1.1.Introduction

The demand for orthopedic implants is growing rapidly due to the growth of diseases like osteoporosis (weakening of bone) and osteoarthritis (inflammation in the bone joints). Human joints are most likely to be affected by degenerative and inflammatory diseases. The degenerative disease directly degrades the mechanical property of bones by excessive loading or absence of normal biological self healing process. It has been estimated that about 90% of the population over the age of 40 years are suffering from some degree of degenerative joint disease [1]. Replacement of diseased joint surface by means of metallic, ceramic or polymeric material through Arthroplasty surgery is the ultimate solution of this problem [2]. Arthroplasty can be simply explained as the surgical technique of replacing affected degenerated natural surface with materials specially designed for implantation purpose with an aim of relief in pain and creating new prosthetic joint to increase mobility among joints. It is well known that bone is made up of three cells which are responsible for growth and resorption of bones. These cells are Osteoblast, Osteoclast, and Osteocytes and are derived from bone marrow. Inspite of their capability of physiological remodelling and self-healing bones are unable to cope with negative effects of extensive defects such as “critical sized defects (CSDs)” [3]. The common techniques which are used for the treatment of CSDs are autograft (bone harvested from patients), allograft (donor bone) and xenograh [4, 5]. These methods are reasonably effective in many cases but they are associated with certain limitations. The application of autograft is limited in cases where the defect area is large for example spinal arthrodesis [6]. Similarly immunologic problems, low osteogenicity and higher resorption rate are some of the limitations of allograft [7].

The aforementioned problems and limitations encourage scientists and researchers to do vigorous search and develop reliable bone substitute with biological and physical properties comparable to human bone. The development of biomaterials starts with the first meeting on biomaterials held at Clemson University, South California in 1969 [8]. The vigorous research results in the development of various metallic alloys, ceramic and polymeric material having ability to mimic the property of healthy bone. As the above-mentioned materials belongs to different class they posse's different physical, mechanical, chemical and biological properties. It can be easily understood by some examples like materials having good mechanical properties like metallic alloys are generally used in load-bearing joint prostheses. Similarly, ceramic materials have excellent wear resistance property and bioactive property therefore they are generally used for coating of implants. Polymers are biodegradable and generally used as for biomaterial because they have a tendency to degrade inside the body without causing any harm to the body and thus, they are used for bone repairing.

In spite of lots of advantages there are certain different areas in which all the above-mentioned material fails to provide adequate support for which they are applied like the use of polymers is limited to the repair of small bone fractures and soft tissues due to their low strength. Similarly, due to the poor mechanical properties prostheses of pure ceramics are not preferred for weight-bearing long bone prostheses, as they fail in load-bearing applications. According to Radha et al. and Zha et al. [9, 10] metallic materials such as metal screws, nails, plates, rods or wires are generally used at injury sites for fastening bone pieces together and after the surgery, these implants are left in the body even after healing of the injury. Due to the non-degradability of these metallic materials in the body, it causes infections which leads

to revival surgical procedures for the removal of implants after healing of the bone. Many scientists and researcher believes that the major cause of failure of metallic biomedical implant is "Stress shielding effect" [10]-[15]. Stress shielding effect which can be simply explained as the mismatch of the mechanical property of natural bone and metallic alloy under in vivo condition. This effect is responsible for the resorption of bone tissues surrounded by the implant material [12]. Along with this low wear and corrosion resistance and lack of biocompatibility are some of the reasons for failure of metallic implants. Thus, to mitigate the limitations associated with metals, ceramic and polymers development of advanced materials that can mimic the property of natural bone is highly desired.

In order to develop appropriate material with high durability and excellent biocompatibility researcher found that Titanium (Ti) and its alloy are one of the materials which have ability to resolve aforementioned problem associated with medical implants. The commercial production of Ti comes in light with the invention of Kroll process in 1946 [13] and within a year Ti alloy based implants were introduced [14]. Since their introduction, Ti alloys have been employed in a wide range of biomedical application like spinal fusion, skeletal repair and dental implants [8,19,20,21]. It was found that Ti and its alloy are superior to medical grade Stainless Steel, Cobalt-Chromium alloy and Magnesium based alloys in terms of mechanical strength, biocompatibility and corrosion resistance. But at the other end higher young's modulus of Ti and its alloy (110GPa) was the main problem causing its limited use in biomedical application. Development of second-generation Ti alloys commonly known as β alloys was an approach to reduce Young's modulus. Low moduli Ti alloys (e.g. TNZT, a β alloy with composition of Ti-35Nb-7Zr-5Ta) were specially developed to better match the modulus of different types of human bones [18]. Weber and

White in 1972 [19] introduced the concept of using porous biomaterials for osseointegration and later on, this led to a large number of research studies to develop porous implant materials using various processing methods. The concept behind the use of porous material commonly known as scaffold for implant application is it posses comparatively low effective young's modulus with respect to conventional Ti based implants. Along with this porous scaffold posse several other benefits like ability of better biological fixation by promoting bone tissue ingrowth into the pores of the implant resulting in uniform stress transfer between natural bone and the implant. Also porous scaffold promote colonisation and subsequent substitution by biological cells [24, 25].

1.1. Characteristics of Biomaterials used for Implant applications

According to Williams, 1987, "Biomaterials can be defined as a nonviable material used in a medical device, intended to interact with biological system". In simple words biomaterials are artificial or natural material which have ability to restore the integrity and functionality of lost or diseased biological structure. In order to produce implant with longevity and higher compatibility with body the biomaterial should posses' certain property which makes it suitable for biomedical applications, the properties include biocompatibility, osseointegration, mechanical property, wear resistance and corrosion resistance. **Table 1.1** lists all the basic requirement of implant which should be considered before design and development of implants.

Table 1.1 Basic requirements of implants for orthopedic applications

Basic Requirements for Implants		
Compatibility • Tissue Reaction • Changes in Properties 1. Mechanical 2. Physical 3. Chemical • Degradation leads to 1. Local deleterious changes 2. Harmful systemic effects	**Mechanical Properties** • Elasticity • Yield Stress • Ductility • Toughness • Time-Dependent Deformation • Creep • Ultimate Strength • Fatigue Strength • Hardness • Wear Resistance	**Manufacturing** • Fabrication methods • Consistency and conformity to all requirements • Quality of raw materials • Superior techniques to obtain excellent surface finish or texture • The capability of a material to get safe and efficient sterilization • Cost of product

1.1.1. Mechanical Properties

The main aim of bone is to provide structural support during mechanical loading and to protect vital organs inside the body. In some cases, if there is any trauma or diseased biological structure the role of diseased organ is performed by implant. Currently the majority of implants used in orthopedic traumas and arthroplasty are of metallic composition,

due to their excellent mechanical strength [22] therefore, the mechanical analysis of metallic implant is of prime concern. In other words, mechanical properties play a major role in selecting the type of material to be appropriate for a particular application. Properties like Young's modulus, fracture toughness, hardness, yield strength, ultimate tensile strength, stiffness, ductility, time dependent deformation and creep are some of the important mechanical properties which should be considered before design and development of orthopedic implants. Wolff's law of bone remodelling (1892) states that mechanical load can affect bone architecture [23] and hence can encourage the process of bone remodelling and fracture healing [28, 29]. Fatigue strength is also an important mechanical property as it determines the response of material under cyclic load conditions, thus it determines the long-term success of the implant subjected to cyclic loading condition [8]. Young's modulus of material plays an important role in success rate of implants because the significant mismatch between the modulus of bone and load bearing implant material cause detrimental effect on load transfer rate from the implant to the bone and within the bone resulting in bone resorption and implant loosening this biomechanical incompatibility is known as stress shielding effect [8, 30, 31, 32]. The mechanical properties of materials used for orthopedic implant application along with human bone are listed in **Table 1.2**.

Table 1.2 Physical and Mechanical Properties of Metallic, Ceramic and Polymeric Biomaterials

Tissue/ Material	Density (g/cm^3)	Young's Modulus (GPa)	Yield Strength (MPa)	Tensile Strength (MPa)	Compressive Strength (MPa)	Fatigue Strength (MPa, 10^7 cycles)	Ref.

