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Synthesize and Fabrication of Artificial Human Bone Developed by using Nanocomposite

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ABSTRACT

Hydroxyapatite is the main component in hard tissues such as bone. It has been studied extensively for the use of prosthetic application. The main disadvantage of this material is it has very poor mechanical strength which is unsuitable for load-bearing applications. On the other hand, zirconia has high strength and fracture toughness, and great biocompatibility. Therefore, addition of a zirconia into a hydroxyapatite one may lead to an improvement of the mechanical properties and will not affect its biocompatibility. Also coefficient of friction is observed is less in Hap-Zr composite than pure hydroxyapatite bone sample.

Keywords: *Biocompatibility, Hydroxyapatite*

1. INTRODUCTION

Bone is an amazing and a true nanocomposite. It is a complex and a highly specialized form of connective tissue pertaining to the formation of the skeleton of the body. Bone, not only provides mechanical support but also serves as a reservoir for minerals, particularly calcium and phosphate. It is a good example of a dynamic tissue, since it has a unique capability of self-regenerating or self-remodeling to a certain extent throughout the life [1]. However, many circumstances call for bone grafting owing to bone defects either from traumatic or from non-traumatic destruction. With reference to statistical reports [2-4], about 6.3 million fractures occur every year in the United States of America (USA) itself, of which about 550,000 cases require some kind of bone grafting. It was also noticed that the fractures occur at an annual rate of 2.4 per 100 population in which men seem to experience more fractures (2.8 per 100 population) than women (2.0 per 100 population).

The most frequently occurring fractures are, in decreasing order, hip, ankle, tibia, and fibula fractures. It is reported that the total number of hip replacements was about 152,000 in the year 2000, which is an increase of about 33% compared to the year 1990 in the USA alone and it is expected to increase to about 272,000 by the year 2030 [5], indicating that there is still a great need for synthetic bone grafts. The need for synthetic bone grafts depends on the complication of the bone defects. For example, if the defect is minor, bone has its own capability to self regenerate within a few weeks. Therefore, surgery is not required. In the case of severe defects and loss of volume, bone would not heal by itself and grafting is required to restore function without damaging living tissues.

There are multiple methods available for the treatment of bone defects, which includes the traditional methods of autografting and allografting. Although autografting and allografting are clinically considered as good therapies, they have limitations. Accordingly, there is a great need for the use of synthetic bone grafts. Nowadays, numerous synthetic bone graft materials, Although there is good progress in bone grafting using synthetic bone grafts, the way in which they execute their functions in vivo is quite different and most of them differ from natural bone either compositionally or structurally. Further, a single-phase material (also called monolithic) does not always provide all the essential features required for bone growth, therefore, a great need for engineering multi-phase materials (also called composite) with structure and composition similar to natural bone. Bone grafts provide mechanical or structural support, fill defective gaps, and enhance bone tissue formation. They are widely used in orthopedic surgery, plastic surgery, oral and maxillofacial surgery, and dental surgery. It should be noted that bone is the second most transplanted tissue in humans. The graft materials not only replace missing bone but also help the body to regenerate its own lost bone. By this method, bone healing time is reduced and new bone formation strengthens the defective area by bridging grafted materials with host bone. Autografting is a method in which tissue or organ is transplanted from one site to another site of the same individual. Allografting can be defined as tissue transplantation between individuals of the same species but of non-identical genetic composition. Xenografting is a process of transplanting tissue from one species to another (e.g., bone from animal to human). As an alternative to the above three types of bone grafts, synthetic substances are gaining much interest for use as bone graft materials. A surgical method that uses synthetic substances to repair or regenerate defective bone tissue is known as alloplastic or synthetic bone grafting (6-10). The benefits of synthetic grafts include availability, sterility, cost-effectiveness, and reduced morbidity. The synthetic grafts eliminate some of the shortcomings of autografts or allografts associated with donor shortage and the chance for rejection or transmission of infectious disease. Over the past four decades, several biomaterials have been developed and successfully used as bone grafts. Evolution of biomaterials in bone grafting Bone and joint substitutes are commonly made of metals, ceramics, polymers, and their composites [7, 11-16]. In most of cases metals and ceramics are used in hard tissue applications. While polymer in soft tissue applications due to their mechanical properties. Currently for synthetic replacement of bone injuries steel alloys, Ti alloys, Cr alloys are using but when these materials are in contact with blood they can produce toxic cells (16-19).

