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Short Communication

Fabrication and Characterization of Strengthened BCP Scaffold Through Infiltration of PCL in the Frame

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Abstract Bioceramics based on calcium phosphate have been widely used due to their excellent biocompatibility and bioactivity as well as chemical composition. Sponge replica method has been developed because of its interconnected pores which are similar to cancellous bone. Porous Biphasic Calcium Phosphate (BCP) scaffold was fabricated with the synthesized powder by the replica method. BCP slurry was coated 3 times and then PU was burnt out at 800 °C. The scaffolds were prepared infiltrated with PCL slurry and sintering at 1200 °C for 3 hours to improve the compressive strength. Bending strength and relative density were measured. Microstructures of the composites were observed using SEM. The biocompatibility and one cell morphology of the fabricated porous body were investigated by MTT assay and SEM observation.

Keywords biphasic calcium phosphate; scaffold; PCL; infiltration; cell adhesion

1 Introduction

Calcium phosphate ceramics, especially hydroxyapatite (HAp, $Ca_3(PO_4)_6(OH)_2$) and β-tricalcium phosphate (β-TCP, $Ca_3(PO_4)_2$), have been developed in tissue engineering because of its chemical similarity to natural bone as well as excellent biocompatibility and bioactivity [2,6,15]. Comparing two kinds of calcium phosphate composite, hydroxyapatite has higher mechanical properties and β-tricalcium phosphate has higher bio-resorption. Recently, biphasic calcium phosphate ceramic, which is consisted of HAp and β-TCP, has received attention for the application of bone substitutes and scaffolds for tissue engineering. BCP ceramics show different mechanical properties and biological response depending on the component ratio of HAp and β-TCP [3,4,12].

Porous scaffold has been widely used for hard tissue engineering due to its similar structure to the natural cancellous bone. There are several attempts to fabricate the scaffold materials like sponge replica method, gel-casting method, and organic material as a pore forming agent. Among them, using sponge replica method can fabricate desirable structure containing interconnected pores and a large surface area which can induce bone formation [7, 14,16]. However, the highly porous structure has too low mechanical properties to be used in medical applications with good reliability [9,10].

Polycaprolactone (PCL) is well known for biocompatible and biodegradable polymer and can be easily formed into thin layer with higher mechanical properties [8,13]. Because of those properties, PCL is highly considered for soft and hard tissue engineering fields. Several applications of PCL have been approved by FDA [1,5].

In this study, porous BCP scaffold was fabricated by sponge replica method which had a similar structure to cancellous bone. Various amounts of PCL were infiltrated into micro-channel pores of scaffold's frame through the residual pores which were remaining during the sintering process. Compressive strength was measured and XRD analysis was performed. The microstructure was characterized in detail using SEM. Furthermore, the BCP scaffold was evaluated for the cytotoxicity of human osteoblast-like cell (MG-63). Also, with human osteoblast-like cell (MG-63), cell attachment was investigated too.

2 Materials and methods

BCP scaffold was fabricated by sponge replica method. As a starting materials, nano-sized biphasic calcium phosphate (BCP) powder which is synthesized by microwave hydrothermal method [11], poly vinyl butyral (PVB, Acros, USA) as a binder, and PU foam (45 ppi, 3 M, USA) were used. To prepare BCP slurry, 50 wt% of synthesized nano powder was ball-milled in ethanol for 24 hours and 5 wt% of PVB was subsequently added into the slurry, and stirred for 5 hours more. Prepared PU foam was immersed in the prepared slurry to cover the frame with BCP slurry. Then, compressed air blew to the foam for keeping the interconnected pores. The coated foam was dried at 80 °C for 1 hour. These dipping and drying steps

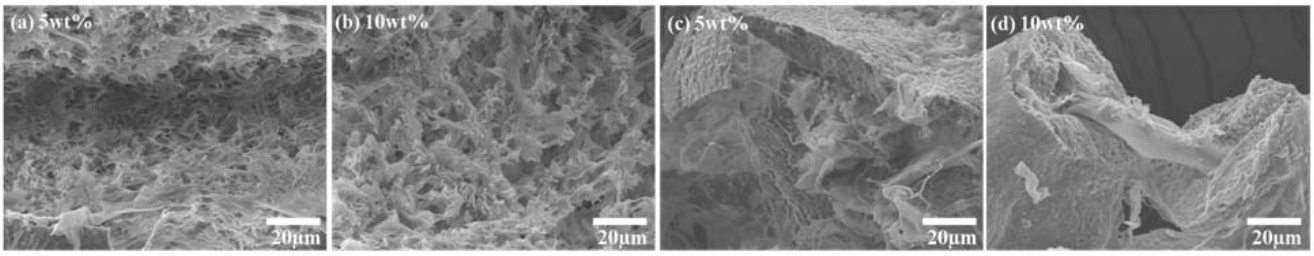

Figure 1: SEM micrographs of PCL depending on the amount of PCL; (a), (c) 5 wt% and (b), (d) 10 wt%.