Natural Bone							
Cortical Bone	1.8-2.0	7-30	NR	164-240	100-230	27-35	[10]
Cancellous Bone	1.0-1.4	0.01-3.0	NR	NR	2-12	NR	[10]
Metals and Alloys							
Ti-6Al-4V (Casted)	4.43	114	760-880	895-930	NR	600-700	[10]
Ti-6Al-4V (Wrought)	4.43	114	827-1103	860-965	896-1172	500-800	[10]
Ti-6Al-7Nb	4.52	105	880	900	NR	NR	[8]
SS316L	8.0	193	170-310	540-1000	480-620	240-480	[10]
Fe20Mn	7.73	207	420	700	NR	NR	[29]
Zn-Al-Cu	5.79	90	171	210	NR	NR	[11]
Co-Cr-Mo alloy	8.3	240	500-1500	900-1540	NR	500-900	[11]
CoCr20Ni15Mo7	7.8	195-230	240-450	450-960	NR	NR	[11]
Pure Mg (casted)	1.74	41	21	87	40	NR	[11]
Pure Mg (Wrought)	1.74	41	100	180	100-140	NR	[11]

AZ31 (Mg Alloy)	1.78	45	185	263	NA	NR	[11]
AZ91 (Mg Alloy)	1.81	45	160	150	NA	NR	[11]
Ceramics							
Alumina Ceramics	4	260-410	NA	400-580	NA	NR	[11]
Synthetic Hydroxyapatite	3.15	70-120	NA	40-200	NR	NR	[33]
Zirconia	3.98	210	NA	800-1500	1990	NR	[30]
Polymers							
PLGA	1.3-1.34	1.69	3.8-26.6	13.9-16.7	NR	NR	[11]
PCL	1.145	281-686	8.37-14.66	68.45-102.7	NR	NR	[11]
PLA	1.8	3750	70	59	NR	NR	[11]

*** NR- Not Reported**

1.1.2. Corrosion and Wear Resistance

Corrosion and wear are inevitable problems associated with orthopedic implants. When a metallic implant is introduced inside the body they come in contact with biological fluid. This biological fluid consists of various kinds of cations and anions due to this there is a tendency of electrochemical corrosion on the metallic implant surface. There are generally four types of corrosion observed in orthopedic implants they are galvanic corrosion, pitting corrosion, crevice corrosion and fretting corrosion. Galvanic corrosion occurs due to the

electrochemical potential difference between two different or same metal surfaces when introduced in biological fluid. Studies showed that when titanium and cobalt alloy are coupled the rate of corrosion was low to 0.02 $\mu A/cm^2$ and no instance of corrosion were found on metallic interface [31]. Pitting corrosion is a localised form of corrosion which creates cavities on the material. In case of metallic implants chloride ion breakdown the protective passive oxide films resulting into the formation of pits at the site [32]. Crevice corrosion is similar to pitting corrosion in terms mechanism of propagation but differ in mechanism of initiation. Crevice corrosion generally occurs in confined spaces with low oxygen tension and high chloride concentration causing destruction of passivation layer [33].The mechanism of fretting corrosion is different from all the above type of corrosion, in this type of corrosion the passivation layer is broken due to micro-motion between the parts of an implant. Whenever there is continuous relative motion between two surfaces under loading condition the surface releases wear debris around the areas surrounding implant bone interface. It is found in studies that low wear resistance results in implant loosening and deposited wear debris cause several reactions with the tissues [34]. Therefore, high corrosion and wear resistance is required to avoid premature failure of implant.

1.1.3. Effect of Porosity

Porosity plays an important role in development of suitable implant in bone tissue engineering. Interconnectivity of pores, porosity volume and pore sizes are some of the important factors that should be considered before designing a implant for bone tissue engineering. The porous structure should be designed in such a way that the pores are interconnected and of suitable size. The importance of interconnectivity of can be easily understood by the fact that the interconnectivity of pores helps in creating an environment to

promote cell infiltration, in vivo vascular formation, Osteogenesis, cell proliferation, flow of oxygen and nutrients, also interconnected pores helps in removal of waste materials [38, 39]. It is also reviewed that interconnectivity of the pores creates a scope of formation of number of vessels because interconnectivity provides a way for the ingrowth of blood vessels] [37]. It is reported that minimum interconnection size over 50 µm is suitable for bone ingrowth [38]. The porosity of the implant enhances the bone integration property because porous surface provides an interlocking medium to surrounding tissue and implant, resulting in good biomechanical compatibility and high resistance to fatigue loading [42, 43]. It has been reported that pore size ranging from 100 to 200 µm is suitable for osseointegration [8].

It is important to mention that as the porosity of the implant increases, the mechanical property of the system decreases [41]. Therefore, without compromising with the mechanical properties, the porosity of the system should be optimized and the pore size should be strictly controlled. There are different techniques like addition of space holder materials of defined size, densification of the green compacts, sintering conditions, etc which can be employed to incorporate pores in the final structure(add more techniques for making porous Ti) [42]. A detailed discussion regarding production of porous structures using different technique will be discussed in subsequent paragraphs.

1.1.4. Biocompatibility

The biocompatibility of any material is the basis of understanding the host response to implants. According to US Food and Drug Administration biocompatibility is defined as effect that the materials induce no measurable harm to the host [43]. It is observed that improper biocompatibility leads to dysesthesia (loss of sense), discomfort, pain, infection,

resorption of bone [44], etc. The ions released from the metallic implant may induce hypersensitivity and leads to implant failure [45]. The success of the biomaterials is mainly dependent on the reaction of the human body to the implant, and this measures the biocompatibility of material or in other words biocompatibility of any implant material shows the positive response when they are subjected to a biological environment [46]. The two main factors that influence the biocompatibility of any material are the host response induced by the material and the degradation of materials in the body environment [8]. Hence, for good biocompatibility non-toxic and excellent corrosion and wear resistance alloying element should be selected during design and development of metallic implant.

1.1.5. Osseointegration

According to the American Academy of Implant Dentistry (1986), Osseointegration may be explained as contact established without the interposition of non-bone tissue between normal remodelled bone and an implant, entailing a sustained transfer and distribution of load from the implant too and within the bone tissue. In other words, osseointegration can be defined clinically as a process in which clinically asymptomatic rigid fixation of alloplastic materials is achieved, and maintained, in bone during functional loading [47]. Factors such as design, surface chemistry and roughness, chemical composition and loading conditions should be considered before fabrication of implant for good osseointegration [48].

1.2. Bone Metabolism

Bone may be simply defined as a rigid living tissue of the body whose primary function is to provide support during mechanical loading and protect vital organs inside the body [49]. It is commonly known that when baby borns their body consists of about 300 soft bones but as

they grow the soft bones are replaced by hard bones and the number of bones decreases to 206. In order to understand the metabolism of bone, it is necessary to understand the physiology and mechanical property of bone. The different structural levels of bone are shown in **Figure 1.1**.

