



An Experimental Study of Porous Hydroxyapatite Scaffold Bioactivity in Biomedical Applications

Auday A. Mehatlaf ^{a*}, Alaa A. Atiyah ^b, Saad B. H. Farid ^c

^a Materials Engineering Department, University of Technology, Baghdad, Iraq,
audaymehatlaf@uokerbala.edu.iq

^b Materials Engineering Department, University of Technology, Baghdad, Iraq,
alaa.a.atiyah@uotechnology.edu.iq

^c Materials Engineering Department, University of Technology, Baghdad, Iraq,
130004@uotechnology.edu.iq

*Corresponding author.

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ABSTRACT

Hydroxyapatite is one of the most bioactive materials used in tissue engineering due to its excellent biocompatibility and chemical composition which is equivalent to the mineral element of bone. In this study, polymer sponge replication method was used to fabricate porous hydroxyapatite scaffolds. Pure phase of hydroxyapatite scaffolds and the chemical bonding were verified via Fourier Transform Infrared and X-ray diffraction. Emission scanning electron microscopy (FESEM) examination showed that the proposed scaffold has high interconnected pores that were achieved just after sintering at temperatures 1350 °C for 2 hours. The percentage porosity values were estimated to be between 75–78 percent. The bioactivity of porous scaffolds was also investigated. They were submerged in a slurry of simulated body fluid (SBF) for seven, fourteen, and twenty-one days, respectively. Both FESEM and XRD analysis have confirmed the bioactivity of the prepared porous hydroxyapatite scaffold through the formation of a dense layer of apatite on its surface. Based on the results, the porous hydroxyapatite scaffolds could be recommended as a critical option for bone defects as well as replacement applications.

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1. INTRODUCTION

By medical evolution, bone regeneration is recently recognized as a new medical technique for tissue engineering scaffolds including bioavailability, sufficient mechanical strength, strong interconnection, and biodegradability. Macro/ nanoporous connected morphology and Composition are thought to be important in affecting cellular responses to tissue engineering scaffolds [1]. Biomaterials are useful in making equipment to substitute apart or function of the body when needed, economically physiologically and reasonable, adequate. Diverse equipment and materials are being used in the treatment of diseases and injuries [2].

Bone grafting has become a surgical technique that replaces damaged bone either with patient-derived material or, synthetic, an artificial, or natural replacement. Bone is a metabolism tissue that can adapt its structure to mechanical stimulation and repair structural damage via the healing process [3]. A number of bone grafting scaffolds now accessible have developed the utilization of bone graft for treatment, repair, either strengthen skeletal fractures and broken bones. Even so, biologically active, the precise mixture of sufficient mechanical, Low-cost scaffolding materials is constantly being studied for such purposes [4]. In the manufacturing of scaffolds, both natural and synthetic biomaterials are used. Systems for bone tissue engineering include bone regeneration following tissue loss owing to degenerative surgical procedures [5].

The scaffolds act mainly for osteoconductive moieties, allowing new bone to be deposited via creeping replacement with neighboring living bone, and secondarily as a promoter for the creation of new centers with bone regeneration by osteogenesis, which happens before implantation via cell seeding [6]. A scaffold for bone tissue regeneration must meet the following requirements to conduct such a role, include biocompatibility, biodegradability, and porosity, as well as comparable mechanical properties to that of the tissue regeneration site substituted hydroxyapatite and Collagen, which are the most common components of the human bone (a type of natural bioceramics that can be contained in the tooth)[7].

