Fabrication and Investigation of Bioceramic Scaffolds by a Polymer Sponge Replication Technique

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Abstract. Initially, hydroxyapatite powder was produced using the wet participation method. The x-ray diffraction and particle size analysis were performed after the preparation method of the powder. Using polymer replication technique to produce scaffolds of HAP with structural and physical properties appropriate to human trabecular bone. The microstructure and chemical bonding of the porous scaffolds were investigated by using XRD spectroscopy, scanning electron microscopy (SEM) and Fourier Transform Infrared (FTIR) after the sintering at temperature 1300 °C for 2 hours. The synthesized scaffold was soaked in medium of the simulated body fluid (SBF) in order to evaluate bioactivity. From the results, it was observed that the scaffolds were contained open and interconnected pores with porosity range between 72-75 %. In addition, SEM morphology analysis was indicated high bioactivity of pores scaffolds due to the creation of apatite layer on its surfaces were observed after soaking 7 days in SBF media.

Keywords: Scaffold, hydroxyapatite, bioactivity, bone regeneration.

1. Introduction
Tissue engineering (TE) field is a combined discipline makes utilization of precept from together natural sciences and engineering so as to complete the repair, replacement and advance a functionality of the associated malfunctioning and diseased cells of tissues thru the contribution of biological materials [1]. A commonly curriculum is to separate determiner cells over a tiny biopsy from the patient and allow them growing on the scaffold below controlled requisites. This incloses the requirement for differentiation and migration of the indigenous cells with defect regions and then in turn quickens tissue regeneration applications [2].

The challenge directed in the tissue engineering pitch is that the biological performance of the cell must ordinate in an isolated pattern to configuration the physico-chemical natures of the material surface form the functional tissue. The choice of the proper biomaterial is an important, made with properties such as surface topography, pore structure, pore size, hydrophilicity, chemical composition, charge and degradation and surface energy [3]. At orthopedic applications, an extent of the bioactive ceramics, i.e. bioglass, hydroxyapatite (HAP), glass ceramics, and tricalcium phosphate (TCP) have been used because of their excellent bone bonding ability and their composition is similar to the mineral of human bone [4,5]. Hydroxyapatite [Ca₁₀(PO₄)₆(OH)₂], the important one of the well-known...
bioactive and biocompatible ceramic. Also, HAP has important physical and chemical similarity to the phase mineral components of the human teeth and bones [6]. Hydroxyapatite was known as the important special materials because of its osteoconductive nature, compatibilities, and porous structure. Thus, porous structure scaffolds that produce from calcium phosphate were usually used for the replacement and repair of that of bone tissues which are damaged over diseases or accidents. Important conditions for exactly margining of any pored matrix to bone tissue engineering (BTE) include biocompatibility, porosity, osteoconductivity and bioactivity. The biomaterial must have suitable mechanical strength and porosity whereby its porous nature permits to the cell to easy penetration, and so creating a good environment that corroborates permeation of tissues, nutrient conveyance, and finally lead to vascularization [7].

Pore geometry and pore size distribution must be modified to the tissue that respective, also, the scaffolds must have an enough stability to handle through implantation, that supplied by the bioceramic scaffolds. So, a variety number of manufacture techniques for the preparation of the porous bioceramic materials have advanced. The polymer sponge replication technique among all of these techniques, that involves saturating of the sponge by slurries comprising ceramics powders and suitable additives, has been received a specific attention. The increasing concern in porous bio ceramics is linked mainly within a few particular characteristics, such as high permeability, low density and high specific surface area [8]. The spongy bodies manufacturing by this technique introduce a number of properties, like must have complex shapes suitable for many of applications and controlled pore size. These pors scaffolds are predictable to have biocompatible, high interconnection among pores, and can be controlled on the rat of degradation to enhancement the bone tissues ingrowth and supporting bone cells linking [9]. The objective of this study was prepared pours scaffolds of hydroxyapatite with interconnected pores by using a polymer sponge replication method which was suitable for applications of hard tissue engineering.

2. Experimental Work

2.1. Synthesis of hydroxyapatite powder

Hydroxyapatite powder was synthesized by the wet precipitation method using diammonium hydrogen phosphate (NH$_4$)$_2$HPO$_4$ and calcium nitrate tetrahydrate Ca(NO$_3$)$_2$.4H$_2$O as raw materials. 0.167 M of Ca(NO$_3$)$_2$.4H$_2$O solution and 0.1 M of (NH$_4$)$_2$HPO$_4$ solution were prepared by dissolving the desired amount of the salt in deionized water according to equation (1), using magnetically stirring for 30 min at room temperature. The solution of Ca(NO$_3$)$_2$.4H$_2$O was slowly added the (NH$_4$)$_2$HPO$_4$ solution. The pH of the resulting solution was measured using digital pH-meter. It was adjusted to 10 by adding concentrated of ammonium hydroxide (NH$_4$OH) with continuous stirring [10].