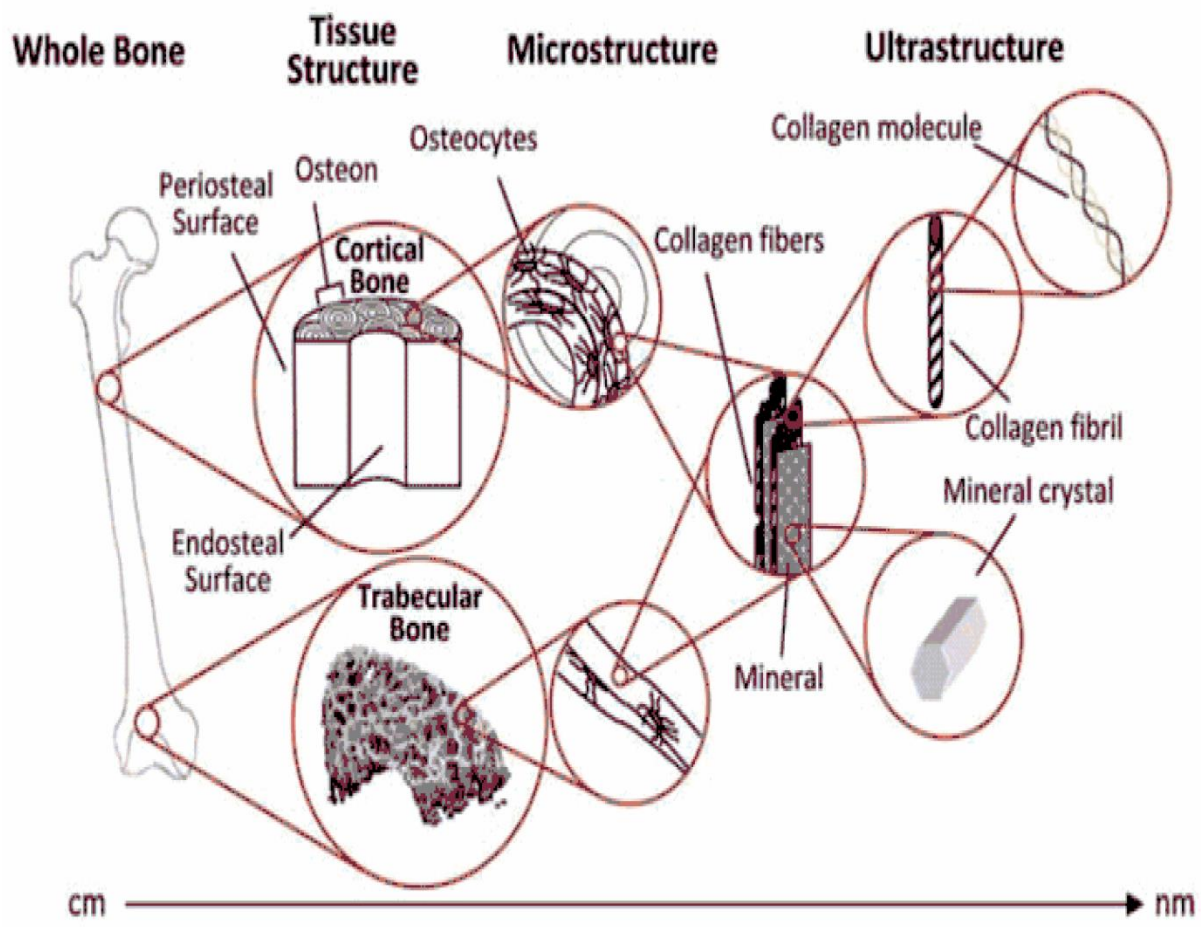

Fig.1.1 The structural levels of bone. Cortical bone is made up of longitudinally oriented osteons, and the trabecular bone within the metaphyses is made up of connected struts and plates. In both bone types, the bone is laid down in layers (lamellae). Both tissue types contain identical components, and their properties are dependent on the amount, morphology, and interaction of these components at each level [50].

1.2.1. Bone Physiology

At microscopic level, on the basis of the arrangement of fibers the structure of bone may be classified into two parts such as woven bone and lamellar bone as shown in Figre 1.2.

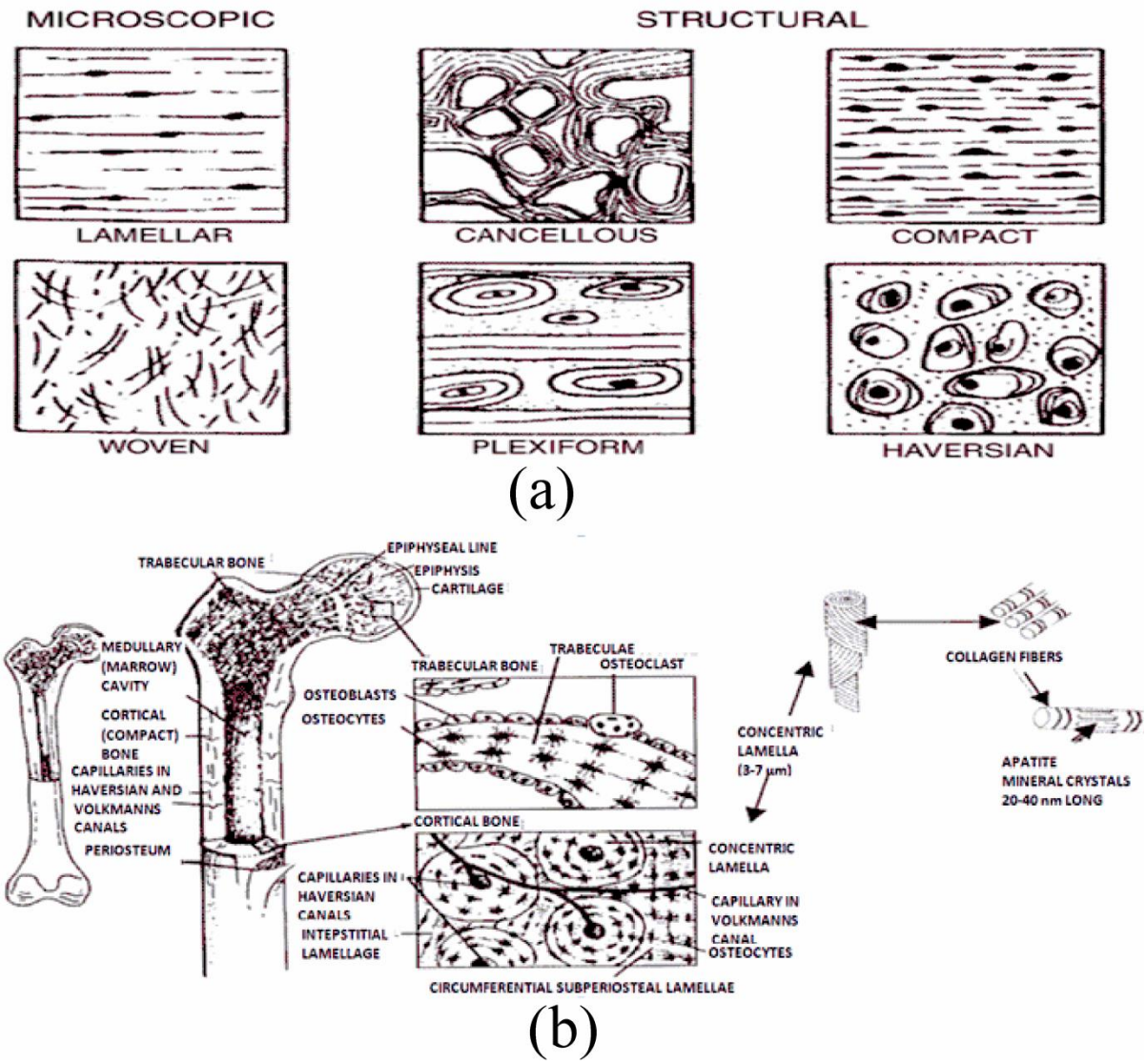

Fig.1.2 Microarchitecture and structural classifications of physiological bone. (a) Schematic of microscopic and structural classifications of bone, (b) Schematic of the microarchitecture of the femur, adopted from [51]

1.2.1.1. Woven Bone

The average mineral grain size of woven bone is about 10 to 15 nm. These bones are the immature form of bone and generally found in the metaphyseal region of bone and in fracture callus [55, 56]. These are coarse-fibred and collagen fibers and are randomly oriented throughout the structure. It can also be conferred that direction independent mechanical behavior of woven bone is the result of nonuniform collagen bone [54].

1.2.1.2. Lamellar Bone

Lamellar bone is another structure of bone that can be distinguished at the microscopic level. Unlike woven bone, it consists of the mineralized matrix which is commonly known as hydroxyapatite having chemical formula $Ca_{10}(PO_4)_6(OH)_2$. These hydroxyapatite crystals are 20 to 80 nm long and 2 to 5 nm thick in the human femur [55, 56]. As the name suggests lamellar bones are organized in the lamina and contain stress oriented collagen fibers which result in anisotropic mechanical property [54].

Further on the basis of structural organization, woven and lamellar bones are organized into trabecular and cortical bone [55, 56]. Trabecular bone is highly porous having porosity in the range of 50 to 90% and large pores with size up to several millimeters in diameter. Therefore, these bones are also known as spongy bone or cancellous bone. Trabecular bone is responsible for bearing compressive forces under physiological loading conditions [57]. These bones are generally found at the metaphyses and epiphyses of both long and cuboidal bones [57]. Distal end radius is an example of trabecular bone.

Similarly, cortical bones are less porous and possess small pores of size up to 1mm in diameter. Therefore, it is also known as compact bone and it contributes to 80% of the

weight of human skeleton. It is harder, stronger and stiffer than trabecular bone due to less porosity. Humerus and Femur are examples of cortical bone.