Hydroxyapatite (HA) was perhaps the majority of essential thermodynamic stability calcium phosphate inorganic constitute under the human skeletal structure in the pathological environment. Synthesized HA has many main advantages, including deliberate biodegradability in physiological conditions, biocompatibility, osteoconductive and osteoinductive abilities [8]. The interconnected pore could provide good conditions for bone regeneration and osseointegration, porous scaffolds have received a lot of attention to the application of tissue engineering applications. Porous hydroxyapatite would be a more easily bioabsorbable and osteoconductive component than bulk HA, and it was utilized as a material for artificial bone grafts in many research and field testing [9–11]. To satisfy the requirements, to attempt to develop porous scaffolding, many processing techniques have been used. Sponge replication method [12], Slip casting [13, 14], solvent casting [15], gas foaming [16] and freeze casting [17] are among the most common techniques of all.

Bioactive ceramics may produce an alike bone similar to the apatite layer if they are, then they will be soaked in simulated body fluid (S B F). SBF is nearly a media with the same components as human extracellular fluid concerning inorganic elements. There are no cells and proteins in SBF, This means that the biomaterials' chemical reactions with the fluid surrounding create the layer of the apatite. Therefore, it is predicted that new biomaterials will be produced by controlling the chemical properties of body fluid materials [18].

The purpose of this research was to fabricate porous scaffolds of the HA that have connected pores via utilizing the methods of foam replication, based on the restrictions related to many other techniques. To investigate bone regeneration support from this form of HA, these scaffolds, which were constructed similar to that of human trabecular bone with the micro-structure, then were used.

2. EXPERIMENTAL WORK

1. Hydroxyapatite Scaffolds Synthesized

The polymer replication process was used to produce a hydroxyapatite scaffold utilizing a polyurethane (PU) foam as an organic template. Parts of commercial PU sponge with cube form form (1x1x1 cm³) are utilized in the preparation. A weight percentage for HA slurry was prepared via stirring for one hour to obtain the homogeneous slurry including 60 wt. % HA powder and 3 wt. %

polyethyleneglycol as just a binder. The PU sponges also were immersed throughout the slurry until all the void spaces had been removed. A body specimen was dried for 10 hours in an oven of about 80 ° C. Finally, the dried cubic samples were treated via the sintering in the furnace for 2 hours at 1350 ° C to achieve the HA scaffolds with the required properties for biodegradable implant material as shown in Figure 1.

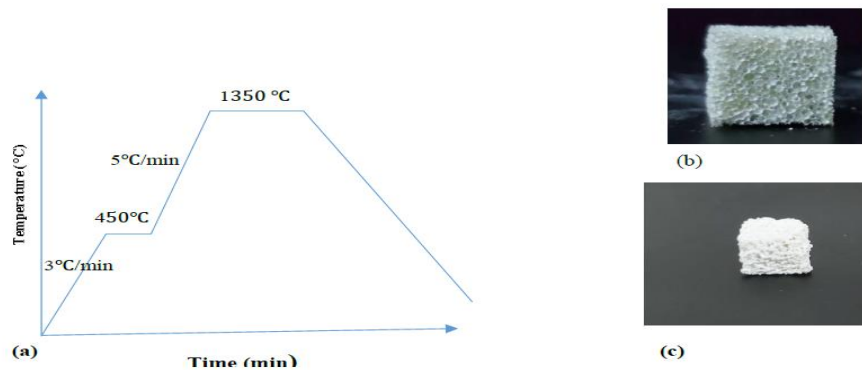


Figure 1: Heating cycle of the sintering process at 1350°C (a), HA scaffold before sintering (b), after sintering (c).

3. CHARACTERIZATIONS

I. Spectroscopy Analysis

The scaffolds' Fourier Transform Infra-Red (FTIR) spectra were discovered to use a BRUKER, Germany, scanner with such a scanning range from 450-4000 cm^{-1} to acquire crucial information's about so many different chemical bonds.

II. X-ray diffraction Analysis

Utilized the XRD diffractometer, a designed HAP scaffold was characterized via x-ray diffraction (XRD-6000, NF type). Cu-K radiation has been utilized with an X-ray over even a two-degree range of 10° to 90° at a rate of five degrees per minute. The phase identification was done by matching a database of diffraction to JCPDS standards.