In order to overcome above theories the existing process for repairing or regenerating bone tissues is replaced by the materials which possesses similar performance parameters. The artificial bone can be implanted alone at the site of injury without affecting its biocompatibility. During the repair/regeneration process, the artificial bone provides structural and mechanical restoration of the damaged tissues. Bone is a composite material consisting of both fluid and solid phases. Its specific gravity is about 2.0 but varies depending on the type of bone. Bone obtains its hard structure because the organic extracellular collagenous matrix is impregnated with inorganic materials, principally hydroxyapatite C10(PO4)6(OH)2 consisting of the minerals calcium and phosphate. Calcium and phosphate account for roughly 65 to 70 % of the bone's dry weight.

Collagen fibers compose approximately 95 % of the extracellular matrix and account for 25 to 30 % of the dry weight of bone.

The organic material gives bone its flexibility while the inorganic material gives bone its resilience. Water accounts for up to 25 % of the total weight of bone with about 85 % of the water being located in the organic matrix around the collagen fibers and ground substance. The other 15 % is located in canals and cavities that house the bone cells. At the gross level bone exists in a variety of shapes and sizes. The general shapes of bones are conserved across vertebrate skeletons, but the specific morphology varies considerably (e.g. a rat femur has the same general shape as a human femur, but it differs greatly in its size and specific architectural details). Bone is identified as either cancellous (also referred to as trabecular or spongy) or cortical (also referred to as compact). Cortical bone represents approximately 4 times the mass of cancellous bone in any long bone. The basic material comprising cancellous and compact bone appear identical, thus the distinction between the two is the degree of porosity and the organization.

Table 1.1 Bone composition

Sr. no	Component	Wt. %
1.	Hydroxyapatite	60-70
2.	Collagen	10-20
3.	Water	9-20
4.	Non-collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin)	3-5
5.	Carbonate	4
6.	Sodium	0.7
7.	Magnesium	0.4
8.	Other inorganic ions (Cl^-, F^-, K^+ Sr^{2+}, Pb^{2+}, Zn^{2+})	traces
9.	Other organic material (polysaccharides, lipids)	traces

Table 1.2 Structural and Material Properties of bone

Sr.No	Properties	Cortical bone	Cancellous bone
1.	Youngs modulus (GPa	14–20	0.05–0.5
2.	Tensile strength (MPa)	50–150	10–20
3.	Compressive strength (MPa)	170–193	7–10
4.	Strain to failure	1–3	5–7
5.	Density (g/cm3)	18–22	0.1–1.0
6.	Surface/bone volume (mm2/mm3)	2.5	20

2. MATERIALS AND METHODS

While selections of materials for artificial human bones the following specifications are taken into consideration.

2.1 Biocompatibility is the first and foremost important criteria while selection of material. It is used to describe the adaptability of material for exposure to the body. Biocompatible materials are generally non-toxic, non-inflammatory and do not produce any adverse reaction and have other suitable physical and mechanical properties. Currently metallic materials, such as Ti alloys, steel alloys, and chromium alloys is widely used as bone implants in orthopedic applications. The use of materials harder and stiffer than bone tissue may be produce mechanical mismatch problems i.e. stress shielding between implant material and adjacent bone tissues. on the other hand metallic material can be produce toxic cells or cancer cells when it will contact with blood. So the main challenge is to select biocompatible material with suitable physical properties.

2.2 The mechanical properties of artificial bone is need to match those of surround tissues i.e. tensile strength, compressive strength, hardness, density etc.

2.3 The artificial bone composition should have appropriate structure and surface that support cell function and tissue regeneration. For example the artificial bone structure permit the transport of oxygen and nutrients and the surface nanotopographgy affects proliferation and cell adhesion.

The following compositions are selected while considering above criteria.

Hydroxyapatite (100%) and Hydroxyapatite (60%) + Zirconia (40%)

Hydroxyapatite is a class of calcium phosphate based bioceramic, frequently used as a bone graft substitute owing to its chemical and structural similarity with natural bone mineral The stoichiometric HA has a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ with Ca/P ratio of 1.67. The HA derived either from natural sources or from synthetic sources is regarded as bioactive substance, since it forms a strong chemical bond with host bone tissue, and hence it is recognized as a good bone graft material. HA is not only bioactive but also osteoconductive, non-toxic, nonimmunogenic, and its structure is crystallographically similar to that of bone mineral with adequate amount of carbonate substitution. A compilation of physiochemical, mechanical, and biological properties of HA are given in below which makes HA an appropriate bone graft material.