1.2.1.3. Chemical Composition of Bone

Generally, bone is made up of 70% inorganic content, 20% organic component and the remaining 10% is water. The inorganic phase of bone is crystalline hydroxyapatite $Ca_{10}(PO_4)_6(OH_2)$ and the 90% of organic phase of bone is Type I collagen. The remaining 10% of organic phase consist of noncollagenous proteins, such as lipids and other macromolecules [55-57]. A.L. Boskey and R.Coleman [50] suggested that the mineral component of bone also changes with age. Various changes in bone mineral composition which alters with age are given below [53]:

- Increasing mineral content
- Increasing carbonate substitution
- Decreasing acid phosphate substitution
- Increasing hydroxyl content
- Increasing Ca/P molar ratio
- Increasing crystal size and perfection

1.2.1.4. Types of Cells in the Bone

Bone is generally made up of three cells and they are Osteoblast, Osteoclast, and Osteocytes.

Osteoblast

These cells are also known as bone forming cells because they are responsible for the synthesis of new bones. In the phenomenon of bone forming osteoid (a protein mixture secreted by osteoblast) plays an important role, which mineralizes to form bone.

Osteocytes

Osteocytes are the bone cells which are originated from osteoblasts and therefore they are mature bone cells. The basic difference between osteoblast and osteocytes is their location in bone. Osteoblasts are located on the periosteal and endosteal surfaces of bone and Osteocytes are arranged concentrically around the central lumen of an osteon and in between lamellae.

Osteoclast

Bone having a physiological grain size of less than 100 nm in diameter is generally termed as healthy bone. A healthy bone is continuously re-modeled through the life span by a process which involves the formation of a bone modeling unit and activation of bone cells. The phenomenon of resorption of bone is performed by osteoclast. The osteoclast is derived from pluripotent cells of bone marrow. Pluripotent cells have a tendency to discriminate different cells including monocytes and macrophages. These pluripotent cells resorb bone by forming disordered cell membrane edges and thus increase the surface area of attachment on the bone surface. Carbonic anhydrase system produces hydrogen ions which decreases the pH of the local environment and due to which the solubility of hydroxyapatite (major inorganic component of bone) crystal increases. After this process, protolytic digestion take place to remove an organic component and the removal of organic and inorganic component results in the formation of resorption pits [51].

1.3. Types of Material Used for Orthopedic Implant Applications: Advantages and Disadvantages

Traditional materials like metals, polymers and ceramics are generally used for implant application. Selection of material depends upon area of application; a brief description of advantage, disadvantage and application is described in Table 1.3.

Ceramics are an inorganic oxide of non-metals and they are known for their good corrosion resistance, biocompatibility, and bioactivity. Ceramics are made up of purely ionic bonds with some covalent character which made them brittle with low fracture toughness and higher value of elastic modulus. Generally, there are two types of Bio-ceramics i.e. Bio-inert ceramic and Bio-resorbable ceramic. Bio-inert ceramics are those ceramics that show high chemical inertness and this made them fit for the biological application. Zirconia and alumina are examples of bio-inert ceramics. Hydroxyapatite and β-tricalcium phosphate is the example of Bio-resorbable ceramics. Due to the poor mechanical properties prostheses of pure ceramics are not preferred for weight-bearing long bone prostheses, as they fail in load-bearing applications. However, ceramics are being used in bone filling and dentistry applications successfully [55]. Similarly, polymers are organic oxides of non-metals; they have great potential to be used as an implant material in low load bearing fracture sites. Polymers are novel material for orthopedic application as they posses suitable mechanical property close to trabecular bone, its biodegradability tendency had increased attention [56]. Polymer like ultra-high-molecular-weight polyethylene (UHMWPE) owns property like high impact strength, low friction coefficient, and low density. These properties made UHMWPE a popular choice for joint replacement. However, the application of UHMWPE is limited due to presence of long term radical in the bulk resulting from the ionizing radiation employed in

sterilization process [57]. Natural polysaccharide polymer like starch, cellulose and alginate are also used in biomedical application [61,62,63]. The main problem associated with use of polymer in orthopedic application is overproduction of wear debris which leads to inflammatory reaction between adjacent tissue and implant. This adverse tissue reaction causes osteolysis, bone resorption and implant failure [61].

Metals and alloys due to their excellent mechanical and biocompatibility property are preferred over ceramics and polymers [62]. Metals like titanium and its alloy, medical grade stainless steel, cobalt chromium alloy and magnesium alloy are generally used for implant application. Metals and alloys contain elements like Co, Cr, Al, Cu, V, Ni these elements are classified as allergic element and elements like Cd, Be, Pb, Ba and Th is classified as toxic element [66, 67]. Elements like Ni, Co, Cr is released from 316L stainless steel and Co-Cr alloy due to corrosion in body environment [68, 69]. Ni toxicity causes skin related diseases like dermatitis and release of Co causes carcinogenicity [8]. The Young's modulus of 316L SS and Co-Cr alloy is much higher as compared to natural bone causing non uniform load transfer between bone and implant which leads to resorption of bone and implant loosening. Also stainless steel posse's poor fatigue strength and wear resistance which limits its application in orthopedics. However, due to its low cost compared to all other metallic alloy 316L SS has maintained its demand in fixation devices like bone plate, bone screw, etc. Magnesium and its alloy due to their biodegradability and potential for avoiding revival surgery has increased the attention of general orthopedic community for surgical fixation of injured musculoskeletal tissue. It is believed that when Mg based alloy is introduced in saline environment it degrades to magnesium chloride, oxide, sulphate or phosphate and these ion s does not cause any adverse effect to local tissues [70, 71]. In spite of lots of advantages there

are certain limitations which limits the use of Mg based alloy. The high corrosion rate of Mg based alloy is one of the major problems [69]. Corrosion causes evolution of hydrogen gas which creates balloon effect in vivo [70]. Due to high corrosion rate the pH value of the surrounding surface also increases [71].

Table 1.3 Advantages, Disadvantages and Applications of Diferent Biomaterials

Materials	Advantages	Disadvantages	Applications
316L SS	Easily available and Low cost, Excellent fabrication properties, Accepted Biocompatibility and toughness.	High Modulus, Poor corrosion resistance, Poor wear resistance, Allergic reaction in surrounding tissue, Stress shielding effect.	Bone Plates, Bone screws, and pins, Wires, etc.
Co-Cr alloys	Superior in terms of resistance to corrosion fatigue and wear, High strength, Long term biocompatibility.	Expensive, quite difficult to machine, Stress shielding effect, High Modulus, Biological toxicity due to Co, Cr and Ni ions release.	Shorter-term implants-Bone plates and wires, Total hip replacements (THR)-Stem or hard-on-hard bearing system
Mg alloy	Biocompatible, Biodegradable, Bioresorbable,	Hydrogen evolution during degradation, Less resistance to corrosion.	Bone screws, Bone plates, bone pins, etc.

	Similar density and Young's modulus of bone (E = 10–30GPa), Less stress shielding effect, Lightweight.		
Ti alloy	Excellent resistance to corrosion, Lower Modulus, Stronger than stainless steels, Lightweight, Biocompatible.	Poor wear resistance, Poor bending ductility, Expensive.	Fracture Fixation plates, Fasteners, nails, rods, screws, and wires, Femoral hip stems, Total Joint Replacement (TJR), Arthroplasty-hips and knees.
Alumina (Al_2O_3)	Biocompatible and bio-inert High hardness, high strength, and abrasion resistance The non-adherent fibrous membrane at the interface. Stress shielding		Femoral head, Porous coatings for femoral stems, Bone screws and plates, Knee prosthesis
Zirconia (Zr_2O_3)	High fracture toughness High flexural strength Low young's modulus		Femoral head, Artificial knee, Bone screws Plates

	Bio-inert Biocompatible Non-toxic		
Bioglass	Biocompatible Bioactive Non-toxic Brittle	Cannot be used for load-bearing applications	Artificial bone Dental implants
Hydroxyapatite (HAp)	Bio-resorbable Bioactive Biocompatible Similar composition to bone Good osteoconductive properties		Femoral knee, Femoral hip, Tibial components, Acetabular cup

