Production and characterization of PCL (Polycaprolactone) coated TCP/nanoBG composite scaffolds by sponge foam method for orthopedic applications

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1. Introduction

Pathological degeneration, trauma, tumor resection, and congenital deformities are the main factors affecting orthopedic and dental treatments [1, 2]. One of the most effective ways for bone regeneration is bone tissue engineering (BTE) [3, 4]. To overcome the restrictions of damaged tissues or organs and provide biological alternatives, treatment of BTE has been practiced clinically [5, 6]. Using biomaterials for the production of scaffolds is a promising BTE technique. These scaffolds support new tissue growth by the provision of an artificial extracellular matrix [7, 8]. The role of these scaffolds is to provide daily mastication like a natural bone in order to build support for the regeneration region and simultaneously undergo gradual degradation with the regeneration of tissue. Acceptable mechanical strength, suitable physicochemical properties, proper surface morphology and porosity, biocompatibility, controllable biodegradability, and osteoconductivity are the most important properties that a well-functioning scaffold should possess [9-11]. Common scaffolds for BTE are currently various bioactive glass, calcium phosphate, and hydroxyapatite (HA) [12, 13]. Tricalcium phosphate (β-TCP) exhibits promising biodegradability, osteoconductivity, and biocompatibility [14]. On the other hand, this bioceramic also has drawbacks. Its degradation rate is higher than bone regeneration [15, 16]. Additionally, due to having a porous structure, the mechanical properties of β-TCP as a support for cell growth is relatively low [17, 18]. As composites materials provide the opportunity to combine the properties of incorporated components [19, 20], several research has been focused on the production of composite scaffolds consisting of polymers and β-TCP for improving osteoconductivity and the mechanical properties of the scaffold to overcome these shortcomings. Polymer/ceramic composites have offered promising properties due to benefiting from both materials properties [21-23]. Chitosan and collagen are natural polymers that are used to produce composites [24-26], but these natural polymers do not have sufficient mechanical strength [27]. Polylactic acid and poly-ε-caprolactone (PCL) are synthetic polymers that have been used for overcoming the disadvantages of natural polymers [28, 29]. Owing to biocompatibility, biodegradability, and good mechanical properties, PCL have been widely investigated [23, 30]. Moreover, bioglass materials with different compositions are able to integrate with living bone tissue by creating an intimate bond. These bioglasses that are able to form a bond with the bone are called “bioactive”. Although there are several studies about producing scaffolds of PCL coated TCP, there is no research about PCL coated TCP/nanoBG composite scaffolds synthesized via sponge foam method for orthopedic applications to the best of authors knowledge. Therefore, in this study, we prepared TCP-bioglass composite scaffolds with different compositions. To enhance the mechanical properties of the scaffolds, they were coated by a polycaprolactone biopolymer, and their
mechanical, bioactivity, and biodegradation were evaluated.

2. Experimental

2.1. Materials

In this study, calcium nitrate tetrahydrate (Ca(NO$_3$)$_2$·4H$_2$O), TEOS ((C$_2$H$_5$O)$_4$Si), PCL polymer (molecular weight of 80,000) and tetraethyl phosphate ((C$_2$H$_5$O)$_3$PO) were bought from Aldrich company. Simulated body fluid (SBF), sodium tripolyphosphate solvent, carboxymethyl cellulose powder, nitric acid (63%), and chloroform (99.5%) were obtained from Merck company, Germany.

2.2. Sample preparation

2.2.1. Production of TCP particles

After boiling the cow thigh in water for 2 hours to remove residual tissue, the bone was kept at 60 °C for 24 hours in an oven to dry. Then, the bone pieces were burnt by fire flame at about 400 °C for 3 h to degrade the organic components of the bone. The obtained black ash was heated at 900 °C for 2 h to produce hydroxyapatite powder.

2.2.2. Synthesis of bio glass nanoparticles (nanoBG)

For the preparation of bioactive glass, raw materials including calcium nitrate (28 mol. %), TEOS (63 mol. %), and tetraethyl phosphate (9 mol. %) were used. Ethanol and hydrochloric acid were also used as a solvent and a catalyst, respectively. To prepare the sol, the solution of deionized water and hydrochloric acid was stirred by a magnetic stirrer for 30 min. After adding TEOS and ethanol with a molar ratio of 1, the solution was stirred for another 30 minutes following the addition of tetraethyl phosphate and stirring for 20 min. Finally, it was stirred at medium speed for 2 hours with the addition of calcium nitrate. After gelation of the initial solution, the formed gel was dried at 60 °C for 10 h. To eliminate excess gases, the gel heat-treated for 15 h at 130 °C. Finally, by heating the dried gel at 900 °C for 2 h, bioglass was obtained as the final product of the operation.