$$6\text{(NH}_4\text{)}_2\text{HPO}_4+10\text{Ca(NO}_3\text{)}_2.4\text{H}_2\text{O}+8\text{NH}_4\text{OH} →\text{Ca}_{10}(\text{PO}_4)_{6}(\text{OH})_2+20\text{NH}_4\text{NO}_3+46\text{H}_2\text{O}$$ (1)

The precipitate was aged under magnetically stirring for 1h at room temperature, after that the HAP precipitate was exposed to aqueous washes followed by distilled water washes. The obtaining precipitate was oven-dried at temperature 80 °C for 10 hours, then calcination treatment was at 800°C for 2h.

2.2. Production of Scaffolds

Hydroxyapatite scaffolds were fabricated using polyurethane (PU) sponge as an organic template using polymer replication technique. For the preparation, the commercial PU sponges pieces with cubical shape (1 cm$^3$) were used. The weight percentage of HAP slurry containing 70 wt% from HAP powder and 3% of poly ethylene glycol were prepared by stirring for one hour to form a homogenous slurry. Then, the PU sponges were soaked in the slurry up to complete removal all of the void spaces. The sponges were exited from solution and squeezed within two glass slides to remove the extra slurry as shown in Figure 1. The body samples were dried in oven at 80 °C for 12 h. Finally, the dried samples
were treated by the sintering in a furnace at temperature 1300°C for two hours to obtain a porous scaffold with the required mechanical strength.

![Image of prepared Hydroxyapatite scaffolds](image)

**Figure 1.** Photograph of prepared Hydroxyapatite scaffolds.

2.3. **Characterizations**

2.3.1. **Particle Size Analyzer**
The distribution of particle size was determined by using a 90 Plus laser particle size analyzer. The sample was put in the device, after ultrasonically dispersion in water for 10 min.

2.3.2. **X-Ray diffraction XRD**
The prepared powders and the scaffolds of hydroxyapatite were also characterized by the x-ray diffraction using XRD diffractometer (XRD-6000, NF type). X-ray was used over 2θ range from 10° to 90° with a rate of 5 deg/min and used Cu-Kα radiation. For identification of the phase was obtained by comparing the diffraction database with standards JCPDS.

2.3.3. **Measurement of Porosity**
The scaffold porosity was measured from the obtained density by using equations. (1) and (2) [11]

\[
\text{porosity } \% = 1 - \left( \frac{\rho_{\text{relative}}}{\rho_{\text{Bulk}}} \right) \\
\rho_{\text{relative}} = \frac{W_d}{V} 
\]

Where: \( \rho \) relative is relative density which was calculated as shown in equation (2) and \( \rho \) bulk is bulk density (g/cm³); \( W_d \) is the weight of dried scaffold (g); \( V \) is the volume of scaffold (cm³).

2.3.4. **Spectroscopy Analysis**
The Fourier Transform Infra-Red (FTIR) spectra for the scaffolds was found by the using BRUKER, Germany with the scanning range of 600-4000 cm⁻¹.

2.3.5. **Microstructural Analysis**
Microstructural analysis was obtained by using Scanning Electron Microscopy (SEM), model (VEGA3 TESCAN). The surface of the porous scaffolds of hydroxyapatite were coated by thin layer of the gold using ion sputtering instrument to prevent charging.
2.3.6. Vitro Bioactivity Tests

The solution of simulated body fluid (SBF), that has inorganic ions concentration like those of human extracellular fluid, which was fabricated according to the procedure described by Takadama and Kokubo [12]. Table 1 shows the ions concentration of SBF medium nearly equal to the solution of human blood plasma. After the preparation method of SBF solution, in vitro bioactivity tests were achieved by soaking the pours scaffolds of hydroxyapatite in the solution of SBF for 7 days at temperature 37 °C static conditions. The solution was replaced every 48 h with fresh SBF for simulate fluid transmission in the physiological conditions, also the pH meter was checked in order to estimate the ionic exchange process between the scaffold and the SBF solution. Finally, the scaffolds were washed by distilled water, then dried at 37 °C and kept in a closed plastic container for further examinations.