1.4. Titanium and Its Alloy: Material of Ultimate choice for Implant Application

Titanium (Ti) is the ninth most abundant element in the lithosphere as it is a constituent of practically all crystalline rock. It was discovered by Reverend William Gregor in 1798 [44]. It is a transition metal with atomic number 22 with an incomplete valence shell. It has the ability to form a substitutional solid solution with an element having a size factor of ±20%. The melting point of titanium is around 1678ºC and the crystal structure of Ti is Hexagonal Closed Packed structure (hcp) α up to the beta transus temperature (882.5ºC), transforming to a body-centred cubic structure (bcc) β above this temperature [72]. The nature of the alloying element decides the alpha (α) to beta (β) transformation temperature of pure

titanium alloy. Elements like Aluminium, Oxygen, Nitrogen, etc are commonly known as α stabilizer because they tend to stabilize the alpha phase as the addition of these elements increases the β transus temperature. Similarly, elements like Vanadium, Molybdenum, Niobium, Iron, Chromium, etc. stabilizes beta transus temperature and therefore known as a β stabilizer. The addition of this element depresses the β transus temperature. Titanium alloys are generally classified on the basis of Alpha (α) and Beta (β) phases present in them. They are classified as α Alloy, near α alloy, (α + β) alloy and Metastable β alloy. The alpha alloys are those which consist of only α phase and α stabilizer i.e. Aluminium, Oxygen, Nitrogen, etc. Near alpha, alloys are a special class of alpha alloys which are made up of 1–2% of β stabilizers and about 5–10% of the β phase. Similarly, α + β alloy are those which contains 10 to 30% of the beta phase and alloys with higher beta stabilizer where the beta phase is formed by fast cooling are known as metastable beta alloys. Generally, α + β or metastable β alloys are employed in the biomedical application. Among all these alloys α and (α + β) alloys are considered as first-generation Ti alloy and they posses high value of Young's modulus (110GPa). The development of second generation Ti alloy i.e. β aFlloy came in existence since 1990 [73]. Due to its ability of possessing lower value of Young's modulus (55-90GPa) β alloys are material of choice for orthopedic application. Different Ti alloys along with their mechanical property used for biomedical application is mentioned in Table 1.4. Due to appropriate mechanical and biocompatible properties Ti and its alloys are material of ultimate choice for implant application.

Table1.4 Mechanical Properties of Ti and Alloys

Material	**Standard**	**Modulus (GPa)**	**Tensile Strength**	**Alloy Type**

			(MPa)	
First Generation biomaterials (1950-1990)				
Commercially pure Ti (Cp grade 1-4)	ASTM 1341	100	240-550	α
Ti–6Al–4V ELI wrought	ASTM F136	110	860-965	α+β
Ti–6Al–4V ELI Standard grade	ASTM F1472	112	895-930	α+β
Ti–6Al–7Nb Wrought	ASTM 1295	110	900-1050	α+β
Ti–5Al–2.5Fe		110	1020	α+β
Second generation biomaterials (1990-till date)				
Ti–13Nb–13Zr Wrought	ASTM F1713	79-84	973-1037	Metastable β
Ti–12Mo–6Zr–2Fe (TMZF)	ASTM F1813	74-85	1060-1100	β
Ti–35Nb–7Zr–5Ta (TNZT)		55	596	β
Ti–29Nb–13Ta–4.6Zr		65	911	β
Ti–35Nb–5Ta–7Zr–0.40 (TNZTO)		66	1010	β
Ti–15Mo–5Zr–3Al		22	NA	β
Ti–Mo	ASTM		NA	β

	F2066			

1.4.1. Methods of Preparing Titanium based foams Using Powder Metallurgy Technique

Ti based materials have low thermal conductivity and high reactivity with surrounding environment due to this it's machining and melting as well as casting becomes difficult. Therefore the Ti components are generally machined from forged Ti blanks at a low speed, in this procedure about 95% of the raw materials are lost as a scrap and recycling of these scrap is still a challenge [74]. In order to reduce the stress shielding effect in Ti based implants incorporation of pores is a promising solution but manufacturing porous Ti structure is not technically easier or simple. However, researcher communities have developed number of manufacturing technique to develop porous Ti structure. In order to reduce the effect of stress shielding, elastic moduli of the implant should be reduced. The use of porous material is suggested to mitigate this problem. The use of porous material in artificial joint replacement is an attractive field of research as it includes different methods and materials which can be used to reduce stiffness mismatch. In the next chapter i.e. **Chapter 2- Literature Survey**, a brief study on different methods of synthesis of porous Titanium alloy scaffold are described.

1.5. Hydroxyapatite: Ideal Material for Biomedical Application

Hydroxyapatite (HAp) with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ has drawn great interest of researchers in the field of development of biomaterials. HAp has wide applications like

development of bone scaffold for tissue engineering [75], bioactive coatings [76], repairing of soft tissues [77]–[80], drug delivery [81]–[87] etc. HAp have hexagonal crystal structure with the lattice parameter of a=b= 9.432 an c= 6.881 Å and $\gamma = 120°$. The Ca/P molar ratio of HAp is about 1.67 and its pH ranges in between 4 to 12 [88]. It is found that HAp can also exist in other crystal form i.e. monoclinic with space group of $P2_1/B$, the major difference between the hexagonal and monoclinic form is orientation of hydroxyl group (OHs) [89]. The HAp synthesized by precipitation from supersaturated solutions at low temperraures generally hav hexagonal crystal structre while HAp synthesized by heating abov 850 °C shows monoclinic crysture structure. HAp was first clinically used in 1975 for the filling of periodontal defect [90] followed to this the commercial use of HAp was started and it was introduced in market in 1981 as a granuals for alveolar ridge augmentation and to fill periodontal lesions [91]. HAp has potential to be used as a coating material on metallic implant to imrove osseointegration in orthopedic implant which was explained by Soballe et al. in 2003 [92].

1.5.1. Advantages and Disadvantages of HAp

HAp is a material of choice in the field of development of synthetic biomaterials used as bone graft for the purpose of bone regeneration. The main advantage of HAp is its properties like biocompatibility and osteoconductive properties but inspite of number of advantages there are certain disadvantages like lack of mechanical strength, ease of degdration in the biological environment, relative lower loading capacity, absence of targeting efficiency as delivery system, etc, are also associated with the application of HAp in the field of biomedical applications.

1.5.2. Examples of Applications of HAp

There are numbers of field in which HAp can be utilized as a potential candidate for bone and tooth repair. HAp has similar composition with that of human bone and teeths which makes it an ideal material for hard tissue repairing. HAp powder and granules are applied in bone and tooth defect fillings, they are generally used as a material for developing porous scaffold for cell growth and new bone development. HAp based ceramic coatings over metallic implant are quite obvious for dental and orthopedic implants [76], [93]. HAp has ability to solve large size bone defects. It can be used as a fabricating scaffold which may act as temporary substrate which allows cell in-growth and proliferation. It can be employed in the field of soft tissue repairing like gums, muscles and skins. Okabayashi et al. in their study showed HAp has ability to activate fibroblasts and accumulate vessels endothelial cells and thus, promoting healing of skin injuries [77]. HAp due to its excellent biocompatibility can also be utilized drug, gene and protein carriers. Thus, it is worth to mention that HAp is a ideal biomaterial candidate for biomedical applications.

1.6. Biocompatibility of Materials

According to Williams 1987 [94] the biocompatibility of any material can be defined as "ability of material to perform with an appropriate host response in a specific application" means a biocompatible material should be bioactive and bio-functional. According to B.D. Retner [95] no material can be biocompatible if it leaches cytotoxic substance when they are implanted. Thus, Bio-implant should be Bio-active and Bio-compatible which means that it should be highly innocuous i.e. they don't cause any allergic reaction in the human body. The success of implant surgery generally depends on the reaction which undergoes between the

implant and the human body**.** There are some factors like Toxicology (study and measurement of the effect of material leaching from biomaterials), Mechanical effects (such as rubbing, compression modulus mismatch, and irritation), size of the implant and broad range of bio-specific interaction with surrounding protein and cells should be considered before designing a biocompatible biomaterial. Detailed study of biocompatibility of Titanium and Hydroxyapatite are discussed in next chapters.

1.7. Thesis Outline

The main aim of the presented chapter is to create an insight and understanding to the basics of biomaterials employed for biomedical applications. Characteristis of biomaterials are briefly explained and the major focus was on explaining the factors which are responsible for designing of bio-implant. Apart from this a dedicated section on bone metabolism is added in order to conceptualize and visualize the interaction of synthetic biomaterials with the natural system. The subsequent chapters will explain the below mentioned content to achieve the objective as mentioned.

