IOSR Journal of Mechanical and Civil Engineering (IOSR-JMCE)
ISSN : 2278-1684, PP : 33-37
www.iosrjournals.org

Development of Hydroxyapatite Bio-Scaffold

Vikram Kumar[1], Dr. Uma Batra[2]
Amit Kadam[3] Prashant Mulik[4],

[1,2](Metallurgy, Punjab Engineering College / PEC University of Technology, India)
[3,4](Mechanical, Dr. JJ Magdum College of Engineering / Shivaji University, India)

ABSTRACT : *Hydroxyapatite (HAP) are particularly attractive materials for bone and tooth implants since they closely resembles human tooth and bone material and have proved to be biologically compatible with these tissues. Porous hydroxyapatite exhibits strong bonding to the bone, the pores provide a mechanical interlock leading to a firm fixation of material. It is more resorbable and more osteoconductive than its dense counterpart and in porous form the surface area is greatly increased which allows more cells attachment in comparison with dense hydroxyapatite. In present work, hydroxyapatite powder has been prepared via sol-gel technique using calcium nitrate tetrahydrate [$CaNO_3.4H_2O$] and potassium dihydrogen phosphate [KH_2PO_4] as precursors for calcium and phosphorous respectively. Porous scaffold was prepared by polymeric sponge media soaked in slurry of hydroxyapatite powder mixed with 5 wt% polyvinyl alcohol. After drying the soaked sponge was burnt at 1250°C, which resulted into a porous scaffold. Porous hydroxyapatite had about 63% porosity. The pore diameter is ~400-500µm and pores were interconnected. It is reported that porosity, pore size and pore inter connectivity depends upon the slurry concentration and the amount of pore size of sponge media.*

Keywords – *Hydroxyapatite, Polymeric Sponge, Scaffold, Sol-gel, Sintering.*

I. INTRODUCTION

A key component in tissue engineering for bone regeneration is the scaffold that serves as a template for cell interactions and the formation of bone-extracellular matrix to provide structural support to the newly formed tissue [1]. Bone is a structure composed of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals deposited within an organic matrix. The morphology is composed of trabecular bone which creates a porous environment with 50–90% porosity [2]. Scaffold properties depend primarily on the nature of the biomaterial and the fabrication process. The nature of the biomaterial has been the subject of extensive studies including different materials such as metals, ceramics, glass, chemically synthesized polymers, natural polymers and combinations of these materials to form composites.
Porosity is defined as the percentage of void space in a solid and it is a morphological property independent of the material [3]. Pores are necessary for bone tissue formation because they allow migration and proliferation of osteoblasts and mesenchymal cells, as well as vascularization [4]. In addition, a porous surface improves mechanical interlocking between the implant biomaterial and the surrounding natural bone, providing greater mechanical stability at this critical interface. The minimum pore size required to regenerate mineralized bone is generally considered to be ~100 µm [5].

1.1 Hydroxyapatite (HAP):

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is one of the most biocompatible ceramics because of its significant chemical and physical resemblance to the mineral constituents of human bones and teeth. It is a bioactive ceramics widely used as powders or in particulate forms in various bone repairs and as coatings for metallic prostheses to improve their biological properties. It has excellent biocompatibility, bioactivity and osteoconduction properties. HAP is thermodynamically the most stable calcium phosphate ceramic compound. It has an exact stoichiometric Ca/P ratio of 1.67 and is chemically very similar to the mineralized human bone. However, despite chemical similarities, mechanical performance of synthetic HA is very poor compared to bone. In addition, the bone mineral present a higher bioactivity compared to synthetic HA [5]. Porous HAP exhibits strong bonding to the bone; the pores provide a mechanical interlock leading to a firm fixation of the material. Bone tissue grows well into the pores, thus increasing strength of the HA implant. The ideal bone substitute is a material that will form a secure bond with the tissues by allowing, and even encouraging, new cells to grow and penetrate. When pore sizes exceed 100 µm, bone grows through the channels of interconnected surface pores, thus maintaining the bone's vascularity and viability [4].

