these diseases or injuries requires synthetic materials which have better biocompatibility, bioactivity, ability to augment damaged organs, blood vessels, and replacement of hard and soft tissues. Materials possessing such properties and have the ability to contact and bond with the living tissue or biological fluids are called biomaterials (Hench 1991). The biomaterials can be classified as biomedical, with an artificial origin (metals, ceramics, polymers and composites) and biological, which have a natural (vegetal, animal or human) origin (Figure 1.1).

The world wide market for all types of biomaterials was estimated at over $23 billion. With the recent emergence of the field known as tissue engineering, including its strong biomaterials segment, the rate of market growth has been estimated at about 12 to 20 % per year (Ratner 2004). Particularly, Indian and Chinese markets for dental, bone graft substitutes and other biomaterials have grown over 53.2 % by 2009. The market is expected to grow rapidly as the need for the number of bone, hip, knee and dental implants increases owing to people affected by the hard tissue diseases.
Prostheses may be secured in the bone using cement or by bony ingrowth onto the implant’s surface.

**Figure 1.1 Classification of biomaterials**

Surface properties and size of a material plays an important role in the field of biomaterial science and engineering. The engineering of functional systems at the molecular scale is termed as nanotechnology. The typical molecular scale spans from subnanometer to several hundred nanometers. A nanometer (nm) is described as one billionth of a meter, or $10^{-9}$ m. Nanotechnology is an inter-disciplinary field with cooperation from researchers in several fields of studies including physics, chemistry, biology, material science, biomedical, engineering, and computer science. Materials in the micrometer scale mostly exhibit physical properties, same as that of the bulk form; however, materials in the nanometer scale may exhibits physical
properties distinctively different from that of bulk. Nanomaterials possess a large fraction of surface atoms per unit volume. Recently, nanobiomaterials and nanomedicine are emerging area in the field of biomedical research. Most of the nanomaterials used in medicine are used for various applications, e.g. polymers (drug delivery), metallic nanoparticles (diagnostic), iron oxide nanoparticles (hyperthermia) and CdS quantum dots (targeted drug delivery). Nanomaterials of metals, polymer, ceramics and its composites have been fabricated for replacement of hard tissues in human body (Vallet-Regi 2001).

1.1.1 Metals

Metals and their alloys are used as biomaterials because of their strength and toughness. Most important in this class are stainless steel, cobalt-chromium alloy, titanium, nickel, aluminum, zinc, and their alloys. Metals and alloys are utilized in replacement devices for joints such as hips and knees, internal fixation devices such as bone plates, screws and pins for hard tissues, and stents for the opening of blood vessels, the oesophagus, and urinary tracks. After severe trauma in the head region, the surgical removal of part of the cranium is often the only possibility to save the patient (Brady et al 1979). The best available material for this particular purpose is pure titanium, which is used in the manufacturing of the required implants. Dental applications employ gold and its alloys. Tantalum is used in special cases such as wire sutures in some plastic and neurosurgical applications. Alloys of platinum and other non corrosive metals in the group are used in pacemakers as conducting leads and other components. Nickel containing stainless steel has been used in early hip implants for its good strength and pitting corrosion resistance. Co-alloys are advantageous for the fabrication of the parts of medical devices subjected to wear like the heads of artificial hip joints. The ability to form an inert thin oxide layer on the surface of aluminum and titanium is also advantageous in many applications. While the widely used
Implant materials are generally biocompatible, they may release ions (Heimann 2002). For example, stainless steels could potentially release Ni\(^{2+}\), Cr\(^{3+}\) and Cr\(^{6+}\) ions on a long-term basis into the body due to which their use is restricted to temporary devices.

1.1.2 Polymers

Polymers are the most versatile class of biomaterials, being extensively applied in medicine. Polymers are very large molecules made up of smaller units, called monomers or repeating units covalently bonded together. Compared with metal and ceramics, polymers offer the advantage of cost-effective synthesis of desirable compositions with appropriate physical, interfacial and biomimetic properties. The polymers are used for soft tissue applications. However, the very strength of a rigid metallic implant used in bone fixation can lead to problems with stress shielding whereas a bioabsorbable polymer implant can increase ultimate bone strength by slowly transferring load to the bone as it heals. Biopolymers are usually used for their flexibility, biocompatibility, sterilizability, chemical resistance, rigidity, and capable of controlled stability or degradation in response to biological condition. Biopolymers can be classified as, (i) natural, synthetic, or combination of both (semi-synthetic), (ii) degradable or non-degradable and (iii) structural or non-structural. Natural derived polymers are abundant and are usually biodegradable. For example, polysaccharide chitin is the second most abundant natural polymer in the world after cellulose. Natural polymers such as collagen, fibrin, alginate, agarose, hyaluronic acid, chitosan etc., are used as scaffolds. The disadvantage of natural polymers lies in the development of reproducible production methods because their structural complexity often renders modification and purification difficult. Additionally, significant batch-to-batch variations occur because of their preparation in living organisms.
Synthetic polymers are available in a wide variety of composition with readily adjusted properties. Synthetic non-resorbable polymers such as polytetra fluoroethylene (Teflon®), poly vinylidene fluoride, poly(glycolic acid), poly(lactic acid), poly(caprolactone), poly(aminog acids), and poly(phosphazenes) are used for vascular grafts, barrier membranes, long term drug delivery, orthopedic implant, tissue engineering, and blood contacting device. Synthetic resorbable polymers such as poly(propylene fumarate), collagen, gelatin, cellulose, chitin, chitosan, alginate, fibrinogen, and fibrin are used for orthopedic implant, artificial skin, capsule coating for oral drug delivery, adhesion barrier, hemostat, wound dressings, and tissue engineering. Commonly used polymers are ultrahigh molecular weight polyethylene (UHMWPE), polypropylene, polymethyl methacrylate (PMMA), poly(urethanes), poly(dimethy siloxane), poly(ethylene vinyl acetate), poly(vinyl alcohol), polystyrene, and aromatic polyesters are used for hip joint total prostheses, bone and dental cement, Intraocular lens, artificial organs, cosmetic implants, non-degradable drug delivery devices, tissue regeneration, breast implant, implantable biosensor, ophthalmic prostheses, tissue culture plastic, and permanent vascular graft (Williams 2003). The main advantage of degradable polymer implants is avoiding the second surgery whereas the main disadvantage is a tendency to release harmful acids, organometallics used as polymerization initiators, chemical reactivity and other toxins during polymer/tissue interactions (Oh et al 2006).