2.2.3. Preparation of ceramic slurry and scaffolds

The obtained tricalcium phosphate powders with a mean particle size of 85 nm were used as the base material to fabricate the scaffold.

Fig. 1. SEM images TCP/bioglass/PCL composite scaffolds of (a) TC-B10, (b) TC-B20, (c) TC-P, (d) TC-B10-P, (e) TC-B20-P, and (f) TC-B30-P
Hydroxyapatite and nanoBG powders with 0, 10, 20, and 30 wt. % bio-
glass was added slowly to deionized water. After homogenization of the
mixture, 1 wt. % sodium tripolyphosphate (STPP) was used to increase
the solid loading on the sponge disks. The slurry was then stirred for 30
min at 300 rpm and then 1 wt. % of carboxymethyl cellulose was added
as the binder. Finally, stirring was continued at 60 °C until complete
homogenization.

Commercial polyurethane foam was cut into 10×10×10 mm cubes
and immersed slowly into the ceramic slurry. Then, the samples were
dried in a vacuum for 24 hours to prevent sponge pores from closing.
The samples were subjected to heat treatment in the furnace during 4
stages to prepare the scaffolds: 1 and heated at 600 °C for an hour to
completely burn the polymer foam (heating rate of 3 °C/min). 2. Heating
from 600 °C to 1350 °C with a heating rate of 5 °C/min. 3. Keeping the
samples at 1350 °C for 2 hours. 4. Cooling the samples in the oven to
room temperature with a 5 °C/min cooling rate. The uncoated scaffolds
containing 10, 20, and 30 wt. % bioglass is noted as TC-B10, TC-B20,
and TC-B30, and P denotes the polymer coating in composites scaffolds.

2.2.4. Composite coatings on scaffolds

The PCL polymer (M_w=80000) was dissolved in chloroform (10%
(w/v)) and stirred for 30 min. 10 wt. % of nanoBG particles were son-
icated in 10 cm^3 chloroform for 15 minutes and then added to the PCL
solution. The final solution was then stirred at room temperature for fur-
ther homogenization for 24 h. After sterilizing the surface of the scaf-
dfold with acetone and ethanol, the scaffolds were soaked in the prepared
solution for 1 minute. Finally, the scaffolds were dried in an oven for 7
days at 37 °C.

2.3. Characterization

2.3.1. Measurement of scaffold porosity and density

For the calculation of density and porosity of scaffolds, the liquid
displacement method was employed. In this study, ethanol 96% was
used to diffuse into scaffold porosities. The scaffold density was calcu-
lated from the following equation:
and the amount of open porosity of the scaffold is obtained from the following relationship:

\[ \rho = \frac{W}{(V_2 - V_3)} \]  

and the amount of open porosity of the scaffold is obtained from the following relationship:

\[ \iota = \frac{(V_1 - V_3)}{(V_1 - V_2)} \]  

where \( W \), \( V_1 \), \( V_2 \), and \( V_3 \) denote the scaffold weight, the volume of ethanol contained in the cylinder, volume of ethanol after immersion of the scaffold in the cylinder for 5 min, and the volume of ethanol remaining after removal of the scaffold from the cylinder, respectively. The amount of \( \iota \) and represent the volume of scaffold and adsorbed ethanol, respectively. Thus, the final volume of the scaffold is calculated through the equation 3.

\[ V = \frac{(V_1 - V_2)}{(V_1 - V_3)} \]  

2.3.2. SEM and EDS analysis

The microstructure and morphology of the prepared scaffolds were investigated via Zeiss 00947B scanning electron microscope and EDS analysis. Prior to microscopic observations, the surface of the composite scaffolds was gold-coated.

2.3.3. Measurement of pH in SBF solution

After immersion of the scaffolds in the SBF solution, the pH of the solution was measured by Aqbus Model 2000 pH meter after 2, 3, 7, 14, 21, and 28 days.

2.3.4. Bioactivity assessment

In vitro bioactivity evaluation was performed by immersion in SBF for 28 days. The cubic scaffolds were immersed in SBS solution and incubated at 37 °C and after 1, 7, 14, and 21 days, the composite scaffolds were taken out, washed with water, and dried in a vacuum oven at 60 °C and taken for further characterization.

2.3.5. Plasma coupling optical emission spectroscopy (ICP)

ICP method was used to measure the number of ions released from the scaffold immersed in SBF solution. In this study, a Zaies 110394 device was used.