Chapter 2 explains a literature review designed to provide a summary of previous work done by the researchers in the field associated with the desired are of interest

Chapter 3 will expain the objective of the present work, material selected for the prepration of composites, the method adopted to synthesis the composite

Chapter 4 explains about the various characterization techniques adopted to study physical, mechanical and biological properties of porus composite

Chapter 5 explains about mechanical and biological behaviour of porous Ti-SiO2 scaffold for tissue engineering application

Chapter 6 explains about low-cost approach to develop HAp/SiO2 based scaffold by valorizing animal bone waste and rice husk for tissue engineering applications

Chapter 7 provides a summary of the findings of this research work and outlines specific conclusion drawn from both the experimental and analytical efforts and suggests ideas and direction for future research.

References

[1] J. Banhart, "Manufacture, characterisation and application of cellular metals and metal foams," *Prog. Mater. Sci.*, vol. 46, no. 6, pp. 559–632, Jan. 2001, doi: 10.1016/S0079-6425(00)00002-5.

[2] B. Dabrowski, W. Swieszkowski, D. Godlinski, and K. J. Kurzydlowski, "Highly porous titanium scaffolds for orthopaedic applications," 2010, doi: 10.1002/jbm.b.31682.

[3] M. Nabiyouni, T. Brückner, H. Zhou, U. Gbureck, and S. B. Bhaduri, "Magnesium-based bioceramics in orthopedic applications," *Acta Biomater.*, vol. 66, pp. 23–43, Jan. 2018, doi: 10.1016/J.ACTBIO.2017.11.033.

[4] H. Zhou, J. G. Lawrence, and S. B. Bhaduri, "Fabrication aspects of PLA-CaP/PLGA-CaP composites for orthopedic applications: A review," *Acta Biomater.*, vol. 8, no. 6, pp. 1999–2016, Jul. 2012, doi: 10.1016/J.ACTBIO.2012.01.031.

[5] M. M. Stevens, "Biomaterials for bone tissue engineering," *Mater. Today*, vol. 11, no. 5, pp. 18–25, May 2008, doi: 10.1016/S1369-7021(08)70086-5.

[6] M. M. M. J. F. Keating, "SUBSTITUTES FOR AUTOLOGOUS BONE GRAFT IN ORTHOPAEDIC TRAUMA," *J. Bone Jt. Surg.*, vol. 83, no. B, 2001.

[7] C. J. Damien and J. R. Parsons, "Bone graft and bone graft substitutes: a review of current technology and applications.," *J. Appl. Biomater.*, vol. 2, no. 3, pp. 187–208, 1991, doi: 10.1002/jab.770020307.

[8] M. Geetha, A. K. Singh, R. Asokamani, and A. K. Gogia, "Ti based biomaterials, the ultimate choice for orthopaedic implants - A review," *Prog. Mater. Sci.*, vol. 54, no. 3, pp. 397–425, 2009, doi: 10.1016/j.pmatsci.2008.06.004.

[9] R. Radha and D. Sreekanth, "Insight of magnesium alloys and composites for orthopedic implant applications – a review," *J. Magnes. Alloy.*, vol. 5, no. 3, pp. 286–312, 2017, doi: 10.1016/j.jma.2017.08.003.

[10] D. Zhao, F. Witte, F. Lu, J. Wang, J. Li, and L. Qin, "Current status on clinical applications of magnesium-based orthopaedic implants: A review from clinical translational perspective," *Biomaterials*, vol. 112, pp. 287–302, 2017, doi: 10.1016/j.biomaterials.2016.10.017.

[11] S. C. P. Cachinho and R. N. Correia, "Titanium scaffolds for osteointegration: mechanical, in vitro and corrosion behaviour," *J. Mater. Sci. Mater. Med.*, vol. 19, no. 1, pp. 451–457, Jan. 2008, doi: 10.1007/S10856-006-0052-7.

[12] Jochem Nagels, Marie¨lle Stokdijk, Piet M. Rozing, "Stress shielding and bone resorption in shoulder arthroplasty," *J Shoulder Elb. Surg*, 2003, doi: doi:S1058-2746(02)00002-2.

[13] M. J. Donachie, *Titanium: A Technical Guide*. Materials Park, OH: ASM International, 2000. [Online]. Available: https://www.asminternational.org/search/-/journal_content/56/10192/06112G/PUBLICATION

[14] S. H. A. Sanan, "Repairing holes in the head: a history of cranioplasty," *Neurosurgery*, vol. 40, no. 3, pp. 588–603, 1997, doi: https://doi.org/10.1097/00006123-199703000-00033.

[15] E. D. Spoerke, N. G. Murray, H. Li, L. C. Brinson, D. C. Dunand, and S. I. Stupp, "A bioactive titanium foam scaffold for bone repair," *Acta Biomater.*, vol. 1, no. 5, pp. 523–533, Sep. 2005, doi: 10.1016/J.ACTBIO.2005.04.005.

[16] U. Müller, T. Imwinkelried, M. Horst, M. Sievers, and U. Graf-Hausner, "Do human

osteoblasts grow into open-porous titanium?," *Eur. Cells Mater.*, vol. 11, pp. 8–15, 2006, doi: 10.22203/eCM.v011a02.

[17] O. E. M. Pohler, "Unalloyed titanium for implants in bone surgery," *Injury*, vol. 31, no. SUPPL. 4, pp. D7–D13, Dec. 2000, doi: 10.1016/S0020-1383(00)80016-9.

[18] S. Yang, K. F. Leong, Z. Du, and C. K. Chua, "The design of scaffolds for use in tissue engineering. Part I. Traditional factors," *Tissue Eng.*, vol. 7, no. 6, pp. 679–689, 2001, doi: 10.1089/107632701753337645.

[19] J. N. Weber and E. W. White, "Carbon-metal graded composites for permanent osseous attachment of non-porous metals," *Mater. Res. Bull.*, vol. 7, no. 9, pp. 1005–1016, 1972, doi: 10.1016/0025-5408(72)90092-X.

[20] R. Langer and J. P. Vacanti, "Tissue Engineering." [Online]. Available: https://www.science.org

[21] D. W. Hutmacher, "Scaffolds in tissue engineering bone and cartilage," *Biomaterials*, vol. 21, no. 24, pp. 2529–2543, Dec. 2000, doi: 10.1016/S0142-9612(00)00121-6.

[22] M. Niinomi, M. Nakai, and J. Hieda, "Development of new metallic alloys for biomedical applications," *Acta Biomater.*, vol. 8, no. 11, pp. 3888–3903, Nov. 2012, doi: 10.1016/J.ACTBIO.2012.06.037.

[23] H. M. Frost, "A 2003 update of bone physiology and Wolff s law for clinicians," *Angle Orthod.*, vol. 74, no. 1, pp. 3–15, 2004, doi: 10.1043/0003-3219(2004)074<0003:AUOBPA>2.0.CO;2.

[24] M. J. Gardner, M. C. H. Van Der Meulen, D. Demetrakopoulos, T. M. Wright, E. R. Myers, and M. P. Bostrom, "In Vivo Cyclic Axial Compression Affects Bone Healing in the Mouse Tibia," *J Orthop Res*, vol. 24, pp. 1679–1686, 2006, doi:

10.1002/jor.20230.

[25] L. Bozec and M. A. Horton, "Skeletal tissues as nanomaterials," *J. Mater. Sci. Mater. Med.*, vol. 17, no. 11, pp. 1043–1048, 2006, doi: 10.1007/s10856-006-0442-x.

[26] M. Long and H. J. Rack, "Titanium alloys in total joint replacement—a materials science perspective," *Biomaterials*, vol. 19, no. 18, pp. 1621–1639, Sep. 1998, doi: 10.1016/S0142-9612(97)00146-4.

[27] van R. B. Huiskes R, Weinans H, "The relationship between stress shielding and bone resorption around total hip stems and the effects of flexible materials.," *Clin. Orthop. Relat. Res.*, vol. 274, pp. 124–134, 1992.

[28] D. R. Sumner, T. M. Turner, R. Igloria, R. M. Urban, and J. O. Galante, "Functional adaptation and ingrowth of bone vary as a function of hip implant stiffness," *J. Biomech.*, vol. 31, no. 10, pp. 909–917, Oct. 1998, doi: 10.1016/S0021-9290(98)00096-7.