Hydrogels are also polymers which are used as scaffold for tissue engineering (Tabata et al 2003). It can swell without dissolving when placed in water or other biological fluids. At equilibrium, hydrogels typically comprise of 60-90% fluid and only 10-30% polymers. The structural changes are versatile and repeatable upon additional changes in the external environment. Hydrogels are attractive because of their high water content,
tissue-like mechanical properties, and ability to be polymerized in vivo under physiological conditions.

1.1.3 Ceramics

Ceramics are inorganic, nonmetallic, refractory, polycrystalline compounds. Ceramics are generally used for their hardness, high wear resistance, high modulus (stiffness), high compressive strength, low coefficient of friction, good electrical insulation and dielectric properties. Ceramics and glasses are used as components of hip, dental, middle ear implant, heart valves, and implantable electronic sensors. The bioceramics (ceramics used for biomedical applications) are classified into two categories, namely inert and bioactive. Bioinert (non-absorbables) is compatible to body fluid but lack interactions with the surrounding tissue. These ceramics are non-toxic, non-carcinogenic, non-allergic, relatively non-inflammatory, and have excellent corrosion resistance. They are made up of metal oxides such as alumina, zirconia, silicone nitrides, and pyrolytic carbon. Highly pure alumina can be used for replacing the metal femoral heads of hip prostheses. Zirconia is used for load bearing application due to the high mechanical strength. Carbon composites have found use as implants especially for blood interface applications such as heart valves. With the advance of nanotechnology these inert bioceramics have gained again an active role. Through nanostructuring their mechanical properties, biocompatibility and chemical homogeneity are enhanced (Catledge et al 2002).

Bioactive ceramics are placed within the human body where they interact with the surrounding tissue through an ion exchange reaction at the surface. These favorable interactions result in the direct chemical bonding with the host biological tissue. Further, it is classified as resorbable and non-resorbable dependant upon the level they are absorbed by the living tissues
Non-resorbable bioactive materials bring out a specific biological response at the interface, which results in the formation of a biological bond between the adjacent tissues and the material itself. They include calcium phosphate ceramic (hydroxyapatite), bioactive glasses, bioactive glass-ceramics and calcium sulphates. Hydroxyapatite is a naturally occurring mineral and is present in the structural component of bone. It is stable at physiological pH and actively takes part in forming strong chemical bonds with the surrounding bone. It is most widely used for dental implants, periodontal treatment, alveolar ridge augmentation, and maxillofacial surgery (Ducheyne and Cuckler 1992).

Resorbable bioactive ceramics degrade gradually over a period of time and are replaced by the natural host tissues. Examples include a variety of phosphates (calcium, tricalcium, aluminum-calcium, zinc sulfate-calcium), oxides (zinc-calcium-phosphorous, ferric-calcium-phosphorous), corals (calcium carbonate), and calcium sulphate dihydrate. They are useful in the replacement or repair of damaged bone by trauma or disease, coating of metal implants to promote bone in-growth, repair and fusion of vertebrate, repair of herniated disks, repair of maxillofacial and dental defects, and drug delivery. Complications in the development of resorbable bioceramics are the maintenance of the strength and stability of the interface during the degradation and replacement period by the natural host tissue and the matching resorption rates to the repair rates of the body tissues (Ducheyne et al 1993).

1.1.4 Composites

In many applications, a single biomaterial may not provide all the necessary properties. For example, metallic or ceramic for the bone has some limitations as the modulii of metals and ceramics are very high. To overcome
some of the limitations, composites are developed by combining two or more components. In a composite, one component forms the continuous phase in which other components are embedded as discontinuous inclusions in the shape of plates, fibers or particles. Polymer based composites possess a wide spectrum of properties, which allow them to be used in a diverse range of medical applications.

For example, titanium mesh is used to reconstruct craniofacial defects where contours suitable to the patient have to be formed. Poly(etheretherketone) (PEEK) is used for cortical bone implants. Hip stems are made from carbon filled PEEK compounds that demonstrate elastic properties similar to the surrounding bone and that reduce the effects of stress shielding. Some of the composite biomaterials include dental composites (acrylic polymer matrix with inclusions of inorganic such as quartz, barium glass and colloidal silica) and orthopedic components (high density polyethylene matrix with inclusions of carbon fibers). Biodegradable composites is formed for use as bioactive matrices to guide and support tissue in-growth. Composites are prepared using polyhydroxy-butyrate (PHB) a naturally occurring β-hydroxyacid linear polyester, and hydroxyapatite (HAp) or tricalcium phosphate (TCP). One of the goals is to achieve a reasonable homogeneous distribution of the HAp/TCP particles in the PHB matrix, as this uniformity would provide an anchoring mechanism when the materials would be employed as part of an implant. It is observed that microhardness increased with an increase in bioceramic contents for both the HAp and TCP compounds. The material is composed of bioactive HAp particles uniformly dispensed in a dense polyethylene matrix. The composite is widely used clinically as “HAPEX” for middle ear reconstruction (Rea and Bonfield 2004). HAp/(agarose/gelatin) is also used as bone scaffold (Sivakumar and Rao 2002).
1.2 CALCIUM PHOSPHATES

Calcium phosphates (CaP) are most important material in the bioceramics group. CaP are major inorganic mineral constituents of biological hard tissues. In the form of carbonated hydroxyapatite, they are present in bone, teeth and tendons to give these organs stability, hardness and function. In addition, various phase of calcium phosphates exist in our body. Apatite \(((\text{Ca},\text{Z})_{10}(\text{PO}_4,Y)_6(\text{OH},X)_2))\) occurs in enamel, dentine, bone, dental calculi, stones, urinary calculi, and soft-tissue calcifications. Octacalcium phosphate \((\text{Ca}_8\text{H}_2(\text{PO}_4)_6\cdot5\text{H}_2\text{O})\) is found in dental and urinary calculi. Brushite and dicalcium phosphate dihydrate (DCPD) \((\text{CaHPO}_4\cdot2\text{H}_2\text{O})\) occurs in dental calculi, decomposed bones, crystalluria, and chondrocalcinosis. Whitlockite, tricalcium phosphate, and \(\beta\)-TCP \((\text{Ca},\text{Mg})_9(\text{PO}_4)_6\) are found in dental and urinary calculi, salivary stones, dentinal caries, arthritic cartilage, and soft-tissue calcifications. Amorphous calcium phosphate (ACP) \((\text{Ca},\text{Mg})_x(\text{PO}_4)_y\) occurs in soft tissue calcifications. Calcium pyrophosphate dihydrate \((\text{Ca}_2\text{P}_2\text{O}_7\cdot2\text{H}_2\text{O})\) is observed in pseudo-gout deposits in synovium fluids (LeGeros 1991).