1.2 Applications:

Porous HA have been applied for cell loading, drug releasing agents, chromatography analysis, and the most extensively for hard tissue scaffolds [6]. In drug delivery systems, it has been recognized that a system for the

slow, local and continuous release of drugs would be a decided advantage for the treatment of many ailments. For example, chronic disease or localized surgical intervention, relying on a sustained local drug delivery needs ceramic capsule suitable to release drugs at a controlled rate. Porous HA has been extensively applied for artificial bone substitutes. The primary purpose of tissue engineering is repair, regeneration, and reconstruction of lost, damaged or degenerative tissues [7].

1.3 Bioceramic Scaffolds

Wolf's law dictates that the bone remodels itself as a function of those forces acting on it, hence preserving its shape and density. The mechanical loads of stress, compression, flex and torsion in bones and the interstitial fluid contained in them generate stresses and deformations at the microscopical level, which in turn stimulate the cells [8]. The fabrication of scaffolds for tissue engineering requires choosing a conformation method that yields pieces with interconnected porosity and pores in the 20 to 400 micron range [9]. The main purpose now is to obtain porous ceramics that act as scaffolds for cells and inducting molecules, able to drive self-regeneration of tissues. Polymer scaffolds were used as negative of the desired ceramic piece. After conforming the piece, the polymer is removed by an acid or basic attack, or using mild temperatures. These pieces, with designed porosity, preserve their bioactive behavior, where apatite has grown throughout all the free surfaces [10, 11]. An important challenge is to design materials that can help the human body to improve its regeneration features, not only recovering the structure of the damaged tissue, but also its function [12]. Tissue engineering aims to restore the structure and function of the tissues or damaged organs. The repair starts by in vitro techniques on scaffolds cultured with cells, to be then implanted in the host [13].

2 EXPERIMENTAL PROCEDURE

2.1 Synthesis of HAP

2.1.1 Materials:

For synthesizing HAP through sol-gel route with a composition of $Ca_{10}(PO_4)_6(OH)_2$, following chemicals were used.

Table 2.1 : Chemicals used for the preparation of Hydroxyapatite

CONSTITUENTS	CHEMICAL FORMULA	MAKE	Molecular Weight (gm)	MOLES	QUANTITY (gm) (for 1000 ml solution)	QUANTITY (gm) (for 500 ml solution)
Calcium Nitrate Tetrahydrate (Ca-precursor)	$CaNO_3.4H_2O$	MERCK	236.16	1	236.16	118.08
Potassium Dihydrogen Phosphate (PO^{3-}_4 precursor)	KH_2PO_4	MERCK	136.09	0.6	81.654	40.887
Ammonia	NH_3	-	-	-	-	-
Deionised Water	H_2O	-	-	-	-	-

2.1.2 Sol-gel synthesis

Sol-gel method has been used for synthesis of Hydroxyapatite (HAP). 0.6M of static solution of KDP was prepared in distilled water separately. The solution of KDP was kept in beaker on the stirrer and the solution of CNT was kept in a burette above it fixed in a stand. In another burette Ammonia was kept. The two burettes were opened, then the CNT and Ammonia was added to KDP drop wise accompanied by continuous stirring of the mixture. Each composition ratio in HAP was adjusted to have Ca/P ratio=1.67. Ammonia was added to mixture to maintain a pH level of 10. Stirring and mixing was carried out for 1 hr. The solution was kept for aging for 24 hours. Washing was done till the ammonia was removed from the mixture. After washing a gel was obtained and this was kept for drying for 48 hrs at 70°C. After drying a dried sample was obtained this was crushed to obtained powder.

2.1.3 Calcination

After the successful formation of powdered samples of HAP, samples were heated at 900°C for 3 hrs in a furnace. Then samples were allowed to cool.

2.2 Synthesis of Hydroxyapatite Scaffold

2.2.1 Materials:

For synthesizing scaffold through polymeric sponge method following chemicals were used.