were repeated 3 times to cover the whole surface without any remaining uncoated space. The PU foam was burnt out at 800 °C for 2 hours in air atmosphere, and then pressureless sintering was carried out at 1200 °C for 3 hours. Various amounts (2.5 wt%, 5 wt%, 7.5 wt%, and 10 wt%) of PolyCaprolactone (PCL) were dissolved in acetone by ultrasonication. The sintered scaffold was placed in the vacuum container, and then, the dissolved PCL solution was injected into the container and kept for 10 minutes for infiltration. Next, the infiltrated BCP scaffold was kept in the vacuum oven to remove solvent for 24 hours.

The relative densities of the porous BCP scaffolds were measured using the Archimedes' principle. To identify the crystal structure and phases of the samples which were crushed into powder, X-ray diffraction (XRD, D/MAX-250, Rigaku, Japan) was employed. To investigate the pore size and microstructure of the BCP scaffold, scanning electron microscopy (SEM, JEOL, JSM-7401F) was used. The specimens, with a dimension of $7\times7\times7\,\mathrm{mm}^3$, were subjected to a compression test using a universal testing machine (UnitechTM, R&B, Korea) with a crosshead speed of 0.5 mm/min in ambient conditions. The obtained stress-strain curve was used to determine the mechanical properties. The cytotoxicity evaluation of the BCP scaffold was carried out on 96 wells based on human osteoblast-like cell (MG-63). Extraction media was prepared by immersing the scaffold in DMEM during 1 day. MG-63 were seeded at a density of 1×10^4 cells/well then were incubated at 37 °C for 24 hours. After 1day, the cell culture media was removed by aspirator, and the extraction media from BCP scaffold was added on 96 wells. After that, the incubation was done for 72 hours. A solution of 20 μL of MTT was added to each well, and covered with aluminum foil and then incubated for 4 hours. After careful removal of the media, 200 μL of dimethyl sulfoxide (DMSO; Sigma) was added to each well to extract the formazan crystals under shaking. The absorbance intensities were measured at 595 nm with ELISA (EL 312e, Bio-Tek). The cell morphology of MG-63 cells cultured on BCP scaffold was observed. First of all, the BCP scaffold was immersed in DMEM for 2 hours. After immersing, the sample was moved to another well for seeding cell. MG-63 cells (1×10^5 cells/mL) were seeded on the top surfaces of the BCP scaffold in 24 wells plate. The attachment of cells was observed after 30 minutes and 60 minutes using a scanning electron microscope (SEM).

3 Results and discussion

Figure 1 shows SEM micrographs of PCL depending on the amount of PCL; (a), (c) 5 wt% and (b), (d) 10 wt%. Figures 1(a) and 1(b) show microstructures of PCL on the surface area of the frame. The morphologies of PCL were almost same in both cases, but the volume of PCL which was coated on the surface area was increased when 10 wt% of PCL used. The inside of the hollow frame was filled with PCL as shown in the fracture surface images of Figures 1(c) and 1(d).

Figure 2 shows stress-strain curves of BCP scaffold with/without infiltration of PCL after compressive strength measurement. In the case of sintered scaffold, the value of compressive strength was around 0.25 MPa at nearly 20% of the strain. However, infiltration process made scaffold strengthened. The values of compressive strength were increased to 0.45 MPa and 0.78 MPa when the amount of PCL were 5 wt% and 10 wt%, respectively.

From a bioanalytical point of view, human osteoblast-like MG-63 cells were adhered definitely. Depending on the condition, cell adhesion was similar at 60 minutes. As shown in Figure 3, the filopodia and lamellapodia activity was significant in all the cases. But the cell attachment was more vigorous in the case of higher PCL coated surface.