Synthetic CaP has been used for the past 50 years in the clinical applications (Kalita et al 2007). There are various phases in CaP family, many of which are used in the biomedical applications. Several calcium phosphates differentiated according to their Ca/P ratio (0.5-2.0) are as shown in Table 1.1 (Fernandez et al 1999, Vallet-Regi and Gonzalez-Calbet 2004). The solubility is given as the logarithm of the ion product of the given formula (excluding hydrate water) with concentrations in mol/L (Fernandez et al 1999). Nowadays, research is focused in the production of biomaterial which can be used as an implant along with clinical aspects.
Table 1.1 Various CaP phases

<table>
<thead>
<tr>
<th>Name</th>
<th>Formula</th>
<th>Ca/P ratio</th>
<th>Solubility at 25 °C -log(K_{sp})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium phosphate monohydrate (MCPM)</td>
<td>Ca(H₂PO₄)₂·H₂O</td>
<td>0.5</td>
<td>1.14</td>
</tr>
<tr>
<td>Dicalcium phosphate (DCP)</td>
<td>CaHPO₄</td>
<td>1.0</td>
<td>6.90</td>
</tr>
<tr>
<td>Dicalcium phosphate dihydrate (DCPD)</td>
<td>CaHPO₄·2H₂O</td>
<td>1.0</td>
<td>6.59</td>
</tr>
<tr>
<td>Octacalcium phosphate (OCP)</td>
<td>Ca₈H₂(PO₄)₆·5H₂O</td>
<td>1.33</td>
<td>96.6</td>
</tr>
<tr>
<td>Tricalcium phosphate (TCP)</td>
<td>Ca₃(PO₄)₂</td>
<td>1.5</td>
<td>25.5</td>
</tr>
<tr>
<td>Amorphous calcium phosphate (ACP)</td>
<td>Ca₁₀₋ₓH₂ₓ(PO₄)₆(OH)₂(x=2)</td>
<td>1.2-2.2</td>
<td>25.7-32.7</td>
</tr>
<tr>
<td>Hydroxyapatite (HAp)</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>1.67</td>
<td>116.8</td>
</tr>
<tr>
<td>Tetracalcium phosphate (TTCP)</td>
<td>Ca₄O(PO₄)₂</td>
<td>2.0</td>
<td>38-44</td>
</tr>
</tbody>
</table>

Among these calcium phosphate phases, hydroxyapatite, tricalcium phosphate and its mixed phase are discussed below.

1.2.1 Tricalcium Phosphate

TCP is an inorganic compound which is used as a bioresorbable ceramic for repairing bone tissue. Bioresorbable ceramics are promising candidates for biomaterials as they show biodegradability through bone remodeling as well as high biological affinity allowing direct contact with living bone (Rejda et al 1977). There are typically two crystalline phases of tricalcium phosphate, the alpha-phase (α-TCP) and the beta-phase (β-TCP). Crystal structure of α-TCP is monoclinic (a = 12.88 Å, b = 27.28 Å and c = 15.21 Å, β = 126.20°) with P2₁/a space group. β-TCP is rhombohedral,
R3cH space group and unit cell parameters $a = b = 10.43 \, \text{Å}$ and $c = 37.37 \, \text{Å}$ with $\gamma = 120^\circ$ (Dorozhkin and Epple 2002). β-TCP ceramics are used as bioresorbable bone substitutes, while α-TCP powders are the main component of bioactive pastes used as bone fillers called calcium phosphate cement. α-TCP is thermodynamically stable at above 1100 °C, while β-TCP is stable below 1100 °C (Metsger et al 1982, Fujishiro et al 2001). The solubility of α-TCP is higher than that of β-TCP, which leads to a higher rate of degradation. The high solubility of α-TCP is desirable for a scaffold in drug releasing systems (Yuan et al 2001) than β-TCP. Recently, α-TCP ceramic was developed with continuous pores by a conventional sintering processing (Kitamura et al 2004). Porous α-TCP ceramics were easily degraded after implantation in bone defects.

1.2.2 Hydroxyapatite

The most used calcium phosphate in implant production is HAp, because it is similar in composition to the mineral component of bones and teeth (Elliott 1973, 1994). It exhibits valuable properties as a biomaterial including biocompatibility, bioactivity, osteoconductivity, direct bonding with bones etc. HAp ($\text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2$) is the most stable and least soluble compared to other calcium phosphates (Table 1.1). Pure HAp crystallizes in the monoclinic space group P2$_1$/b ($a = 9.84 \, \text{Å}, b = 2a \, \text{Å}, c = 6.88 \, \text{Å}$ and $\gamma = 120^\circ$) (Dorozhkin and Epple 2002). However, at temperature above 250 °C, there is a monoclinic to hexagonal phase transition in HAp with space group P6$_3$/m. The most common crystal structure is hexagonal with space group P6$_3$/m having cell parameters $a = b = 9.43 \, \text{Å}$ and $c = 6.88 \, \text{Å}$, $\alpha = \beta = 90^\circ$ and $\gamma = 120^\circ$, where ‘P’ is a primitive hexagonal system, P6$_3$/m refers to a space group with a six-fold symmetry axis with a three-fold helix and a miller plane and density is 3.16 g/cm$^3$ (Dorozhkin and Epple 2002). Some impurities, like partial substitution of hydroxide by fluoride or chloride
ions, stabilize the hexagonal structure of HAp at ambient temperature. HAp has been used for many biomedical applications such as orthopedics, dental implants and bone cements (Vallet-Regi 2006). It acts as a reinforcement material in hard tissues which is responsible for the stiffness of bone, dentin and enamel. In addition, nanosized HAp can be used as a drug carrier to deliver drugs (anticancer and antibiotic) to the infected sites (Yamashita et al 1998).