Table 1. The comparison between human blood plasma and SBF solution [12].

| Ion         | Ion concentrations (mM) |
|-------------|-------------------------|
|             | Human Blood Plasma      | Simulated Body Fluid (SBF) |
| Na⁺         | 142                     | 142                        |
| K⁺          | 5                       | 5                          |
| Mg²⁺        | 1.5                     | 1.5                        |
| Ca²⁺        | 2.5                     | 2.5                        |
| Cl⁻         | 103                     | 147.8                      |
| HCO₃⁻       | 27                      | 4.2                        |
| HPO₄²⁻      | 1                       | 1                          |
| SO₄²⁻       | 0.5                     | 0.5                        |
| pH          | 7.2 - 7.4               | 7.4                        |

3. Results and discussions

3.1. Particle Size Analyzer Results

Figure 2 shows the results of particle size analysis of the prepared hydroxyapatite powders that were produced by wet precipitation method. It is clear that the size of the hydroxyapatite particle aggregates distribute is (1385.5 nm) with mean size about (1613.9 nm).

![Figure 2. Particles size analysis of prepared hydroxyapatite powder.](image-url)
3.2. XRD of HAP powder results

Figure 3 shows the XRD patterns of the hydroxyapatite particles prepared by the wet precipitation method. The observed peaks of calcined HAP are narrow peaks which indicate obtaining highly crystalline powder. These patterns are in agreement with standard file (JCPDS, Card for HAP, NO. 74-0565). This prepared high crystalline particles of HAP was considered the gestures for preparation of scaffolds.

![XRD spectrum of prepared powder of hydroxyapatite.](image)

**Figure 3.** XRD spectrum of prepared powder of hydroxyapatite.

3.3. SEM results

The interconnected pores in the sintered scaffolds of hydroxyapatite were shown in Figure 4. Bigger pore sizes and higher porosity of the scaffolds which may lead easily cells proliferation and migration in these pores. Due to the replicating of scaffolds, pores were interconnected, and nutrients transfer can happen within these scaffolds.

![SEM showing pours nature of hydroxyapatite scaffolds after sintering process.](image)

**Figure 4.** SEM showing pours nature of hydroxyapatite scaffolds after sintering process.

3.4. Porosity analysis

Using equations (1) and (2), porosity of hydroxyapatite scaffolds was measured. Highly porous scaffolds of HAP can be obtained reaching interconnected and open porosity in the ranges about 72-75 %. It is clear that the results in better cell proliferation and tissue growth, therefore, an ideal. This
is a significant achievement supporting the potential suitability of the HAP scaffolds for bone tissue engineering, since their porosity is certainly in the range of human trabecular bone [9].

3.5. XRD of HAP scaffold results
The XRD patterns of the pours scaffolds prepared using polymer sponge replication technique, followed by the sintering process at temperature of 1300 °C for 2 hours were showed in Figure 5. It was observed from the analysis of the XRD pattern comparable to that hydroxyapatite standard file (JCPDS card for HAP No. 09-0432), the sharp reflection peak at the 2θ of 31.7 identify the phase of hydroxyapatite.

![Figure 5. XRD spectrum of porous HAP scaffold after sintering process.](image)

3.6. FTIR results
The analysis by FT-IR were presented the characteristics chemical bands of HAP scaffolds, as shown in Figure 6. The stretching and bending band ν₃ of PO₄ group is the most sharp peaks was observed at 1043 cm⁻¹, also, the ν₁ stretching band of PO₄ was established by the presence of very small peak at 961 cm⁻¹, which approve the existence of the phosphate groups (PO₄) in the pours scaffolds of HAP [1]. The stretching bands of the hydroxyl group (OH) were observed at 3400 and 3566 cm⁻¹. The band at 1457 cm⁻¹ attribute to components of the of CO₃ group. The 2361 cm⁻¹ band can be accredited absorption of the CO₂ group [13].
3.7. Bioactivity analysis
In vitro evolution of the scaffolds’ bioactivity was achieved through examining changes in the composition of the soaking in simulated body fluid (SBF) medium. The scanning electron microscopy micrographs of the HAP scaffolds after soaking in SBF for 7 days were shown in Figure 7. It is clear that the HAp aggregates layer with ideal cauliflower morphology entirely covered the pores in to scaffolds which suggesting the continuous development of reaction layer as the result of the ion exchange occur between the SBF and HAP scaffold [14].

Figure 6. FTIR spectra of the prepared HAP pours scaffold.

Figure 7. SEM images of scaffolds showing spherical aggregates of apatite after immersion in SBF

4. Conclusions
This study deals with the applicability porous HAP scaffolds for bone repair and regeneration. The established scaffolds been a highly porous structure about (72-74%) and entirely open and interconnected micro-pores. The results analysis by SEM were showed scaffolds with varies sizes of interconnected pores depending on the density in the poly urethane sponge used in the preparation
method. The bioactivity was observed formation of apatite layer on the surface pours scaffold after 7 days immersion in SBF solution, these indicate that the prepared HAP scaffolds could be used as biological material for bone tissue applications.

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