III. Measurement of Porosity

Equations (1) & (2) were used to calculate the scaffold porosity form of the density retained [19].

$$\text{Porosity \%} = 1 - \frac{\rho_{\text{relative}}}{\rho_{\text{bulk}}} \quad (1)$$

$$\rho_{\text{relative}} = \frac{W_d}{V} \quad (2)$$

Where: ρ_{relative} is the relative density measured in eq. (2), ρ_{bulk} is the bulk density (g/cm^3); W_d is the dry weight of HA scaffold (g); and V is the total volume of the HAP scaffold (cm^3).

IV. Microstructural Analysis

Felid Emission Scanning Electron Microscopy has been used to achieve microstructural analysis (FESEM). To avoid charging, the surface morphology of the hydroxyapatite pours scaffolds were coated with a thin film of gold utilizing an ion sputtering tool.

V. SBF Soaking

The following procedure is how SBF was produced, in Table I, the reagent-grade chemicals were dissolved in 700 cm³ of ultrapure water in a 1000 cm³ glass beaker, which was stirred with a magnetic stirrer. Before inserting the next reagent, the previous reagent had to fully dissolve. Diluting a 35-mass percent HCl solution yielded 1.0 M HCl. The pH of the solution was changed to 7.3 by diluting with 1.0 M HCl solution at 37°C. The solution was moved to the volumetric flask after the pH change and ultrapure water was added to make the overall volume of the solution 1000 cm³. Appropriate quantities of scaffold samples were soaked for up to 7, 14, and 21 days in SBF at 37°C. The soaked scaffolds were centrifuged after 7, 14, and 21 days, and the extracted scaffolds were rinsed in ultrapure water and then dried for more than 12 hours at 60°C, which was prepared according to the method explained by Takadama and Kokubo [18].

TABLE I: Regents for preparing SBF

Seq.	Reagent	Amount (g/l)
1	NaCl	8.035
2	NaHCO ₃	0.35
3	KCl	0.224
4	K ₂ HPO ₄ ·3H ₂ O	0.228
5	MgCl ₂ ·6H ₂ O	0.305
6	HCl	40 Ml
7	CaCl ₂	0.278
8	Na ₂ SO ₄	0.071
9	(CH ₂ OH) ₃ CNH ₂	6.057

4. RESULTS AND DISCUSSIONS

I. FTIR results

The chemical bonds of the HAP scaffold were identified using FT-IR analysis, as seen in Figure 2. A stretch and bend band ν_3 of the PO₄ group has the sharpest peaks at 953 cm⁻¹, the ν_1 stretching band of PO₄ was formed by the existence of a very small peak on 860 cm⁻¹, and the 560 cm⁻¹ and 493 cm⁻¹ bands arise from ν_4 PO₄, which confirmed the presence of phosphate groups (PO₄) throughout the HAP pours scaffolds [12]. The hydroxyl group (OH) stretching band appears at 3450 cm⁻¹. Components of a CO₃ group are represented by the band around 1455cm⁻¹. The absorption of a CO₂ group can be attributed to the 2361 cm⁻¹ band [20].