1.2.3 Biphasic Calcium Phosphate

Several attempts have been made to synthesize the mineral component of bones starting from biphasic mixtures of calcium phosphates (Daculsi 1998). Hence, bone replacing materials based on mixtures of HAp and β-TCP has been prepared which under physiological conditions evolve to carbonate hydroxyapatite. The chemical reactions are based in equilibrium conditions between the more stable phase (HAp) and the phase prone to resorption (β-TCP). As a consequence, the mixture is gradually dissolved in the human body, acting as a stem for newly formed bone and releasing Ca\(^{2+}\) and PO\(_4^{3-}\) to the local environment. This material can be injected, used as coating or in any other form suitable for application as bulk bone replacement forming of bulk pieces, filling of bone defects.

At present, a wide range of biphasic mixtures are under preparation, using various calcium phosphates, bioactive glasses, calcium sulphates, etc. (Ragel et al 2002). Currently, there is an increasing interest on the preparation of mixtures of two or more calcium phosphates. These materials are commonly prepared with hydroxyapatite and a more resorbable material such as tricalcium phosphates (α and β) or calcium carbonate in different proportions depending on the characteristics required for a specific application. BCP ceramic control the resorbability of TCP and at the same
time maintains the osteoconductive property of HAp. BCP are widely used for various clinical applications like periodontal bone defects, orthopedics, face and jaw surgery. The main attractive feature of BCP is their ability to form a strong direct bond with the host bone (Ozalay et al 2009).

1.3 CALCIUM PHOSPHATE COATING

At present, for all those clinical applications where load-bearing properties are required, most of the implants used are metallic, with subsequent and serious problems due to: (i) the large differences in mechanical properties between the artificial implant and the natural bone, giving rise to ruptures, (ii) the presence of ions when released from the artificial implant, could be toxic or harmful and provoke pains, and (iii) the impossibility to regenerate natural bone (Balamurugan et al 2008). An alternative option until a more similar material to bone becomes available is to coat the metallic implant with ceramics. This technique is being used nowadays, both for dental implants and hip joint prosthesis. There is still a long way to follow, but several metallic implants with ceramic coatings are commercially available already, and the research in problem solving is under way. An additional advantage when coating a metallic implant with ceramics is the reduction in ion release issues from the metal alloy. The ceramic represents a truly effective barrier that hinders the metallic ion kinetics of release towards the living body.

HAp is being specifically used for this purpose, in order to improve the attachment of hip joint prostheses owing to its excellent biological properties such as non-toxicity and lack of inflammatory response and fibrous and/or immunitary reactions (Yang et al 2005). HAp, TCP and their biphasic combinations are important ceramic materials in the replacement of hard tissues because they can form a strong bond with the bone and favour bone formation. However, the poor mechanical properties of calcium phosphates
limit the use of the bulk material to non-load bearing implants. For this reason, one of the most important uses of these calcium phosphates is to coat inert or bioactive implants with mechanical properties adequate for orthopedic substitutions. In this way, the coated implants will not only have the good mechanical properties of the substrate but also an enhanced osseointegration and bioactivity due to the calcium phosphate layer.

1.4 AGAROSE

Agarose is a biocompatible and biodegradable natural polymer, which belongs to the family of polysaccharide isolated from red algae such as seaweed. It acts as gelling agent leading to strong gels and allowing rapid room temperature polymerization and it behaves like a hydrogel (Suzawa et al 2010). It consists of a galactose-based backbone and is commonly used as a medium for cell culture in the form of agar. Structurally, agarose is a galactan (galactose polymer) in which the linkages alternate 1-4, 1-3, 1-4, 1-3. This arrangement allows two chains to join together and adopt a LH double helix. The two chains wrap together so tightly that gaps are closed, and water is trapped inside the helix. The two chains have four ends and these remain as random chains that are able to link up with other helices. In the case of agarose gels, pore formation is a physical process resulting from a shift in conformation of the molecules composing it. In its powdered form, agarose is composed of loose, random coils of polysaccharide. When mixed with water, melted and cooled, however, these random coils adopt a more orderly helical conformation. Each polysaccharide molecule participates in forming a number of double helical structures with other polysaccharide molecules resulting in a tangled mass of molecules (gel), with the spaces between the helices acting as pores. Agarose are used for tissue engineering application and it mimics the physico-chemical properties of extra cellular matrix with the chemical resemblance and highly hydrated content in three dimensional
structures of the scaffolds. Beads form of microcarriers of agarose is used for drug delivery (Watanabe et al 2007).

1.5 BONE DISEASE

Diseases or injuries caused to bones are the major reason for abnormalities of the human skeletal system. There are several kinds of bone disease such as osteogenesis, pagets, osteoporosis, osteopetosis, osteomyelitis and osteosarcoma, etc. From these kinds of bone disease, most of them are affected by osteomyelitis and osteosarcoma. Osteomyelitis means an infection of the bone or bone marrow. Staphylococcus aureus is the organism which is responsible for osteomyelitis. Osteosarcoma is a cancerous (malignant) bone tumor that usually develops during the period of rapid growth that occurs in adolescence as a teenager matures into an adult. Before major surgery to remove the tumor or infection, chemotherapy (antibiotic or anticancer drug) is usually given to patient. A single dose results in the immediate maximum release of the drug and subsequently drops below the minimum effective level by conventional method (Oral, eye and intravenous etc.). As a result, multiple doses are required to maintain the average drug level within the optimum range. To overcome the need for frequent dosing and to avoid maximum drug release, the localized and controlled release of drug delivery systems has been developed (Nandi et al 2009).

Antibiotic and anticancer drug impregnated biomaterials have been employed in the treatment of osteomyelitis and bone cancer by local drug delivery method. There are several drug carriers such as nano particles of polymethylmethacrylate (PMMA), poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers, calcium phosphate and its composite, and coated on metal implants etc. (Gunatillake and Adhikari 2003, Nandi et al 2009). Among these, calcium phosphate and its composite are potential material for applications such as local drug delivery carrier as well as bone implants.
(Ginebra et al 2006). The release of drugs from any one of the carrier depends on different factors such as particle size, morphology, porosity, degradation rate and the bond between the drug and the matrix. In addition, antibiotic loaded HAp coated metal implants are used for load bearing application. Amoxicillin, gentamicin, ciprofloxacin, vincristine, ifosfamide, doxorubicin, etoposide and 5-fluorouracil are commonly used for the treatment of serious infections caused by bacteria and bone cancers.

1.6 NANO BIOCERAMIC SYNTHESIS

Nano bioceramic powder is synthesized by various methods such as wet precipitation including sol-gel, hydrothermal, microwave, solid state reaction, ball milling, microemulsion technique and etc. Most of the industries are using wet precipitation method like sol-gel, hydrothermal and microwave for synthesis of large quantity of nano bioceramics like hydroxyapatite, β-tricalcium phosphate (β-TCP), alumina, zirconia, carbon and etc.