Table 2.2 : Chemicals used for the preparation of Hydroxyapatite

CONSTITUENTS	CHEMICAL FORMULA	Molecular Weight (gm)	MOLES	QUANTITY (gm) (for 1000 ml solution)	QUANTITY (gm) (for 100 ml solution)
HAP	$Ca_{10}(PO_4)_6(OH)_2$	1004.64	0.5	502.32	50.232
PVA	C_2H_2OH	46.07	0.05	2.3035	0.23035
Water	H_2O	18	-	-	-
Polyurethane Sponge	-	-	-	-	-

2.2.2 Experimental Procedure:

- **Polymeric Sponge Method**

Highly porous hydroxyapatite (HA) scaffolds were produced using polymeric sponges. The polyurethane sponges with dimensions of approximately 1×1×1 cm^3 were stretched to various levels. A hydroxyapatite (HA) slurry was prepared by dispersing HAP powder $Ca_{10}(PO_4)_6(OH)_2$ which was calcined at 900°C for 3 hours, in ethanol as binder. The prepared polymeric sponges were impregnated in the HA slurry and dried in an oven at 80°C for 24 hours. HA impregnated sponges were heated slowly to 800°C at a heating rate of 150°C/hour and maintained at this temperature for 3 hours to burn out the polymeric sponge and the binders used in the HA slurry. This was followed by sintering at 1250°C for 3 hours to densify HA struts.

RESULT AND DISCUSSION

Figure 3.1 Scaffold prepared by Polymeric Sponge Method

2.3 Phase analysis of sintered porous HAP

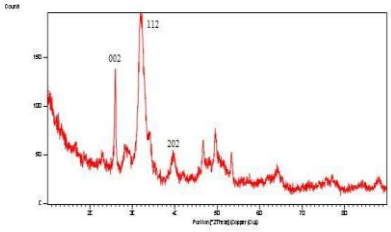


Figure 3.2: **XRD Pattern of as sintered Hydroxyapatite scaffold at 1250°C**

Table 3.1: XRD measurements for HYDROXYAPATITE SCAFFOLD

Pos. [°2Th.]	FWHM [°2Th.]	d-spacing [Å]	Rel. Int. [%]	Area [cts*°2Th.]	Phase identification using JCPDS	JCPDS File No.	hkl
25.9966	0.3264	3.42474	100.00	42.18	HAP	09-432	002
32.0325	0.7103	2.79416	74.81	34.33			112
49.6219	0.2821	1.83720	39.52	10.80			213
46.6094	0.0010	1.94868	33.64	0.03			222
39.6438	0.1579	2.27350	16.01	1..63			202

The XRD pattern shows that the sintered powder contains only hydroxyapatite (the highest intensity peaks being at d = 3.42, 2.79, 2.26) for hkl 002, 112 and 202 peaks. All other peaks also correspond to HAP and no extra peaks were found. Thus the synthesized HAP powders was phase pure at 1250°C with decomposition to β - TCP.

2.4 FTIR Characterization of sintered porous HAP

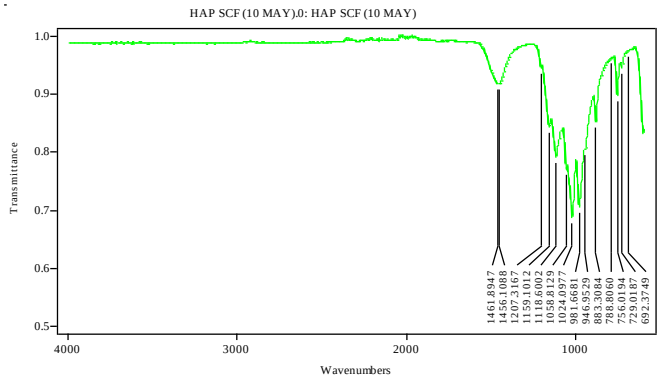


Figure 3.3: FTIR spectra of Hydroxyapatite scaffold sintered at 1250^{0}C

Figure 5.3 presented the FTIR spectrum of sintered hydroxyapatite scaffold powder in the as sintered form that identified band corresponding to PO_4^{3-} group corresponding to (1058.8129, 1024.0977, 981.6681, 946.9529 cm^{-1}) characteristics of the hydroxyapatite. Based on the FTIR results one can affirm that hydroxyapatite formed is pure phase. On sintering OH^- peaks have weakened due to sintering at 1250°C.

