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Development and Characterization of Porous Hydroxyapatite Scaffold by Using Polymeric Sponge Method

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Abstract

In recent years, scrupulous attention has been given to the preparation of porous hydroxyapatite (HA). Porous HA exhibits strong bonding to the bone and provide a mechanical interlock leading to a firm fixation of the material. Bone tissue grows well into the pores, increasing strength of the HA implant. Its high surface area leads to excellent osteoconductivity and resorbability providing fast bone ingrowth. This paper briefly describes the preparation of porous HA scaffold by polymeric sponge method for artificial bone application. The technique of saturating a body of porous polyurethane foam with slurry containing HA powder, water and additives was applied and proved to be successful. The characterization of the hydroxyapatite porous scaffolds was assessed by means of scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transformed infrared spectroscopy (FTIR).

Key Words: Porous; Hydroxyapatite; Bone in growth; Polymeric sponge.

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1. Introduction

Biomaterial research field focus on the synthesis of bio-ceramics for three decades to applications in orthopedic and dentistry and particular attention is paid to the synthesis of hydroxyapatite (HA) ceramics with porous morphology required for vascularization, bone cell invasion and angiogenesis which further improves its biomedical properties. Since porous HA is more resorbable and more osteo-conductive than dense HA, there is an increasing interest in the development of synthetic porous material for the filling of both load-bearing and non-load-bearing osseous defects [1]. Indeed their applications as materials for bone regeneration have been increasing in different fields such as orthopedic surgery, dentistry, maxillofacial surgery and reconstructive surgery as hard tissue scaffold, cell loading and drug releasing agents [2, 3].

Porous matrices, known as scaffolds, to which cells attach and colonize, play a vital role in synthesizing bone-extracellular matrix and associated biological molecules to facilitate the formation of functional tissues/organs. The scaffolds are designed to provide a structural frame work as well as a microenvironment for the seeded cells and to facilitate the formation of new tissues. These scaffolds exhibit strong bonding to the pores and provide a mechanical interlock leading to a firm fixation of the material. Bone tissues grow well into the pores, thus increasing the strength of HA implant [4]. Porous HA can be produced by a number of methods including conversion of natural bones [5], ceramic foaming technique [6], polymeric sponge method [7], gel casting of foams [8], starch consolidation [9], microwave processing [10] and electrophoretic deposition technique [11]. The porous scaffolds obtained from reticulated polymer substrates have a number of distinct properties such as controllable pore size, porosity and complex ceramic shapes suitable for different applications [6, 12]. The polymeric sponge method, as this method named, is performed by impregnating porous polymeric substrates (sponges) with HA slurry.

Polyurethane is the most attractive polymer because of its low softening temperature and ease of burn-off which minimize thermal stress that may fracture the un-sintered ceramic. The scaffolds prepared via the polymeric sponge method have shown well- interconnected pores but poor mechanical strength for load bearing applications therefore it is mainly used as cancellous bone substitute of specific filler due to these properties. Physical characteristics of porous HA which include porosity degree, pore-size distribution, pore interconnectivity, pore morphology and orientation influence bone penetration in implants [13]. Minimum pore size required to enable ingrowth of the surrounding bone together with blood supply is about 100-150 mm for macropores [14,15] and even at pores of as small as 50 mm osteo-conduction is still possible [16].

Another important requirement for porous implants is interconnectivity of the pores for the penetration of the osteoblast-like cells inside the pores and surface roughness for the attachment of cells. This interconnectivity allows circulation and exchange of body fluids, ion diffusion, nutritional supply, osteoblast cell penetration and vascularization [17]. Reference [18] produced porous tricalcium phosphate with a porosity of 55-70% using polyurethane foam.

The polymeric sponge method has been used to fabricate porous hydroxyapatite with desirable properties for different types of applications [12, 19 , 20]. In present study porous hydroxyapatite scaffolds were prepared by

polymeric sponge method using hydroxyapatite powder from egg shells.

Since Pakistan is developing country our strategy in R & D in the biomaterials and tissue engineering field is to design and develop new synthetic materials and structures according to clinical requirement at our country level from indigenous resources and at the same time to improve current accepted biomaterials. Therefore the present work was undertaken to develop porous HA scaffolds via polymeric sponge method using hydroxyapatite powder from egg shells by employing available techniques. However experimental work for practical implementation of these prepared scaffolds in dogs is under progress.