1.6.1 Wet Precipitation Technique

Precipitation of a solid from a solution is a common method for the fabrication of nanoparticles. In the precipitation process, the salts of various elements are taken in the required proportion and are dissolved in water or together with suitable solvents to acquire complete mixing on an atomic scale. A precipitating reagent is added which results in the precipitation of the components at the required ratio. The precipitate is dried and manipulated in the same way as powder, except that normally there is no further need for finer grinding. Particle size and morphology can be controlled by changing different reaction parameters. For obtaining the precipitate of well-defined stoichiometry, the factors that have to be taken into consideration are (i) the chemical conditions like pH and anion concentration, (ii) the hydrodynamic
conditions like vigorous mixing and (iii) the counter ions. Precipitation technique can provide uniform nucleation, growth and aging of the nano particles throughout the solution (Walton 1967). Recently most of semiconductor, magnetic, optical, ferro electric materials at the nano level are synthesized by this technique.

1.6.2 Sol-gel Method

The sol-gel process is known as chemical solution deposition and it is a wet-chemical technique widely used in the fields of materials science and ceramic engineering. The sol-gel process involves a solution or sol that undergoes a sol-gel transition (Klein 1994). It is a useful process of self-assembly for the synthesis of nanoparticles. Colloids are suspensions with molecules of 20 to 100 µm in diameter in a solvent. The colloid suspended in a liquid is the sol and the suspension that keeps its shape is the gel. The gravitational forces of colloids are negligible and interactions are dominated by short range forces, such as van der Waals attraction and surface charges. The inertia of the dispersed phase is small enough that it exhibits Brownian motion, a random walk driven by momentum imparted by collisions with molecules of the suspending medium (Brinker and Scherer 1990). The sol-gels are suspensions of colloids in liquids that keep their shape. This process involves the evolution of networks through the formation of a colloidal suspension and gelation of the sol to form a network in continuous liquid phase.

The sol-gel formation occurs in four stages, (i) hydrolysis, (ii) condensation and polymerization of monomers to form particles, (iii) growth of the particles, (iv) agglomeration of the particles followed by the formation of networks that extend throughout the liquid medium resulting in thickening which forms a gel. These processes are basically affected by the initial reaction conditions. By controlling these factors, it is possible to vary the
structure and the properties of the sol-gel derived inorganic network. An aerosol is a colloidal suspension of particles in a gas and an emulsion is a suspension of liquid droplets in another liquid. When solvent removal occurs under hypercritical (supercritical) conditions, the network does not shrink and a highly porous, low-density material known as an aerogel is produced. A xerogel is a solid formed from a gel by drying with unhindered shrinkage. Xerogels usually retain high porosity (25%) and enormous surface area (150-900 m$^2$/g), along with very small pore size (1-10 nm).

All sol-gel processing methods can be classified as aqueous or alcohol-based. As the names suggest, aqueous-based systems are carried out in the presence of water, while alcohol-based systems generally exclude water build-up until the hydrolysis stage. Similarly, sol-gel precursors can be classified as either alkoxides or nonalkoxides. While alkoxides are the precursors for sol-gel production owing to their volatility, other compounds, such as metal salts can also be used (Gillan and Kaner 1996). The preparation of sol solutions involves the use of solvents usually organic alcohols. The particular solvent employed can influence the particle morphology. This approach is being used extensively in the development of new materials for catalysis, chemical sensors, membranes, optical gain media and biomedical implant.

In the early 1990s bioactive glasses were prepared for the first time by the sol-gel process (Li et al 1991). Porous bioglasses could be prepared from the hydrolysis and polymerization of metal hydroxides, alkoxides and/or inorganic salts. For hydrolysis under controlled conditions, dispersed spherical nanoparticles can be synthesized (Wilson et al 2002). One of the most interesting alternatives for bone regenerative purposes is the association of ostogenic agents with bioactive glasses, intended to form three-dimensional (3D) scaffolds for bone tissue engineering (Vallet-Regi et al 2006). The
bioactive behaviour of many compositions involves not only osteoconduction and osteoproduction, but also osteoinduction processes when implanted in living tissue (Hench 2006). Moreover, sol-gel processes can be combined with supramolecular chemistry of surfactants, resulting in a new generation of highly ordered mesoporous materials for biomedical applications (Anee et al 2003). Mesoporous HAp is excellent candidates for controlled drug delivery systems, and a great research effort has been carried out in this topic recently (Vallet-Regi 2006, Vani et al 2011).

1.6.3 Hydrothermal Method

The term hydrothermal is purely of geological origin. It was first used by the British geologist Sir Roderick Murchison to describe the action of water at elevated temperature and pressure, in bringing about changes in the earth’s crust leading to the formation of various rocks and minerals. By this process, the largest single crystal is formed in nature (beryl crystal of > 1000 kg) and largest quantity of quartz single crystals (1000 kg) was artificially created in single run in the laboratory (Yoshimura and Byrappa 2008). Hydrothermal processing can be defined as any homogeneous (nanoparticles) or heterogeneous (bulk materials) reaction in the presence of aqueous solvents or mineralizers under high pressure and temperature conditions to dissolve and recrystallize materials that are relatively insoluble under ordinary conditions. Chemists prefer to use a term, viz. solvothermal, meaning any chemical reaction in the presence of a non-aqueous solvent or solvent in supercritical or near supercritical conditions. Similarly there are several other terms like glycothermal, alcothermal, ammonothermal, carbonothermal, lyothermal and so on (Yoshimura and Byrappa 2008).

Hydrothermal system mainly consists of a pressure vessel called autoclave. Autoclaves for crystal growth are usually fabricated from steel or
corrosion resistant alloys. These are usually thick-walled steel cylinders with a hermetic seal which must withstand high temperatures and pressures for prolonged periods of time. Furthermore, the autoclave material must be inert with respect to the solvent. Professional equipment consists of pressure gauge, internal stirring and in/out let value for solution removal during hydrothermal process. This method is one of the important technique to grow crystals of different materials. This technique has been used to grow dislocation free, pure and bigger size single crystals (Brice 1986). It can be employed for large-scale synthesis of piezoelectric, magnetic, and optic materials. Rapid convection and very efficient solute transfer results in comparatively rapid growth of larger, purer and dislocation free crystals. It can be used for all inorganic species starting from native elements to the most complex silicates, phosphates and other compounds.