[29] S. Agarwal, J. Curtin, B. Duffy, and S. Jaiswal, "Biodegradable magnesium alloys for orthopaedic applications: A review on corrosion, biocompatibility and surface modifications," *Mater. Sci. Eng. C*, vol. 68, pp. 948–963, 2016, doi: 10.1016/j.msec.2016.06.020.

[30] Tadashi Kokubo, *Bioceramics and their Clinical Applications*. 2008.

[31] L. C. Lucas, R. A. Buchanan, and J. E. Lemons, "Investigations on the galvanic corrosion of multialloy total hip prostheses," *J. Biomed. Mater. Res.*, vol. 15, no. 5, pp. 731–747, 1981, doi: 10.1002/jbm.820150509.

[32] E. McCafferty, *Introduction to corrosion science*. 2010. doi: 10.1007/978-1-4419-0455-3.

[33] W. . Urish, K.L, Anderson, P.A and Mihalko, "The Challenge of Corrosion in Orthopaedic Implants," *J. Am. Acanademy Orthop. Surg.*, 2013.

[34] A. Sargeant and T. Goswami, "Hip implants: Paper V. Physiological effects," *Mater. Des.*, vol. 27, no. 4, pp. 287–307, Jan. 2006, doi: 10.1016/J.MATDES.2004.10.028.

[35] N. Abbasi, S. Hamlet, R. M. Love, and N. T. Nguyen, "Porous scaffolds for bone regeneration," *J. Sci. Adv. Mater. Devices*, vol. 5, no. 1, pp. 1–9, Mar. 2020, doi: 10.1016/J.JSAMD.2020.01.007.

[36] Y. Kuboki *et al.*, "BMP-Induced osteogenesis on the surface of hydroxyapatite with geometrically feasible and nonfeasible structures: Topology of osteogenesis," 1998.

[37] F. Bai *et al.*, "The correlation between the internal structure and vascularization of controllable porous bioceramic materials in vivo: A quantitative study," *Tissue Eng. - Part A*, vol. 16, no. 12, pp. 3791–3803, 2010, doi: 10.1089/ten.tea.2010.0148.

[38] B. T. J X Lu, B Flautre, K Anselme, P Hardouin, A Gallur, M Descamps, "Role of interconnections in porous bioceramics on bone recolonization in vitro and in vivo," *J Mater Sci Mater Med*, vol. 10, no. 2, pp. 111–120, 1999, doi: https://doi.org/10.1023/a:1008973120918.

[39] B. W. Sauer, A. M. Weinstein, J. J. Klawitter, S. F. Hulbert, R. B. Leonard, and J. G. Bagwell, "Role of Porous Polymeric Materials in Prosthesis Attachment.," *J Biomed Mater Res Biomed Mater Symp*, vol. 5, no. 5, pp. 145–153, 1973.

[40] M. Abdel-Hady Gepreel and M. Niinomi, "Biocompatibility of Ti-alloys for long-term implantation," *J. Mech. Behav. Biomed. Mater.*, vol. 20, pp. 407–415, Apr. 2013, doi: 10.1016/J.JMBBM.2012.11.014.

[41] M. M. Dewidar and J. K. Lim, "Properties of solid core and porous surface Ti–6Al–

4V implants manufactured by powder metallurgy," *J. Alloys Compd.*, vol. 454, no. 1–2, pp. 442–446, Apr. 2008, doi: 10.1016/J.JALLCOM.2006.12.143.

[42] A. Laptev, O. Vyal, M. Bram, H. P. Buchkremer, and D. Stöver, "Green strength of powder compacts provided production of highly porous titanium parts," *Powder Metall.*, vol. 48, no. 4, pp. 358–364, 2005, doi: 10.1179/174329005X73838.

[43] W. Jin and P. K. Chu, "Orthopedic implants," *Encycl. Biomed. Eng.*, vol. 1–3, pp. 425–439, 2019, doi: 10.1016/B978-0-12-801238-3.10999-7.

[44] R. Van Noort, "Titanium: The implant material of today," *J. Mater. Sci.*, vol. 22, no. 11, pp. 3801–3811, 1987, doi: 10.1007/BF01133326.

[45] N. J. Hallab, S. Anderson, T. Stafford, T. Glant, and J. J. Jacobs, "Lymphocyte responses in patients with total hip arthroplasty," *J. Orthop. Res.*, vol. 23, p. 384391, 2005, doi: 10.1016/j.orthres.2004.09.0Ol.

[46] D. F. Williams, "On the mechanisms of biocompatibility," *Biomaterials*, vol. 29, no. 20, pp. 2941–2953, 2008, doi: 10.1016/j.biomaterials.2008.04.023.

[47] A. W. Tomas Albrektsson, John Brunski, "A requiem for the periodontal ligament' revisited," *Int J Prosthodont*, vol. 22, no. 2, 2009.

[48] B. G. Mavrogenis AF, Dimitriou R, Parvizi J, "Biology of implant osseointegration," *J. Musculoskelet. Neuronal Interact.*, vol. 9, no. 2, pp. 61–71, 2009.

[49] D. B. B. R. B. Martin, "Structure Function and Adaptation of Compact Bone," *Mater. Res. Bull.*, vol. 7, pp. 14–15, 1972.

[50] A. L. Boskey and R. Coleman, "Aging and Bone," vol. 89, no. 12, pp. 1333–1348, 2015, doi: 10.1177/0022034510377791.

[51] T. J. Webster, "Nanophase ceramics: The future orthopedic and dental implant

material," *Adv. Chem. Eng.*, vol. 27, pp. 125–166, 2001, doi: 10.1016/s0065-2377(01)27005-7.

[52] J. P. I. F.S. Kaplan, W.C. Haye, T.M. Keaveny, A. Boskey, T.A. Einhorn, "Form and function of bone," *Orthop. Basic Sci.*, pp. 127–185, 1994.

[53] R. S. L. J. B. Park, *Biomaterials. An Introduction, Second Edition*. 1993. doi: https://doi.org/10.1177%2F088391159300800307.

[54] Y.C. Fung, *Biomechanics Mechanical Properties of Living Tissues Second ed*. 1993.

[55] H. Mehboob and S. H. Chang, "Application of composites to orthopedic prostheses for effective bone healing: A review," *Compos. Struct.*, vol. 118, no. 1, pp. 328–341, Dec. 2014, doi: 10.1016/J.COMPSTRUCT.2014.07.052.

[56] A. M. S. Ibrahim, P. G. L. Koolen, K. Kim, G. S. Perrone, D. L. Kaplan, and S. J. Lin, "Absorbable Biologically Based Internal Fixation," *Clin. Podiatr. Med. Surg.*, vol. 32, no. 1, pp. 61–72, Jan. 2015, doi: 10.1016/J.CPM.2014.09.009.

[57] H. Lee, F. Zeng, M. Dunne, and C. Allen, "Methoxy Poly(ethylene glycol)-block-Poly(δ-valerolactone) Copolymer Micelles for Formulation of Hydrophobic Drugs," 2005, doi: 10.1021/bm050451h.

[58] H. Yu *et al.*, "Cisplatin loaded poly(L-glutamic acid)-g-methoxy poly(ethylene glycol) complex nanoparticles for potential cancer therapy: Preparation, in vitro and in vivo evaluation," *J. Biomed. Nanotechnol.*, vol. 12, no. 1, pp. 69–78, 2016, doi: 10.1166/jbn.2016.2152.

[59] W. Song, Z. Tang, D. Zhang, H. Yu, and X. Chen, "Coadministration of Vascular Disrupting Agents and Nanomedicines to Eradicate Tumors from Peripheral and Central Regions," 2015, doi: 10.1002/smll.201500324.

[60] C. Shi *et al.*, "Cisplatin-loaded polymeric nanoparticles: Characterization and potential exploitation for the treatment of non-small cell lung carcinoma," *Acta Biomater.*, vol. 18, pp. 68–76, May 2015, doi: 10.1016/J.ACTBIO.2015.02.009.

[61] S. C. Kim *et al.*, "In vivo evaluation of polymeric micellar paclitaxel formulation: toxicity and efficacy," *J. Control. Release*, vol. 72, no. 1–3, pp. 191–202, May 2001, doi: 10.1016/S0168-3659(01)00275-9.

[62] J. Lausmaa, B. Kasemo, H. Mattsson, and H. Odelius, "Multi-technique surface charaterization of oxide films on electropolished and anodically oxidized titanium," *Appl. Surf. Sci.*, vol. 45, no. 3, pp. 189–200, Oct. 1990, doi: 10.1016/0169-4332(90)90002-H.