3. Results and discussion

3.1. Microstructural study of the composite scaffolds

The SEM images of the TCP/bioglass/PCL scaffolds are shown in Figure 1. As seen in SEM images, the cross-section of the synthesized scaffold shows interconnected. The mean pore size of the scaffolds appears to be about 300-550 μm, which is appropriate for cells to infiltrate into the channels and pores of the scaffold. Ideal scaffolds are required to possess a high volume of open and interconnected pores in order to obtain high cell seeding density in the scaffolds, and to facilitate transporting of oxygen as well as nutrients for cell differentiation and cell proliferation. Scaffolds with porosity dimensions of 100 to 500 microns are considered for cell culture, soft and hard tissue growth, and vascularization. In bone tissue engineering, the pore size larger than 300 microns is required. It should be noted that the increase in porosity size leads to a decrease in the mechanical strength of the scaffold; therefore, the balance between these parameters should be considered. As can be seen in the figure, PCL has been successfully coated on the scaffolds.

3.2. Measurement of pH and Released ions in SBF solution

Figure 2 shows the pH variations of coated and uncoated scaffolds in the SBF solution within 28 days. According to the results, the pH change is between 7.4 and 8, which is acceptable for a physiologic environment to prevent damages to cells and tissues.

SBF solution was analyzed after 28 days to measure the released amount of ions from the scaffolds. The obtained results for uncoated samples are presented in Table 1. As shown in the table, most calcium and phosphorus ions were absorbed by the sample’s surface, and the concentration of these ions in SBF decreased, showing the apatite formation. The lowest concentration of Ca and P ions is related to the TC-B20 sample, which means the apatite formation is higher; in other words, the bioactivity of this scaffold is higher than that of other scaffolds.

As observed in Figure 3, the bright precipitates formed on the surface

| Sample | Ca (ppm) | P (ppm) | Si (ppm) |
|--------|---------|--------|---------|
| TC     | 24      | 22     | 0       |
| TC-B10 | 20      | 20     | 50      |
| TC-B20 | 18      | 14     | 40      |
| TC-30  | 20      | 19     | 44      |
| SBF    | 100     | 31     | 0       |
of the scaffolds indicate the deposition and formation of apatite particles. Also, the result of point analysis by EDS on the bright spots of the TC-B10-P scaffold shows the presence of P and Ca, revealing that these precipitates are calcium phosphate. This also indicates the bioactive behavior of this sample.

3.3. Porosity and mechanical properties of the scaffolds

In general, well-functioning implants for tissue engineering are required to have high pore density, good biocompatibility, and sufficient mechanical strength. The porosity percentages of the samples are shown in Figure 4. As evident, polymer coating decreased the porosity of the scaffolds. The porosity of all the prepared samples is in the range of 75-80%, which is in the acceptable range for tissue engineering scaffolds. It can be concluded from the compressive strength results (Figure 5) that the polymer coating inside and on the surface of the porosities caused enhancement of adhesion strength. TC-B20-P shows the highest value of compressive strength. The polymer coating on the surface and inside the pores of the scaffold results in the improvement of the scaffold strength so that in some cases, the strength is multiplied. PCL penetrates the ceramics scaffold, preventing stress concentration and cracking in the scaffold, thus increasing its strength. On the other hand, the highest mechanical strength is related to the sample containing 20 wt. % of bioglass. The bioglass nanoparticles in this sample are homogeneously dispersed in the space between the beta-tricalcium phosphate particles, which result in higher strength.

3.4. Biodegradation

Weight loss of the scaffolds after soaking in SBF for 28 days is depicted in Figure 6. The sample TC-B20 shows the best performance in terms of biodegradation. In the case of coated specimens, the rate of degradation is relatively lower than that of the coated specimens but similar to the coated specimens, TC-B20-P has the lowest degradation. The SEM images of TC-B20-P before and after degradation is shown in Figure 7.

4. Conclusions

In this research, TCP/nano-bioglass/PCL composite scaffolds with different compositions were produced. Adding 20% bioglass improved the mechanical strength more than 2 times (from 0.4 MPa to 1.1 MPa). The coating of the scaffolds also enhanced the mechanical properties as the strength of the TC-B20 sample increased from 1.1 MPa to 2 MPa. All scaffold samples contain 75-80% porosity, which is acceptable for bone scaffolds. The result of the elemental analysis of ICP showed that the highest bioactivity behavior was related to the TC-B20 sample. Evaluation of elemental analysis results of SBF solution on coated scaffold samples showed the positive effect of coating and improved bioavailability. Finally, the degradation results showed that the degradation improved in TC-B20 and also the enhancement was observed by coating.

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