HAp single crystals of size 7×3×3 mm have been grown by hydrothermally (Mengeot et al 1973). Carbonate HAp single crystal of size 12 mm length 200 µm in width and hexagonal prism like crystal (9.5 µm length and 1.5 µm) were grown by this technique (Ito et al 1996, Neira et al 2009). The phase conversion takes place after the hydrothermal treatment, for example, dicalcium phosphate dihydrates, calcite and coral convert to HAp (Xu et al 2001, Yoshimura et al 2004, Ashok et al 2007). For nanomaterial synthesis, hydrothermal method has several advantages over other techniques. Nanosized HAp with uniform morphologies, controllable size, uniform dispersion and narrow size distribution has been synthesized successfully by low-temperature hydrothermal process (Manafi et al 2008). Thermally stable HAp was synthesized by hydrothermal method in the presence of malic acid (Parthiban et al 2009). A novel, porous triphasic calcium phosphate composed of nonresorbable HAp and resorbable tricalcium phosphate (α- and β-TCP) has been synthesized hydrothermally at a relatively low temperature
Hierarchically nanostructured HAp hollow spheres assembled from nanorods have been successfully synthesized using solvothermal method at 200 °C in water/N,N-dimethylformamide (DMF) mixed solvents (Ma et al 2008). HAp coating on various substrates was also done using hydrothermal process. HAp was coated on the plane of (0001) on titanium alloy substrate (Haders et al 2009). The hydrothermal treatment may increase the negative charge of the titanium surface, which is effective for inducing HAp nucleation and improve the attachment of MC3T3-E1 cells (Hu et al 2010).

1.6.4 Microwave Method

Dielectric heating (electronic heating, RF heating, high-frequency heating) is the process in which microwave electromagnetic radiation heats a dielectric material. This heating is caused by dipole rotation. Synthesis of nanomaterials using microwave energy has been an increasing interest in the scientific community (Mohamed et al 2010). The microwave irradiation is considered a fast and easy way to create highly versatile, tailored nanorods and nanowires which can be used in medical applications, drug delivery, sensors, communications and optical devices. Microwave heating provides significant enhancement in reaction rates. The microwave energy is delivered directly to the material through molecular interaction with the electromagnetic field. Microwave heating involves transfer of electromagnetic energy to thermal energy rather than simple heat transfer. Since microwave can penetrate the material and supply energy, heat can be generated throughout the volume of the material resulting in volumetric heating. Hence it is possible to achieve rapid and uniform heating of materials.

Microwave synthesis of nanosized HAp ceramic has recently gained interest. Biphasic calcium phosphate ceramics consisting of a mixture of HAp
and β-TCP was synthesized using microwave method (Manjubala and Sivakumar 2001, Lee et al 2007). The rapid formation of nanocrystalline flower-like HAp was reported Liu et al (2005) by microwave synthesis. Nanocrystalline fluorine-substituted HAp was also successfully synthesized by microwave processing (Rameshbabu et al 2006). Microwave heating method has solved the problems on synthesizing carbonated hydroxyapatite (CHAp) by the conventional heating precipitation method which included the problems such as long reaction and large particle size, poor crystallinity of CHAp etc. (Ran et al 2007). Composites comprising of gelatin with HAp scaffold were fabricated through a novel microwave vacuum drying and cross-linking process (Sundaram et al 2008). Biodegradable poly(lactide-co-glycolide)/β-TCP composites were synthesized through polymerization using microwave energy (Jin et al 2010, Vani et al 2011).

1.7 BIOMATERIALS COATING METHODS

HAp can be coated on biomedical implant and devices using various coating techniques such as plasma spraying, sputtering, pulsed laser deposition, dynamic mixing method, dip coating, sol-gel, electrophoretic deposition, biomimetic coating and hot isostatic pressing. Recently newer techniques are investigated to coat HAp on load bearing implants.

1.7.1 Electron Beam Evaporator (e-beam evaporator)

Vacuum evaporation (including sublimation) is a physical vapor deposition (PVD) process, where the material is thermally vaporized from source and deposited on the substrate without collision with the gas molecules present in the space between the source and substrate (Figure 1.2). Focused high energy electron beam are necessary for the evaporation of refractory material, such as most ceramics glasses, carbon and refractory metals. This
e-beam heating is also useful for evaporating large quantities of materials. When vaporizing solid surfaces of electrically insulating materials, local surface charge buildup can occur on the source surface leading to surface arcing that can produce particulate contamination in the deposition systems.

Figure 1.2 Schematic diagram of electron beam evaporator

Deposition of thin film by vacuum evaporation is a very simple convenient and widely used technique (Figure 1.2). In the deflected e-gun the high energy electron beam is formed using a thermionic-emitting filament to generate the electrons, high voltages (2-20 kV) to accelerate the electrons and electric or magnetic fields to focus and deflect the beam onto the surface of the material to be evaporated. Electron beam guns for evaporation typically operate at 2-50 kW. Using high power e-beam sources deposition rates as high as 50 microns per second have been attained from sources capable of vaporizing material at rates of up to 10-15 kg of aluminum per hour. Electron beam evaporators can be made compatible with ultra high vacuum processing. The e-beam is magnetically deflected through greater than 180° to avoid deposition of evaporated material on the filament insulators. The beam is focused on to the source material which is contained in a water-cooled copper hearth “pocket”. The e-beam can be rastered over the surface to produce
heating over a large area. Electron gun sources can have multiple pockets so that several materials can be evaporated by moving the beam or the crucible, so that more than one material can be vaporized with the same electron source. The high energy electron bombardment produces secondary electrons which are magnetically deflected to ground. The electrons ionize a portion of the vaporized material and these ions can be used to monitor the evaporation rate. The ion can also create an electrostatic charge on electrically insulating substrates. If the fixture is grounded the electrostatic charge can vary over the substrate surface, particularly if the surface is large, affecting the deposition pattern. This variation can be eliminated by deflecting the ions away from the substrate by using a plate at a positive charge above the source or by electrically floating the fixture so that it assumes a uniform potential. Electron beam deposition of dielectric materials can generate insulating surfaces that can build up a charge that cause arcing and particular formation on the deposition system, with the e-beam evaporation of some materials.