[63] X. Gu, Y. Zheng, Y. Cheng, S. Zhong, and T. Xi, "In vitro corrosion and biocompatibility of binary magnesium alloys," *Biomaterials*, vol. 30, no. 4, pp. 484–498, Feb. 2009, doi: 10.1016/J.BIOMATERIALS.2008.10.021.

[64] Y. J. Chen, Y. J. Li, J. C. Walmsley, S. Dumoulin, P. C. Skaret, and H. J. Roven, "Microstructure evolution of commercial pure titanium during equal channel angular pressing," *Mater. Sci. Eng. A*, vol. 527, no. 3, pp. 789–796, Jan. 2010, doi: 10.1016/J.MSEA.2009.09.005.

[65] D. G. Barceloux and D. Barceloux, "Chromium For personal use only," *Clin. Toxicol.*, vol. 37, no. 2, pp. 173–194, 1999, [Online]. Available: www.dekker.com

[66] D. G. Barceloux, "Donald G. Barceloux," vol. 37, no. 2, pp. 239–258, 1999.

[67] G. Eddy Jai Poinern, S. Brundavanam, and D. Fawcett, "Biomedical Magnesium Alloys: A Review of Material Properties, Surface Modifications and Potential as a Biodegradable Orthopaedic Implant," *Am. J. Biomed. Eng.*, vol. 2, no. 6, pp. 218–240,

2013, doi: 10.5923/j.ajbe.20120206.02.

[68] R. Murugan and S. Ramakrishna, "Development of nanocomposites for bone grafting," *Compos. Sci. Technol.*, vol. 65, no. 15–16, pp. 2385–2406, Dec. 2005, doi: 10.1016/J.COMPSCITECH.2005.07.022.

[69] Y. Zhao, G. Wu, J. Jiang, H. M. Wong, K. W. K. Yeung, and P. K. Chu, "Improved corrosion resistance and cytocompatibility of magnesium alloy by two-stage cooling in thermal treatment," *Corros. Sci.*, vol. 59, pp. 360–365, 2012, doi: 10.1016/j.corsci.2012.03.020.

[70] F. Witte *et al.*, "In vivo corrosion of four magnesium alloys and the associated bone response," *Biomaterials*, vol. 26, no. 17, pp. 3557–3563, 2005, doi: 10.1016/j.biomaterials.2004.09.049.

[71] E. Ghali, *Corrosion Resistance of Aluminum and Magnesium Alloys: Understanding, Performance, and Testing*. 2010.

[72] E W Collings, *The physical metallurgy of titanium alloys*. 1984.

[73] R. Singh, P. D. Lee, R. J. Dashwood, and T. C. Lindley, "Titanium foams for biomedical applications: A review," *Mater. Technol.*, vol. 25, no. 3–4, pp. 127–136, 2010, doi: 10.1179/175355510X12744412709403.

[74] M. Qian, W. Xu, M. Brandt, and H. P. Tang, "Additive manufacturing and postprocessing of Ti-6Al-4V for superior mechanical properties," *MRS Bull.*, vol. 41, no. 10, pp. 775–783, 2016, doi: 10.1557/mrs.2016.215.

[75] S. V Dorozhkin, "Calcium Orthophosphate Cements and Concretes," *Materials (Basel).*, vol. 2, pp. 221–291, 1980, doi: 10.3390/ma2010221.

[76] M. Vallet-Regí and J. M. González-Calbet, "Calcium phosphates as substitution of

bone tissues," *Prog. Solid State Chem.*, vol. 32, no. 1–2, pp. 1–31, Jan. 2004, doi: 10.1016/J.PROGSOLIDSTCHEM.2004.07.001.

[77] R. Okabayashi, M. Nakamura, T. Okabayashi, Y. Tanaka, A. Nagai, and K. Yamashita, "Efficacy of Polarized Hydroxyapatite and Silk Fibroin Composite Dressing Gel on Epidermal Recovery From Full-Thickness Skin Wounds," 2009, doi: 10.1002/jbm.b.31329.

[78] Y. Shin, H. Aoki, N. Yoshiyama, M. Akao, and M. Higashikata, "Surface properties of hydroxyapatite ceramic as new percutaneous material in skin tissue," *J. Mater. Sci. Mater. Med.*, vol. 3, no. 3, pp. 219–221, 1992, doi: 10.1007/BF00713453.

[79] D. Y. Ji, T. F. Kuo, H. Da Wu, J. C. Yang, and S. Y. Lee, "A novel injectable chitosan/polyglutamate polyelectrolyte complex hydrogel with hydroxyapatite for soft-tissue augmentation," *Carbohydr. Polym.*, vol. 89, no. 4, pp. 1123–1130, Aug. 2012, doi: 10.1016/J.CARBPOL.2012.03.083.

[80] M. Liu *et al.*, "Effect of nano-hydroxyapatite on the axonal guidance growth of rat cortical neurons", doi: 10.1039/c2nr30072a.

[81] V. U. Uskokovic´, D. P. Uskokovic´2, and U. Uskokovic´2, "Review Nanosized hydroxyapatite and other calcium phosphates: Chemistry of formation and application as drug and gene delivery agents," 2010, doi: 10.1002/jbm.b.31746.

[82] I. Rodríguez-Ruiz *et al.*, "pH-Responsive Delivery of Doxorubicin from Citrate−Apatite Nanocrystals with Tailored Carbonate Content," 2013, doi: 10.1021/la4008334.

[83] K. Lin *et al.*, "Cite this: CrystEngComm," vol. 15, p. 2999, 2013, doi: 10.1039/c3ce26683d.

[84] K. Lin *et al.*, "Strontium substituted hydroxyapatite porous microspheres: Surfactant-free hydrothermal synthesis, enhanced biological response and sustained drug release," *Chem. Eng. J.*, vol. 222, pp. 49–59, Apr. 2013, doi: 10.1016/J.CEJ.2013.02.037.

[85] K. Lin *et al.*, "Biomimetic hydroxyapatite porous microspheres with co-substituted essential trace elements: Surfactant-free hydrothermal synthesis, enhanced degradation and drug release", doi: 10.1039/c1jm12514a.

[86] S. H. Zhu *et al.*, "Hydroxyapatite nanoparticles as a novel gene carrier," *J. Nanoparticle Res.*, vol. 6, no. 2, pp. 307–311, 2004, doi: 10.1023/b:nano.0000034721.06473.23.

[87] J. Li, Y. C. Chen, Y. C. Tseng, S. Mozumdar, and L. Huang, "Biodegradable calcium phosphate nanoparticle with lipid coating for systemic siRNA delivery," *J. Control. Release*, vol. 142, no. 3, pp. 416–421, Mar. 2010, doi: 10.1016/J.JCONREL.2009.11.008.

[88] S. Koutsopoulos, "Synthesis and characterization of hydroxyapatite crystals: A review study on the analytical methods," 2002.

[89] G. Ma and X. Y. Liu, "Hydroxyapatite: Hexagonal or Monoclinic?," 2009, doi: 10.1021/cg900156w.

[90] K. H. M. E. B. Nery,K. L. Lynch,W. M. Hirthe, "Bioceramic Implants in Surgically Produced Infrabony Defects," *J. Periodontol.*, vol. 46, no. 6, pp. 328–347, 1975.

[91] M. T. Manley, "CALCIUM PHOSPHATE BIOMATERIALS A REVIEW OF THE LITERATURE," *Heal. Environ. Res. Online*, pp. 1–23, 1993.

[92] H. B.-R. K Soballe, ES Hansen, "The effect of osteoporosis, bone deficiency, bone

grafting and micromotion on fixation of porous-coated hydroxyapatite-coated implants".

[93] Larry L. Hench, "Bioceramics: From Concept to Clinic," *J. Am. Ceram. Soc.*, vol. 74, no. 7, pp. 1487–1510, 1991, doi: https://doi.org/10.1111/j.1151-2916.1991.tb07132.x.

[94] D. F.Williams, "Definitions in Biomaterials," *Prog. Biomed. Eng.*, vol. 4, 1987.

[95] B. D. Ratner, *The Biocompatibility of Implant Materials*. Elsevier Inc., 2015. doi: 10.1016/B978-0-12-800196-7.00003-7.