Microstructure of scaffold structure

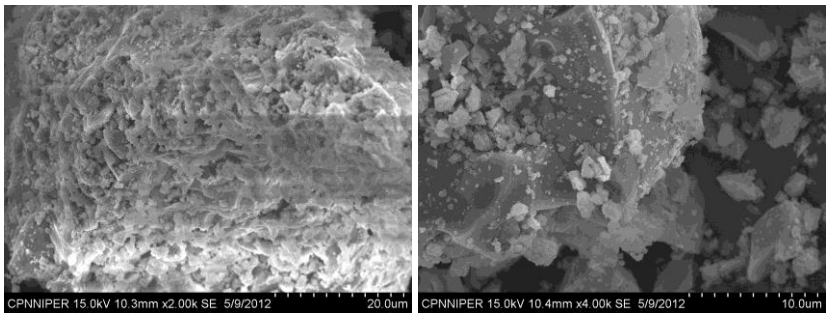
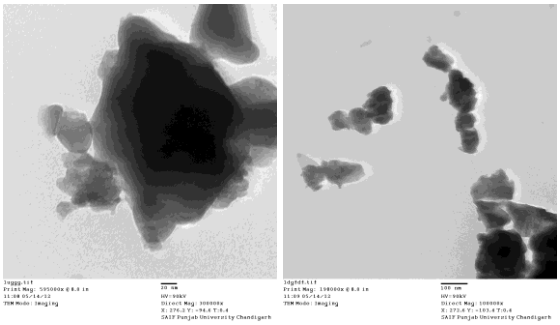


Figure 3.4 SEM images of Sintered HAP scaffold at 1250°C

Figure 5.4 show SEM image of the porous HAP scaffold prepared from polymeric sponge method. It has been observed that macropores were interconnected through cell walls. From the SEM image it was observed that pore size of the HAP scaffold were in range ~400 to 500 µm and micropores were interconnected.

This shows that the polymeric sponge method using 5wt% PVA is a suitable method for producing porous scaffolds of HAP with porosity size suitable for tissue connectivity.

3.4 Transmission electron microscopy (TEM)



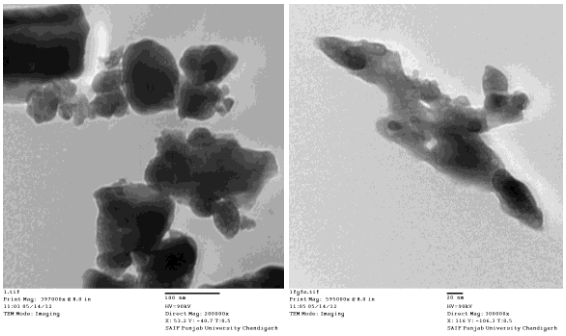


Figure 3.5: TEM micrograph of sintered HAP scaffold powder

Figure 5.4 shows the TEM image taken on the powder produced by grinding sintered HAP scaffold. It is observed that the large agglomerates of sintered particles with pores are present.

