PCL Infiltration into a BCP Scaffold Strut to Improve the Mechanical Strength while Retaining Other Properties

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Abstract A highly porous Biphasic Calcium Phosphate (BCP) scaffold was fabricated by the sponge replica method with a microwave sintering technique. The BCP scaffold had interconnected pores ranging from 80 µm to 1000 µm, which were similar to natural cancellous bone. To enhance the mechanical properties of the porous scaffold, infiltration of polycaprolactone (PCL) was employed. The microstructure of the BCP scaffold was optimized using various volume percentages of polymethylmethacrylate (PMMA) for the infiltration process. PCL successfully infiltrated into the hollow space of the strut formed after the removal of the polymer sponge throughout the degassing and high pressure steps. The microstructure and material properties of the BCP scaffold (i.e., pore size, morphology of infiltrated and coated PCL, compressive strength, and porosity) were evaluated. When a 30 vol% of PMMA was used, the PCL-BCP scaffold showed the highest compressive strength. The compressive strength values of the BCP and PCL-BCP scaffolds were approximately 1.3 and 2 MPa, respectively. After the PCL infiltration process, the porosity of the PCL-BCP scaffold decreased slightly to 86%, whereas that of the BCP scaffold was 86%. The number of pores in the 10 µm to 20 µm range, which represent the pore channel inside of the strut, significantly decreased. The in-vitro study confirmed that the PCL-infiltrated BCP scaffold showed comparable cell viability without any cytotoxic behavior.

Key words BCP (biphasic calcium phosphate), compressive strength, PCL (polycaprolactone), in-vitro study.

1. Introduction

Porous scaffolds have been widely used for hard tissue engineering due to their structural resemblance to human cancellous bone. It is generally agreed that microstructures of highly porous scaffold with interconnected pores and large surface areas are conducive to the growth of hard tissues.3-5 There are several methods for fabricating scaffold materials, such as polymeric sponge replica,6 freeze casting,7 polymer impregnating,8 and fibrous monolithic process.9 Among these, the sponge replica method can fabricate desirable structures containing interconnected pores and large surface area. However, the mechanical property of the highly porous structure is insufficient for medical applications with good reliability;10-12 compressive strength of natural cancellous bone is approximately 2-12 MPa.10 There have been several attempts to enhance the material properties of scaffolds. The strength can be increased with a change in the crystal characteristics. Ramay and Zhang have used HA nanofibers to increase the compressive strength of the porous scaffold,11 but this will produce a stiffer construct. The other approach would be to include a polymer to create a polymer-ceramic composite. However, there has been no remarkable achievement with Ca/P ceramic, and researchers were unable to attain the desirable mechanical properties in previous studies.12-15 (For example, PCL-coated HAp showed 0.57 MPa of compressive strength.12) Especially, polycaprolactone, a well-known biocompatible and biodegradable polymer, has been considered a suitable substrate candidate for tissue engineering.16,17 Due to its properties, PCL has potential for biomedical applications.18,19

On the other hand, calcium phosphate ceramics, especially hydroxyapatite (HAp, Ca10(PO4)6(OH)2) and β-tricalcium phosphate (β-TCP, Ca3(PO4)2), have been used extensively in hard tissue engineering because of their chemical similarity to natural bone and their excellent biocompatibility and bioactivity.20,21 HAp has direct chemical bone-bonding ability, and TCP has a higher bioresorption property. Recently, biphasic calcium phosphate (BCP) ceramic, which consists of HAp and β-TCP, has received attention for applications as bone substitutes and scaffold materials. Also, BCP ceramics exhibit various mechanical properties and biological responses depending on their compositional ratio of HAp and β-TCP.22,23

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In this study, a porous BCP scaffold, which had a structure similar to that of cancellous bone, was fabricated by the sponge replica method. The proper microstructure was optimized using polymethyl-methacrylate (PMMA) for the infiltration process. The PCL infiltrated into the hollow strut of the sintered scaffold, which was formed after the burning out of the organic sponge. Compressive strength measurements, porosity measurements, and XRD analysis were performed. The microstructure was characterized in detail using SEM. Furthermore, the biocompatibilities of BCP and PCL-BCP scaffolds were evaluated by MTT assay. Also, to evaluate the attachment behavior of MG-63 cell, cell morphology was observed by the FE-SEM technique.