2. Materials and methods

Hydroxyapatite (Ca/P ratio = 1.68, density = 3.16g/cm^3, particle size 50-100 µm) prepared from egg shells was taken as raw material for preparation of porous hydroxyapatite scaffolds. The polyurethane sponge was cut into pieces of 15x15x15 mm. The optimum composition of the slurry was obtained when 10 gram of hydroxyapatite powder was mixed with 0.5 gram methylcellulose, 2.5 gram dicalcium phosphate (DCP) and 40 ml distilled water. The pieces of sponge were immersed in this slurry 4-5 times and compressed slightly to facilitate its absorption. Excessive slurry was removed by squeezing. The HA coated sponges were dried at room temperature for 24 hours followed by drying at 75 ºC for 2 hours to remove moisture. Hydroxyapatite coated sponge pieces were heated at 80 0ºC at slow heating rate of 20 ºC/ minute and maintained at this temperature for 5 hours to burn out the sponge and organic binders. Then porous scaffolds were sintered at 1200 ºC for 2 hours. The composition, morphology and porosity of prepared scaffolds were investigated using Scanning Electron Microscope (SEM) (S3700N, HITACHI) and liquid displacement method was used to calculate porosity. The crystalline phase and chemical nature of the scaffold was characterized using PANalytical X-Ray Diffractometer AVATAR 320 and Fourier Transform Infra red (FTIR) Thermo Nicolet spectroscope.

3. Results and discussion

The porous HA scaffolds prepared by

Polymeric sponge method as shown in Fig. 1

were initially analyzed by IR for chemical nature and molecular bond structure. These spectral

were conducted in the range of 4000-400 cm^{-1}.

As shown in Fig. a well resolved band at 1011

cm^{-1} was identical to phosphate band. Appearance of strong band at 542 cm^{-1} and one band at 752

cm^{-1} indicates the presence of phosphate ions in hydroxyapatite phase.

The crystallographic study of the porous scaffold was performed by X-ray diffractometer using CuKα radiations. The intensity data was collected in 0.02^o steps in the 2θ range, 10-60^o as shown in Fig. 3. The

strongest peak in the XRD pattern appear in the range of 20-40, 2θ; characteristic of the apatitic phase (JCPDS #9-432).

Reference [19] also described the same results from FT-IR and XRD studies By using liquid displacement method the porosity was calculated to be 62.67%. The morphology and microstructure of the scaffolds were examined using SEM (Fig. 4).The observations revealed an interconnective macro porous structure but micro pores were also present. This kind of structure is considered to be adequate, as the macroporosity indices osteoinduction and the microporosity allows improved cell adhesion [21].

It can also be seen in Fig. 4 that the pores in the scaffolds are interconnected and their sizes range from 80 to 400 μm. When pore sizes exceed than 100 μm, the bone will grow within the interconnected pore channels near the surface and maintain its vascularity and long term viability. In this manner, the implant serves as a structural bridge or scaffold for bone formation. This shows that the scaffolds' pores are sufficiently large to accommodate cells. Pores of 20-50 μm diameter may give a favourable function for physiological liquid exchange [17], while pores with a diameter 100-350 μm are suitable for cell colonization and vascularization leading to bone penetration into ceramic structure [22].

4. Conclusion

Porous ceramic implant with a wide range of pore sizes is necessary to meet all the function involved in oseointegration. Our objective in this study was to develop porous scaffold of HA with an orderly structure of pores to facilitate bone integration by using polymeric sponge method. The scaffolds can be easily fabricated into a variety of shapes and sizes and malleable to fit irregularly shaped defects. Moreover the surface area of porous bodies is much higher, which guarantees a good mechanical fixation in addition to providing sites on the surface that allow chemical bonding between the bioceramics and bones. The results exposed in this work represent an initial phase of investigation using a waste product (egg shells) as raw material for bone substitute which in later studies will be evaluated in its biological performance.