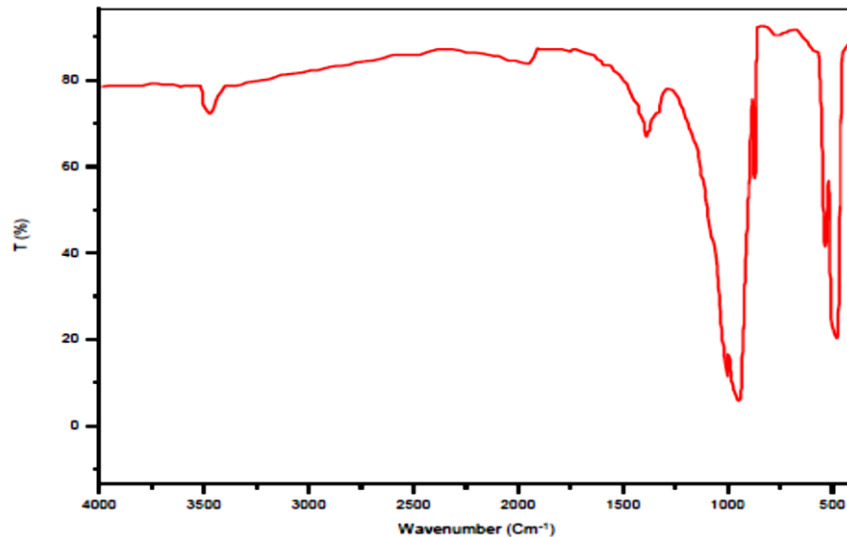


Figure 2: FTIR spectra of the prepared HA porous scaffold.

II. XRD of HA scaffold results

Figure 3 shows the XRD samples of the porous scaffolds synthesized utilizing the polymer sponge replication method, followed also by a sintering process around 1350 °C for 2 hours. The sharp reflection peak at just the 2theta of 31.7 defined the phase for hydroxyapatite, according to the study of the XRD analysis similar to that of the hydroxyapatite standardized file (JCPDS card for HA No. 09-0432).

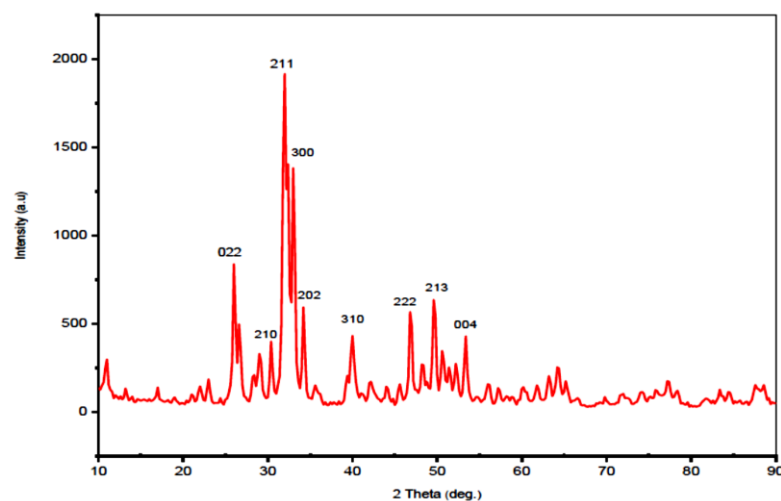


Figure 3: XRD spectrum of porous HAP scaffold after sintering process.

III. Analyzed porosity

Porosity of hydroxyapatite scaffolds was calculated by using Eq. (1) and (2). HA scaffolds with connected and open porosity of 75-78 percent can be made. This leads to increased cell proliferation and tissue regeneration, making it an ideal situation. It is a major accomplishment that supports the HAP scaffolds' possible appropriateness for bone regeneration, as their porosity is similar to that of human trabecular bone [21].

IV. Results of FESEM

Figure 4 depicts the connected pores in sintered hydroxyapatite scaffolds at different magnifications. A successful replica of the polyurethane sponge template was obtained after the optimum sintering process at 1350°C for 2 hours. The microstructure of the HA scaffold consisted of interconnected pores and a dense struts network of this material as shown in Figures 4 (a) and (b). The scaffold struts are well exhibited a diffused surface micro-roughness, which can be useful to promote the osteoblast cells attachment during implantation in vitro [22]. Most of these pores are spherical shapes and uniformly distributed. The microstructures of the scaffold shown in Figures 4 (c) and (d) confirm a densified structure with grain boundaries that are well defined and without any noticeable defect. This confirms the good sintering process of the HA particles. Larger pore sizes and higher porosity of the scaffolds can allow cells to proliferate and migrate more easily through these pores. Pores became connected as a result of scaffold replication, and nutrients could be transferred in these scaffolds.