3.5 Porosity measurement

Dry weight of sample was taken then samples were kept inside a beaker filled with kerosene and it was kept inside a desiccator for half an hour. Then suspended as well as soaked weight of samples was taken. Apparent porosity and density of sample was represented as:

Apparent Porosity = (S-D) / (S-H)

Bulk Density = (D × Density of Kerosene) / (S-H)

Relative Density = (Bulk Density/ Theoretical Density) × 100

Porosity = 1- Relative density

Density of kerosene = 0.81 g/cc

Theoretical density of HA = 3.16 g/cc

Where, D = dry weight
S = suspended weight
H = soaked weight

3.6 In-Vitro Biodegradation

Biodegradation of porous sample were carried out in Tris-HCL solution. HAP samples were soaked in Tris buffer solution at pH 7.4 and temperature 37°C for 7 days. When the porous HAP was soaked in Tris buffer solution, the pH of buffer increases from 7.4 to 7.8 which confirms the biodegradation of scaffolds. The weight loss of the scaffold was approximate 3%.

4 CONCLUSION

Phase pure HAP was prepared from CaNO3.4H2O and KH_2PO_4 by sol-gel method. HAP crystallized at a temperature of 1250°C with the appearance of β-TCP phase. The porous scaffolds were prepared from polymeric sponge method. Porous HAP scaffolds prepared from this method had porosities of around 63%. The scaffolds had pore diameters in the range of ~400-500 μm and the pores were inter-connected. The pore size and pore inter connectivity depended upon the slurry viscosity and solid loading. Increase in PVA led to decrease in porosity of the material. Pore size and the porosity depended upon the amount of sintering temperature.

5 REFERENCES

1. E.H, Van den Bergh J.P, Holzmann P, Ten Bruggenkate C.M, Tuinzing D.B, Burger E.H. *"Mineralization processes in de-mineralized bone matrix grafts in human maxillary sinus floor Elevations*," *J BiomedMater Res,* (1999); 48(4): pp 393–402.
2. Kuboki Y, Takita H, Kobayashi D, Tsuruga E, Inoue M,Murata M, et al. "*BMP-induced osteogenesis on the surface of hydroxyapatite with geometrically feasible and non-feasible structures: topology of osteogenesis*," *J BiomedMater Res,* (1998); 39(2): pp 190–99.
3. Hulbert S.F, Young F.A, Mathews R.S, Klawitter J.J,Talbert C.D, Stelling F.H. "Potential of ceramic materials as permanently implantable skeletal prostheses," *J BiomedMater Res*, (1970); 4(3): pp 433–456.
4. Leony Leon C.A. *"New perspectives in mercury porosimetry," Adv Colloid Interface Sci*,(1998); 76–77: pp 341–72.
5. Itala AI, Ylanen H.O, Ekholm C, Karlsson K.H, Aro H.T. "*Pore diameter of more than 100 micron is not requisite for bone ingrowth in rabbit,." J BiomedMater Res,* (2001); 58(6): pp 679–83.
6. L.L. Hench, J. Wilson, *"An Introduction to Bioceramics," World Scientific, Singapore,* (1993).
7. L.L Hench, *Biomaterials 19,* 1998 : pp 1419
8. Stein H, D'Ambrosia R, "*Effect of Pore Structure of Bioactive Glass Balls on Biocompatibility*," *Wolff's Law Decoded*, http://www.orthosupersite.com/view.aspx?rid=26900.
9. Mann S, Webb J, Williams R.J.P., "*Biomineralization.: Chemical and Biochemical Perspective*," *(Eds.), VCH, USA*, (1989).
10. Cabañas M.V, Peña J, Román J, Vallet-Regí M, "*Calcium Orthophosphates as Bioceramics: State od the art*," *J. Biomed. Mat. Res*., (2006); 78(A): pp 508.
11. Sánchez-Salcedo S, Nieto A, Vallet-Regí M, "*Development and Mechanical Characterization of Hydroxyapatite Micro/Macro Porous Scaffolds by an Innovative Gel-Casting Process*," *Chem. Eng*. J., (2008); 137: pp 62.
12. Hench L.L, Polar J.M, "*Third-Generation Biomedical Materials*," *Science*, (2002); 295: pp 1014.
13. Wise, Transtolo, Altobelli, Yaszemski, Gresser and Schwart., "*Encyclopedic Handbook of Biomaterials and Bioengineering*," *Dekke, New York*, (1995).