Figure 1: Prepared Porous HA scaffolds

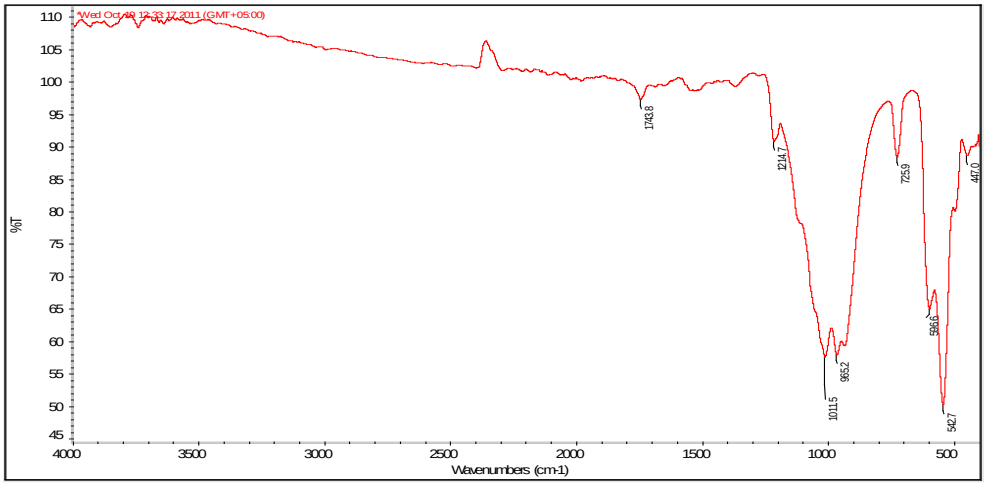

Figure 2: FTIR of porous HA scaffold

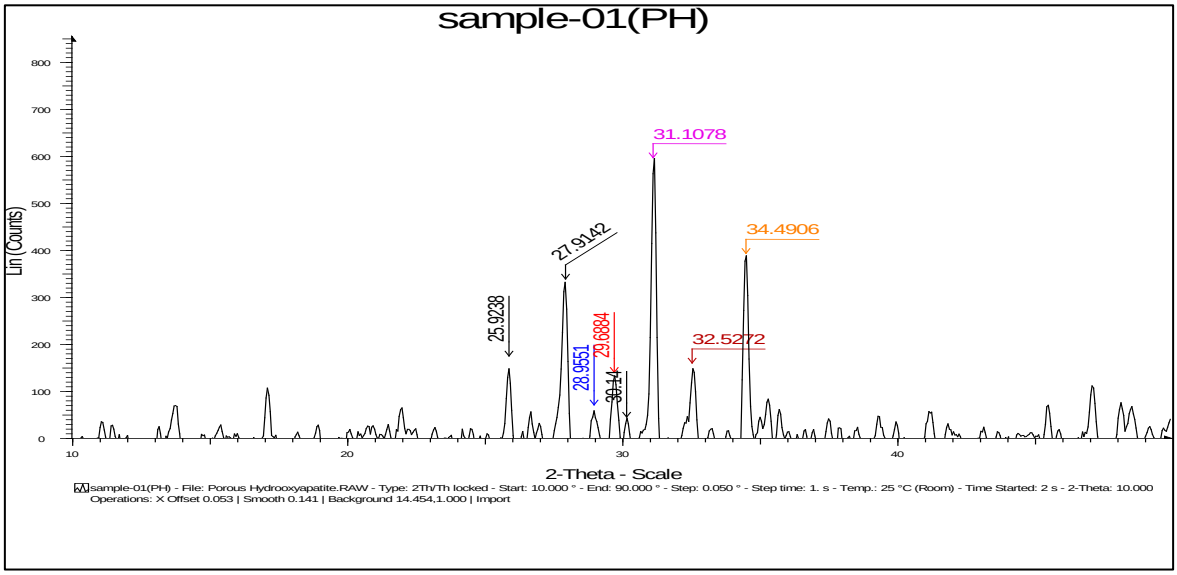

Figure 3: XRD patterns of porous HA scaffold

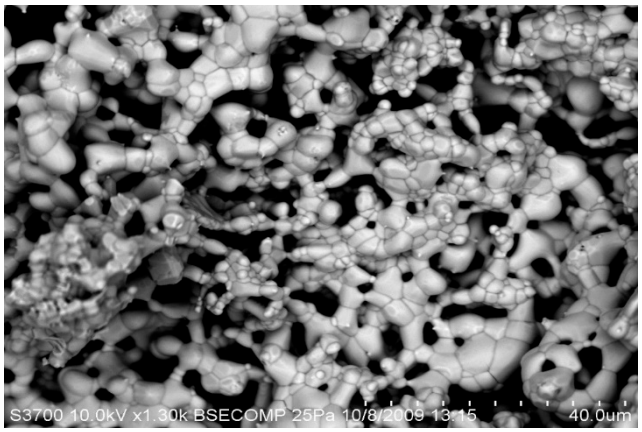

Figure 4: SEM analysis of porous scaffold

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