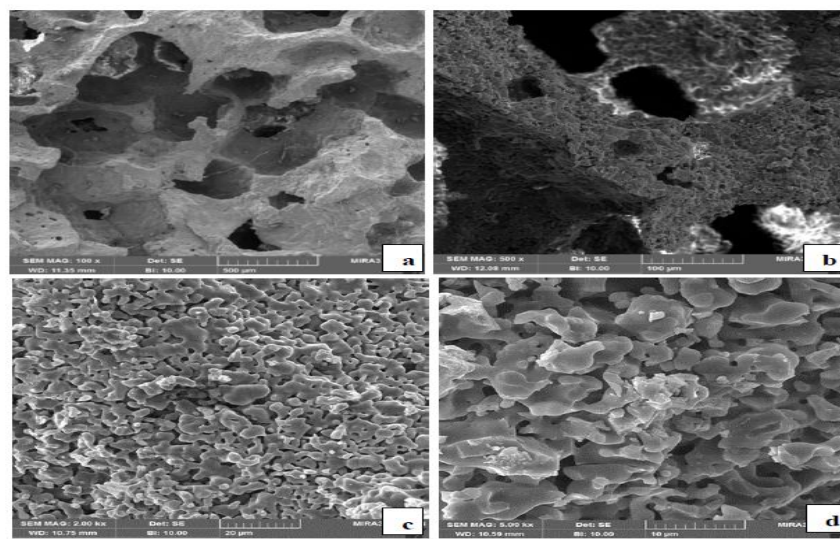


Figure 4: FESEM of sintered HA scaffold with different magnifications

V. . Bioactivity analysis

|FESEM results

Examining changes in a composition of a soaked in simulated body fluid solution allowed for in vitro development of the scaffold bioactivity. It has previously been mentioned that in SBF, bone-like apatite forms on HA ceramics [23]. These results refer to large plate-like crystals with morphology similar to gypsum flowers and significant roughness on a small scale; similar plate-like crystals have been identified to octacalciumphosphate [24]. In biomimetic apatite specimens where it has been acquired from octacalciumphosphate crystallization as just a primary phase which later developed into hydroxyapatite [25]. After 7 and 14 days of immersion, HA plate-like crystal structures covered the surface, as shown in the FESEM pictures in Figure 5. However, HA after 21 days immersion in the SBF was precipitated much more than that after 7- and 14-days immersion.