In this method, evaporation of the material takes place in vacuum environment. Sufficient amount of heat is supplied to the evaporant to attain the desired vapor pressure and the evaporated material is allowed to condense on the substrate. Vacuum evaporation is possible for a wide range of materials, particularly for metals except the refractory metals with low vapor pressure by heating the materials, the vapor pressure of the charge is raised to a level at which evaporation and sublimation occurs. The important process parameters are the substrate material, source and substrate temperature, source-substrate distance, backward gas composition and pressure. Using this method, evaporants with extraordinary range of chemical reactivity and vapour pressure can be deposited. This method leads to a large diversity in source designs including resistance-heated filaments, electron beams, crucible heated by conduction, radiation or RF induction, arcs, exploding wires and lasers.
High voltage electron beam guns are not generally used in a plasma environment because of sputter erosion of the gun filament by positive ions. There are also problems with the reaction of the hot filaments in reactive gases. In order to use an electron beam evaporator in a plasma or reactive gas environment, the electron emitter region can be differentially pumped by being isolated from the deposition environment. This is done by having a septum between the differentially pumped electron emitter chamber and the deposition chamber, the septum has a small orifice for the electron beam to pass from one chamber to the other. This type of configuration is used in e-beam ion plating.

The various coating techniques have been developed to produce much thinner HAp layers with a more homogeneous phase composition and structure. The electron beam deposited HAp films were shown to be very thin and to have dense and homogeneous structures (Choi et al 2000). Furthermore, this technique is versatile, allowing the composition of the coating and properties to be tailored by modifying the target material and equipment variables.

1.7.2 Sputtering Method

Sputter deposition is a physical vapor deposition process for depositing thin films (Figure 1.3). Sputtering means ejecting a material from a target (source material) and depositing it on a substrate such as silicon, titanium and its alloy substrate. Substrates are placed in a vacuum chamber and are pumped down to a prescribed process pressure. Sputtered atoms ejected from the target have a wide energy distribution, typically up to tens of eV (100000 K). Sputtering starts when a negative charge is applied to the target material causing a plasma or glow discharge. Positively charged gas ions generated in the plasma region are attracted to the negatively biased target plate at a very high speed. This collision creates a momentum transfer.
and ejects atomic size particles from the target. These particles are deposited as a thin film onto the surface of the substrate. Sputtering is extensively used in the semiconductor industry to deposit thin films of various materials in integrated circuits processing. Thin anti-reflection coatings on glass, which are useful for optical applications are also deposited by sputtering. Because of the low substrate temperatures used, sputtering is an ideal method to deposit contact metals for thin-film transistors. This technique is also used to fabricate thin film sensors, photovoltaic thin films (solar cells), metal cantilevers and interconnects etc. Magnetron sputtering can be done either in DC or RF modes. DC sputtering is done with conducting materials. If the target is a non-conducting material the positive charge will build up on the material and it will stop sputtering. RF sputtering can be done both on conducting and non-conducting materials. Here, magnets are used to increase the percentage of electrons that take part in ionization of events and thereby increase the probability of electrons striking the argon atoms, increase the length of the electron path, and hence increase the ionization efficiency significantly.

**Figure 1.3 Schematic diagram of RF magnetron sputtering unit**

RF magnetron sputtering has been shown to be a particularly useful technique for the deposition of bioceramic (CaP) thin films, due to the ability of the technique to provide greater control of the coating process and improve the adhesion between the substrate and coating (Boyd et al 2006). The effect
of different annealing temperatures was investigated on thin calcium phosphate coatings fabricated by RF magnetron sputtering technique. By this method, thin uniform crystalline HAp coated on titanium and nickel-titanium were obtained which finds applications as super elastic or shape-memory implant material (Pichugin et al 2008).

1.7.3 Pulsed Laser Deposition (PLD)

In this method, there is a vacuum chamber containing the coating material in a sintered/pressed form which is then bombarded with a laser beam (Figure 1.4). The energy source is located outside the chamber and hence the use of high vacuum as well as ambient gas is essential. This technique uses high power laser pulses to melt, evaporate and ionize material from the surface of a target. The evaporated material is collected on an appropriately placed substrate upon which it condenses and the thin film grows. During the process, one can control laser energy density and pulse repetition rate for getting good quality films. The advantage of PLD technique is small size of target required compared with sputtering techniques. In addition, to produce multi-layered films of different materials, a fine control of film thickness down to atomic monolayer can be achieved by controlling the number of pulses. The other important advantage is that the stoichiometry of the target can be retained in the deposited films. PLD generally can be divided into the following four stages: (i) interaction between laser radiation and the target, (ii) dynamic of the ablation materials, (iii) decomposition of the target materials onto the substrate, and (iv) nucleation and growth of a thin film.
Figure 1.4 Schematic diagram of pulsed laser deposition (PLD)

In the first stage, pulsed laser beam is focused on a target and the laser beam results in a locally very high energy density on the target surface. The electromagnetic energy is converted into thermal energy via electronic processes, and results in evaporation of the target material. This process takes place on a very short time scale, shorter than the pulse duration. The instantaneous ablation rate is highly dependent on the fluences of the laser irradiation on the target. The ablation mechanisms involve many complex phenomena such as collision, thermal and electronic excitation, exfoliation and hydrodynamics. In the second stage, the emitted materials (plume) tend to move towards the substrate according to the laws of gas-dynamic and show the forward peaking phenomenon. The uniformity of the films can be controlled by controlling the laser spot size and the plasma temperature. The target-to-substrate distance is another parameter that governs the angular spread of the ablated materials and a mask placed close to the substrate could reduce the spreading.

The third stage is important to determine the quality of the deposited film. The ejected high-energy species from the target material impinge onto the substrate’s surface. The energetic species sputter some of the surface atoms on the substrate and a collision region is established
between the incident flow and the sputtered atoms. Film grows immediately after the formation of this thermalized region (collision region). The region serves as a source for condensation of particles. When condensation rate is higher than the rate of particles supplied by the sputtering, thermal equilibrium condition can be reached quickly and film grows on the substrate surface at the expenses of the direct flow of the ablation particles. Fourth stage of nucleation and growth of crystalline films depends on many parameters such as the density, energy, degree of ionization, and the type of the condensing material, as well as the temperature and the physical-chemical properties of the substrate.