2. Experimental Procedure

2.1 Fabrication of the BCP scaffold

The BCP scaffold was fabricated by the sponge replica method. BCP nano-powder was synthesized by microwave hydrothermal method using Ca(OH)₂ (99.99%, Aldrich Chemical) and H₃PO₄ (85-87%, Dongwoo Fine Chemicals, Korea) as precursors. The starting materials included in-house synthesized BCP nano-powder; polyvinyl butyral (PVB, Acros, USA) as a binder; PMMA (particle size: ~15 µm, SUNJIN Chemical, Korea) as a pore forming agent for infiltration process; and a PU sponge (60 ppi, HD sponge, Korea). First, powder mixtures were prepared by ball-milling BCP nanopowder with varying amounts of PMMA (20, 30, and 40 vol%). To prepare the BCP slurry, 10 wt% of PVB was dissolved and 10 vol% of powder mixture was subsequently added while stirring. The polyurethane (PU) sponge was immersed in the prepared slurry to coat the strut with BCP. Then, compressed air was blown onto the sponge to maintain the interconnected pores. The BCP slurry-coated PU sponge was dried at 80°C for one hour, and the dipping and drying steps were repeated three times to achieve complete coating. The PU sponge was burned out at 1000°C for 2 hours in air to remove all organic materials (e.g., PMMA, PVB). Microwave sintering was carried out at 1300°C for 10 minutes at a heating speed of 100°C/min.

2.2 Infiltration of PCL

Stirring at 60°C, 15 wt% of polycaprolactone (PCL, Sigma-Aldrich, USA) was dissolved in N,N-dimethylformamide (DMF, JUNSEI, Japan). For the infiltration process, the sintered scaffolds were placed in the pressure vessel and degassed at 90 kPa. The dissolved PCL solution was injected into the vessel, and the pressure of the vessel was slowly increased to 0.5 MPa by a compressor and maintained for 10 minutes. All processes were performed at 60°C. The infiltrated BCP scaffold was removed from the vessel and centrifuged at 500 rpm for 5 minutes to maintain the interconnected pores. PCL-BCP scaffolds were kept in the hood to remove solvent for 24 hours. After infiltration, the samples were designated as BP20, BP30, and BP40 for 20, 30, and 40 vol% of PMMA, respectively.

2.3 Characterization

X-ray diffraction (XRD, DMAX-250, Rigaku, Japan) was used to identify the crystal structure and phases of the scaffold, and scanning electron microscopy (SEM, JEOL, JSM-7401F, Japan) was used to investigate the microstructure of the BCP scaffolds. The specimens (7 × 7 × 4 mm³) were subjected to compression tests using a universal testing machine (Unitech™, R&B, Korea) with a crosshead speed of 0.5 mm/min under ambient conditions. The obtained stress-strain curve was used to determine mechanical properties. A mercury porosimeter (PoreMaster™, Quantachrome Instruments, FL, USA) was used to analyze the pore structure of the scaffolds such as porosity and pore size distribution.

2.4 In-vitro studies

2.4.1 Cytotoxicity test

The cytotoxicity evaluation of the BCP scaffolds and PCL-BCP scaffolds were carried out in 96-well plates seeded with human osteoblast-like cells (MG-63), which were obtained from the Korean Cell Line Bank. Extraction media were prepared by immersing the specimens in Dulbecco’s modification of Eagle’s Medium (DMEM: HyClone, Logan, UT) and placed in an incubator maintained in Dulbecco’s modification of Eagle’s Medium containing 5% CO₂ at 37°C. The MG-63 cells were seeded at a density of 1 × 10⁵ cells/well, and they were incubated at 37°C for 24 hours. After one day, the cell culture media was removed by aspiration, and the extraction media from the BCP and PCL-BCP scaffolds were added to 96-well plates at varying percentages of extraction media (12.5%, 25%, 50% and 100%). Afterwards, they were incubated for 72 hours. Twenty µl of MTT solution was added to each well, and they were incubated for four hours. The absorbance intensities were measured at 595 nm with ELISA (EL 312e, Bio-Tek, USA).