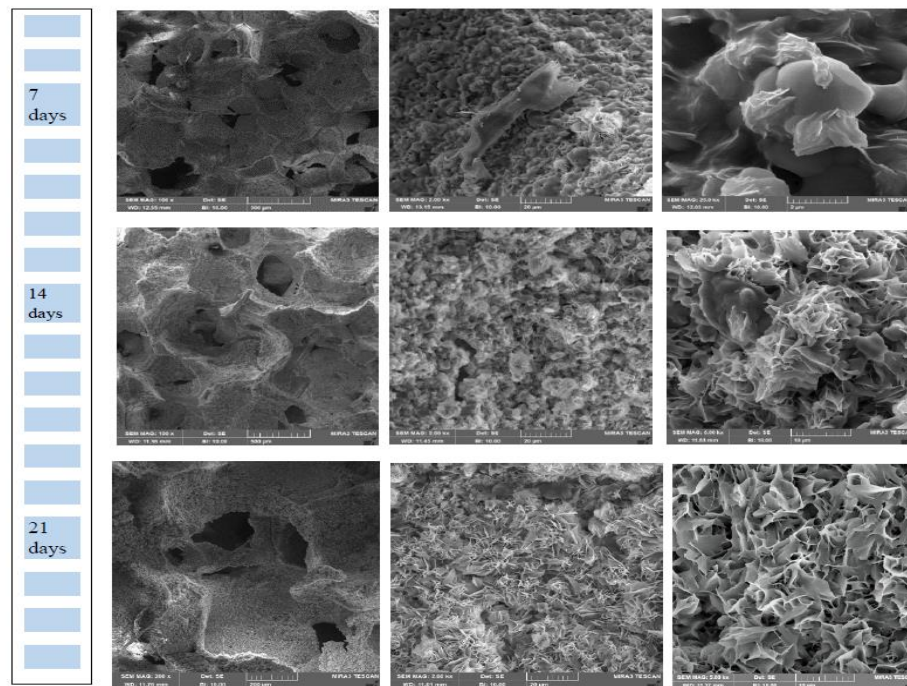


Figure 5: FESEM images of scaffolds showing apatite layer after immersion in SBF.

XRD Results

After soaking in the SBF for 7, 14, and 21 days, the XRD forms of hydroxyapatite samples are shown in Figure 6. All of the samples had diffraction peaks correlated with hydroxyapatite. The peaks of the hydroxyapatite scaffolds have become sharper when the period of immersion increased because the hydroxyapatite was precipitated when the test media (SBF) were refreshed and when aged in the unrefreshed medium. Regardless of variations in aspect ratios of hydroxyapatite crystals soaked in the SBF, no time-dependent changes in diffraction patterns were observed in any sample.

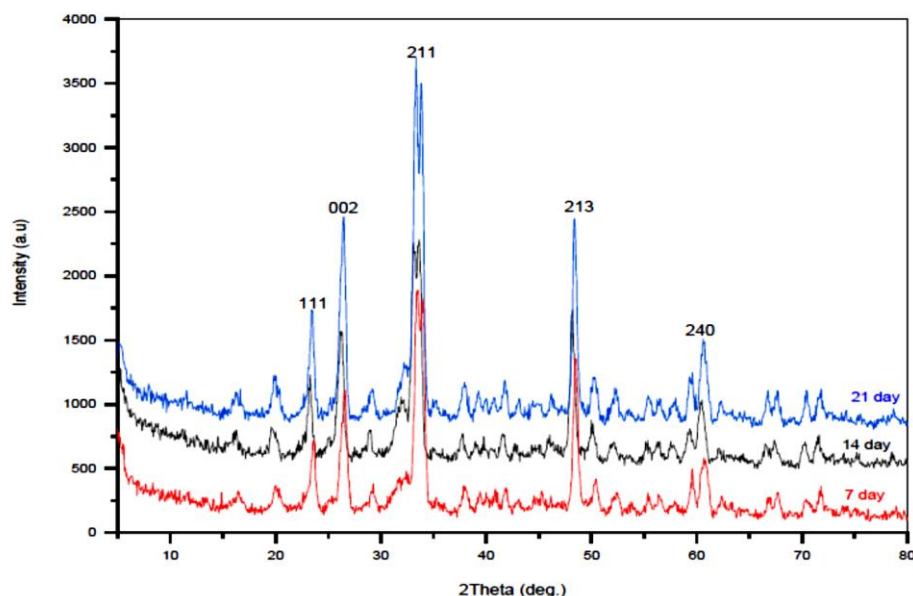


Figure 6: XRD analysis of scaffolds after immersion in SBF.

5. CONCLUSIONS

The sponge replication method was used to effectively fabricate a porous structure body with nanostructure HA in this research. The designed scaffold with spherical pores that are strongly

interconnected. The developed scaffolds had a porous structure (75-78%) with completely open and connected micropores. Based on the density of the polyurethane sponge utilized in a preparation process, FESEM analysis revealed scaffolds with varying sizes of interconnected pores. The preparing scaffold was submerged in SBF for 7, 14, and 21 days in an in vitro test, and the influence of SBF upon this scaffold was examined, revealing good bioavailability of the preparing scaffold. The described properties appear to have made that HA scaffolds a great candidate for tissue engineering applications, particularly applications that do not require any loading, such as drug delivery and cell loading, but more evidence is required through cultured cells and in vivo studies.

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