Table 2.1 Hydroxyapatite properties

Sr. No.	Properties	Experimental data
1.	Chemical composition	$Ca_{10}(PO_4)_6(OH)_2$
2.	Ca/P molar	1.67
3.	Crystal system	Hexagonal
4.	Elastic modulus(Gpa)	114
5.	Youngs modulus (Gpa)	80-110
6.	Compressive strength(Mpa)	400-900
7.	Fracture toughness(Mpa m1/2)	0.7-1.2
8.	Bending strength (Mpa)	115-200
9.	Hardness (HV)	600
10.	Relative density (%)	95-99.5
11.	Melting point (degree celcius)	1614
12.	Biocompatibility	High

Zirconia has emerged as a versatile and promising material among dental ceramics, due to its excellent mechanical properties owing to the transformation toughening mechanism. Zirconia (ZrO_2) is a white crystalline oxide of zirconium. Zirconia is a polycrystalline ceramic without a glassy phase and exists in several forms. Its mechanical properties are very similar to those of metals and its colour similar to tooth colour. The mechanical properties of zirconia are the highest ever reported for any ceramic. These Capabilities are highly attractive in prosthetic bone.

Mechanical properties of zirconia were proved to be higher than those of all other ceramics and similar to those of stainless steel. Fracture toughness Zirconia is between 6 and 10 MPa m1/2, which is almost twice as high all that of aluminum oxide ceramics. This is due to transformational toughening, which gives zirconia its unique mechanical properties. It has a flexural strength of 900-1200MPa and a compression resistance of 2000MPa. An average load-bearing capacity of 755N was reported for zirconia restorations. Fracture loads ranging between 706N, 2000N and 4100N were reported. Both in vitro and in vivo studies have confirmed the superior biocompatibility. They are chemically inert materials allowing good cell adhesion and while no local or systemic adverse reactions have been associated with it. Zirconia ceramics have similar cytotoxicity to alumina (both lower than tiO2). Also starch powder is selected for the binder of this composition. Starch powder is also biocompatible.

3. FABRICATION OF BONE

For synthesis the ball mill is used to mix the powder of hydroxyapatite and zirconia by weight percentage. A ball mill, a type of grinder, is a cylindrical device used in grinding (or mixing) materials like ores, chemicals, ceramic raw materials and paints. Ball mills rotate around a horizontal axis, partially filled with the material to be ground plus the grinding medium. Stainless steel balls are used as grinding medium. Constituents were mixed and blended in Horizontal Ball mill with the speed of 120 rpm.

Fig.3.1 Ball Mill

The total time selected for milling is 4 hours. The ball mill has six no. of balls having 10 mm dia. And two balls having 5 mm dia. Along with composition the binder starch powder is added in ball mill machine.

Fig.3.2 Composite mixed powder

After mixing and milling the mixture is poured into die and quasi-statically compacted up to 300 MPa by using compression molding machine at atmospheric pressure and temperature. Material was kept for 15-20 min under this pressure condition.

Fig. 3.3 Compression Molding Machine

Then material is removed from die and heated up to 300 degree Celsius temp. in melting furnace and cooled at room temperature after cooling the sample is look like that.

Fig. 3.4 Sample bone of Pure Hydroxyapatite

Fig. 3.5 Sample Bone of Hap- Zr Composition

4. RESULTS AND DISCUSSIONS

Wear test on Pin-on-Disc setup.

The pin mentioned above section was mounted on disc by means of holder and while the disc rotated. The disc speed is set to 500 rpm. And 2 kg load is set. And time required for test is 60 sec. set. After all parameter were set the tests are started on dry lubrication condition. The coefficient of friction under dry lubrication condition of pure hydroxyapatite bone and Hap-Zr composition is mentioned below table.

Table 4.1 Coefficient of friction of pure Hap and Hap-Zr composite

Sr. no.	composition	load in kg	rpm	Trk dia. In mm	Coe. Of Friction
1.	Pure HAp	2	500	70	0.215
2.	Hap-Zr	2	500	70	0.145

The coefficient of friction is observed less in Hap- Zr composition as compared to pure Hydroxyapatite bone.

5. CONCLUSION

Two series of powders pure hydroxyapatite and 40 % reinforced zirconia contents have been quasi-statically pressed at 300 MPa and sintered at 300 °C for 15 min. The avg. coefficient of friction of pure hydroxyapatite observed is more than Hap-Zr. Composition.

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