2.4.2 Morphology of cultured cells

Human osteoblast-like MG-63 cells obtained from the Korean Cell Line Bank (KCLB) were used to investigate the attachment of cells to the specimens. The cells were maintained in Dulbecco’s modification of Eagle’s Medium (DMEM: HyClone, Logan, UT) and placed in an incubator containing 5% CO₂ at 37°C. The MG-63 cells (1 × 10⁵ cells/ml) were seeded on the top surfaces of the BCP scaffold in a 24-well plate for 15 min, 30 min and 60 min. For SEM observation, the cell-culture specimens were rinsed with PBS twice, and the cells were fixed with...
2.0% glutaraldehyde. Afterwards, the specimens were dehydrated in ethanol solutions of varying concentration (50, 70, 90, 95 and 100%), each for 5 min. The dehydrated cells were kept in hexamethyldisilazane (Sigma-Aldrich, U.S.A) for 5 min and allowed to air dry.

3. Results and Discussion

Porous BCP scaffolds with interconnected macro pores, which had structures similar to that of cancellous bone, were fabricated successfully by the sponge replica method. SEM micrographs (Fig. 1) show various BCP scaffolds: (a, b) pure BCP, (c) BCP scaffold using PMMA as a pore forming agent, and (d) PCL-infiltrated BCP scaffold. The size of interconnected macro pores ranged from 80 µm to 1000 µm, and the struts of sintered scaffolds were approximately 50–100 µm thick, as shown in Fig. 1(a). After the sintering step, complete densification occurred in the BCP scaffolds without any residual pores on the surface of the strut (Fig. 1(b)). When PMMA was embedded during the fabrication steps, micropores were observed on the surface area due to the burning out of PMMA (Fig. 1(c)). During the infiltration process, PCL homogeneously coated the surface of the strut (Fig. 1(d)) and infiltrated into the internal hollow space.

Since the PU sponge was completely removed during the burning-out and sintering steps, a triangle-shaped hollow space was observed, as shown in Fig. 2(a). The formation of the triangle-shaped hollow space is one of the factors contributing to the low mechanical strength of sintered BCP scaffolds. From the strut area, complete densification occurred, such that we could not achieve

Fig. 1. SEM micrographs show various BCP scaffolds: (a, b) pure BCP, (c) BCP scaffold using PMMA as a pore forming agent and (d) PCL-infiltrated BCP scaffold.

Fig. 2. Fracture morphologies of scaffolds: (a) pure BCP, (b) BCP scaffold using PMMA as a pore forming agent and (c) PCL-infiltrated BCP scaffold.
infiltration throughout the dense strut. To facilitate PCL infiltration into the strut of the BCP scaffold, various amounts of PMMA were used during the fabrication process. Fig. 2(b) shows BCP scaffolds fabricated with 30 vol% of PMMA, which was the optimal condition. The PMMA was completely removed during the burning out process, and spherical micropores formed randomly. Various sizes of interconnected micropores were observed in the fracture surface area. These micropores formed connections from the outside to the inside, and they served as pathways during the infiltration process. Following the degassing step, PCL partially infiltrated into the hollow space during the infiltration process. However, the pressurizing step completed infiltration throughout the BCP scaffolds, homogeneously. The infiltration process did not affect any change to the original structure of the BCP scaffolds. To obtain the fracture surface image (c), scaffolds were frozen in liquid nitrogen for 10 seconds, and they were broken rapidly using a very sharp razor blade. The infiltrated PCL was observed in the internal hollow space, which formed due to the burning out of the PU sponge and due to micropores where the PMMA was embedded in the strut. However, there was remaining space due to evaporation of solvent.

The XRD patterns of (a) the calcined raw powder at 750°C, and (b) the porous scaffold sintered at 1300°C are shown in Fig. 3. The HAp and β-TCP phases were clearly detected in both samples. The intensity ratio was calculated by comparing the two highest peaks of HAp and β-TCP. In the case of calcined powder (a), the intensity percentages of HAp and β-TCP phase were approximately 71% and 29%, respectively. When the scaffold was sintered at 1300°C (b), the intensity percentage of β-TCP phase increased to approximately 32% as a result of the phase transformation of HAp to β-TCP. The extent of change of the phase was very low. This is because the scaffolds were sintered in a microwave furnace, which uses a very fast heating rate and short dwelling time for complete sintering. The primary reason for showing small amounts of phase changes is to demonstrate the merits of short exposure time at high temperature.