4 Conclusions

A new approach combining sponge replica method and infiltration process to increase the mechanical properties was developed. PCL was infiltrated into the micro-channel pore, which was formed after burning-out PU foam, through the residual fine pores on the surface area. PCL had almost the same morphology even though a different ratio was used, but the amount of coated/infiltrated-PCL was increased as higher amount of PCL used. The value of compressive strength was increased as 0.45 MPa and

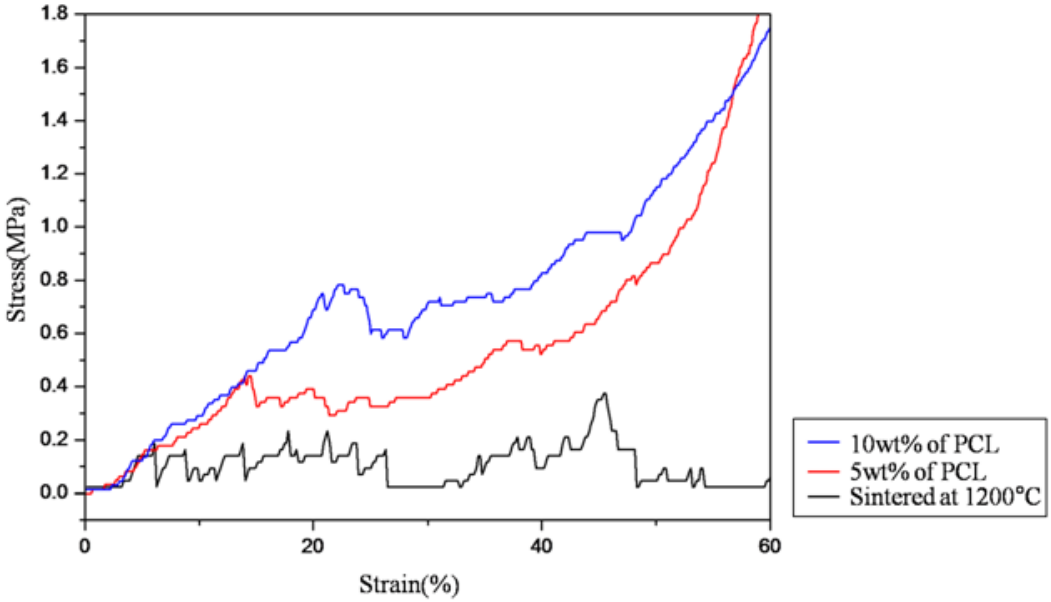

Figure 2: Stress-strain curves of BCP scaffold with/without infiltration of PCL.

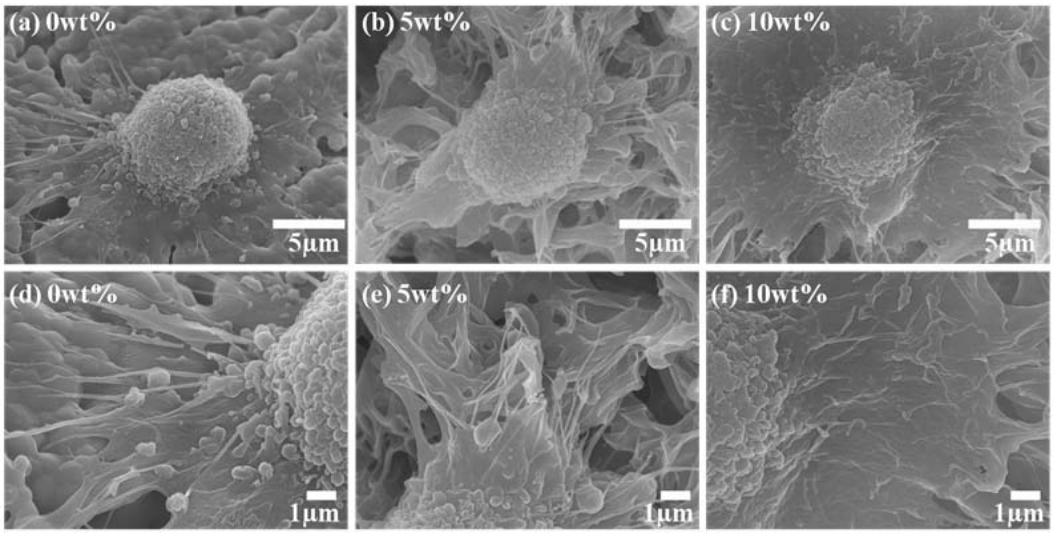

Figure 3: One cell morphologies of BCP scaffold with/without PCL at 60 min; (a), (d) without PCL, (b), (e) 5 wt% of PCL, and (c), (f) 10 wt% of PCL.

0.78 MPa when 5 wt% and 10 wt% of PCL were employed, respectively, compared to sintered scaffold which had 0.25 MPa of compressive strength at nearly 20% of strain in all conditions. The results also proved that BCP scaffold had a suitable condition for cell attachment, adhesion and proliferation.

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