PLD technique is used to deposit the various kinds of materials such as oxides, nitrides, carbides, semiconductors, metals and even polymers or fullerenes can be grown with high deposition rates for different applications. Recently, the adjustment of PLD parameters can also deposit biomaterial (CaP) coatings of different phases and composition. HAp was coat on titanium substrate by PLD technique (Bao et al 2008). Silicon substituted HAp and interlayer of titania, which is used to improve adhesion between HAp layer with Ti substrate were deposited by PLD (Solla et al 2007, Rajesh et al 2011).

1.8 SURFACE MODIFICATION BY ION BEAM IRRADIATION

Currently many researchers are investigating to modify the surface properties of biomedical implants for enhancement of biological response. There are many techniques available to modify the surface such as chemical etching, physical vapor deposition, plasma spraying and ion beam implantation/irradiation (Chu et al 2002, Cui and Luo 1999). Biomaterials modification by the ion beam process including plasma surface treatment has recently become an interesting topic in the field of surface engineering (Sioshansi and Tobin 1996). There are three main reasons that encourages
this trend. First, the importance of surface modified biomaterials to the longevity of medical implants has been recognized by both major medical device companies and more and more patients. In recent years, some specialized companies offered surface treatments to the medical market in several countries. In bone replacement, especially long bone and joint replacement, metal implants are widely used to take the unsubstituted position. Although the metallic orthopedic implants may have excellent bulk properties such as ideal strength and elasticity, it has relatively poor surface properties, e.g. poor wear resistance and limited biocompatibility. It is therefore necessary to make a compromise between the bulk and surface properties. In the case of hip replacement, the wear debris from the implant is one of the essential factors for the aseptic loosening which is a frequent cause of failure of the prosthetic implants. It is generally accepted that improving the wear resistance and biocompatibility of the implants by surface engineering is an optimal option.

Second, the range of biomaterials has been significantly extended from synthesized materials of metals, ceramics and polymers to those including biological materials. For example, the newest definition of biomaterials is either naturally occurring materials in living organisms or materials designed to repair humans. Recently, a number of new effects of ion implantation on biomaterial have been observed and used in improving crops and modifying microbes. However, the interaction mechanism between low energy (keV) ions and biological systems is still unclear due to the complex hierarchical structure of biological systems.

Third, ion-beam processes including plasma treatment which is based on ionized particle bombardment have been particularly successful in biomaterial modification, compared to other available surface treatment processes such as conventional coating, nitriding, and laser process, etc. This
may be a result of the advantage of ion-beam process, example, exact process control, low temperature processing, versatility of ion species, non-equilibrium process and reliability. In general, the cost of the ion-beam process is relatively high, as it involves a vacuum chamber. However, the concerns on the life quality in many cases make the cost of the ion beam process less problematic.

An energetic ion beam penetrates into a solid or thin film, it looses their energy by excitation or ionization of atoms by inelastic collisions, known as electronic stopping and the energy spent in this process is called electronic energy loss. Electronic energy loss is dominant in high energies where the displacement of atoms due to elastic collisions is insignificant. The electronic energy loss process dominates for heavy ions with high energies (> 2 MeV) are referred to as Swift Heavy Ions (SHI). In SHI irradiation the modification of thin films or the near surface region of the bulk samples is due to the electronic excitation. Hence, SHI irradiation promises to produce better surface modification in biomaterials and this provided the motivation to pursue these research.

The irradiation experiment was carried out using a 15 UD Tandem Pelletron Accelerator available at Inter University Accelerator Centre (IUAC), New Delhi, which is able to deliver ion beams of almost all the elements across the periodic table in the range of 10-270 MeV (Kanjilal 2001). The irradiation experiments are performed in the high vacuum chamber, with vacuum maintained above $10^{-6}$ mbar. The vacuum is to avoid any collision of the ion particle with gas molecules. The ion beam is scanned in x- and y- direction (10 mm x 10 mm) over the samples area with the help of a magnetic scanner. A cylindrical enclosure of stainless steel surrounds the sample ladder, which is kept at a negative potential of 120 V. This enclosure suppresses the secondary electrons coming out of the sample during
irradiation. An opening in the suppressor allows the ion beam to fall on the sample. The total number of the particles/charges falling on the sample can be estimated by a combination of the current integrator and the pulse counter from which the irradiation fluence can be measured.

The effect of low energy irradiation on HAp composites has been reported by Suljovrujic et al (2003). Also, the irradiation effect of 30 MeV cluster beams of $\text{C}_{60}$ in producing track formation on FAp was studied by Jaskierowicz et al (2004). Radiation induced amorphization of synthetic silicate apatites results in volumetric swelling and increases in their aqueous dilution rates (Weber 1983).

1.9 THE IMPORTANCE AND SCOPE OF THE THESIS

Bone and teeth is a composite of organic and inorganic (CaP) constituents. Synthetic HAp in the form of powder, solid and coating on metal is used as implants for the replacement of bone and teeth. The pure phase of HAp has poor bioactivity due to low resorbability. For this reason, synthesis of mixed phase of calcium phosphates, polymer scaffolds and organic/inorganic composites are being carried out. For the commercial purpose, the bioactivity of HAp solid block and the coated metal implant surface are modified by various techniques. The ion beam irradiation has been identified as a good method to modify the surface properties of an implant to significantly improve its osseointegration. The synthesis of biomaterials by using such novel techniques promises challenging possibilities in biomedical applications.

Bone diseases (infection and cancer) are major problems associated with the human body. Recently interest has grown in the use of local antibiotic therapy to prevent the bone cancer or infection from the bacteria. Hence, in vitro investigations on the biological and drug delivery behavior of
biomaterials (powder and thin films) are essential for their application in biomedical industry.

The thesis broadly comprises the following

i) Nanosized HAp/agarose composites synthesized by sol-gel technique at low temperature and constant pH, followed by microwave treatment of the prepared composites. The prepared composites were tested for their in vitro antibiotic and anticancer drug releases properties.

ii) Nanosized carbonate substituted HAp/agarose composites synthesized by solvothermal technique was characterized by XRD, FTIR, Raman, BET, SEM and TEM. In vitro biological performance and drug release behavior were analyzed.

iii) Biphasic calcium phosphate (HAp/β-TCP) thin films prepared by electron beam evaporation technique was characterized by XRD, FTIR, SEM, wettability, bioactivity, antimicrobial activity and biocompatibility test.

iv) RF magnetron sputtering technique was used to prepare calcium phosphate thin films and surface modification was carried out by irradiating with swift heavy silicon ion beams.

v) The effect of silver ion irradiation on HAp thin films prepared by pulsed laser deposition (PLD) technique was studied.