Table 1 shows materials properties (porosity and compressive strength) of the scaffolds. The BCP scaffolds exhibited approximately 88% porosity and approximately 1.3 MPa of compressive strength. After the infiltration process of PCL, BP30 porosity decreased slightly to 86%. Although BP30 porosity decreased, the decrease was very small, and the porosity was still acceptable for a scaffold in clinical applications. Furthermore, in BP30 the compressive strength increased to approximately 2 MPa. This result was compared with a previous report concerning PCL-coated calcium phosphate scaffolds that showed approximately 0.57 MPa of compressive strength. As illustrated in Fig. 2, infiltration of PCL into the microchannel pores, which formed due to the burning out of the sponge, greatly improved the mechanical properties compared to coating the surface with PCL.

The typical pore size distribution graphs are presented in Fig. 4: (a) BCP scaffold, and (b) BP30. The majority of the pores ranged from 80 µm to 1000 µm diameter for both
the BCP and BP30 scaffolds. Pores with diameters between 100 \( \mu \)m and 200 \( \mu \)m were most prevalent; however, the BCP scaffold contained some pores with diameters between 10 \( \mu \)m and 20 \( \mu \)m (Fig. 4(a)). This porous structure is attributed to the burning out of the PU sponge. Following the infiltration process, the number of pores in the 10 \( \mu \)m to 20 \( \mu \)m range significantly decreased. This suggests that PCL successfully infiltrated into the triangle-shaped hollow space.

Fig. 5 demonstrates representative stress-strain curves of the BCP scaffold with and without infiltration of PCL, with varying amounts of PMMA; (a) BCP scaffold, (b) BP20, (c) BP30 and (d) BP40. For the BCP scaffold, in which PCL had not infiltrated, compressive strength was approximately 1.3 MPa. After the scaffold was broken, it demonstrated nearly 0.2 MPa of compressive strength. However, following the infiltration process, the compressive strength increased to 1.8 MPa and 2 MPa for 20 vol\% (BP20) and 30 vol\% (BP40) PMMA, respectively. When 40 vol\% PMMA (BP40) was used, the strength decreased to approximately 1.1 MPa; this may be due to the increased PCL infiltration due to excess PMMA. Furthermore, after breakage the strength values were maintained between 0.7 MPa – 1 MPa due to the PCL.

Fig. 6 shows optical microscope and SEM images of BCP scaffolds after compressive strength measurements.

Without infiltration of PCL, the BCP scaffold was completely broken and became almost powdery, as shown in Fig. 6(a). However, following infiltration a compressed shape was formed due to the ductility of PCL, as shown in Fig. 6(b). From the enlarged SEM image (c), it is clearly observed that when the load was applied, PCL was pulled out from the broken struts of the scaffolds as indicated with the arrow. This fact could be a distinct advantage when the scaffold is implanted in the body, because the scaffold can retain its form even after being subjected to a compressive load.

The cytotoxicities of the BCP and PCL-BCP scaffolds were assessed by MTT assay. Osteoblast-like MG-63 cell viability shows no cytotoxicity due to the BCP scaffolds or the PCL-BCP scaffolds (Fig. 7). However, as the concentration of extract increased, cell viability of the PCL-infiltrated BCP scaffold was slightly lower than that of BCP scaffold.

The cell attachments on the specimens were observed by SEM. After seeding the MG-63 cells were incubated for 15, 30, or 60 minutes on the BCP and PCL-BCP scaffolds. The cell attachment on the PCL-BCP scaffold was similar to that on the BCP scaffold at each corresponding time point. However, at the initial stages (15 and 30 min) large numbers of filopodia formed in the BCP scaffold compared to the PCL-infiltrated BCP scaffold. Thus, at
60 minutes, as shown in Fig. 8(c) and (f), osteoblast-like MG-63 cell spreading was better on the BCP scaffolds than on the PCL-BCP scaffolds because of surface morphology and properties of PCL.24) The surface roughness and hydrophobic nature of the polymer affect cell attachment and growth.25) PCL is a hydrophobic polymer, and cell attachment is not as good as that for hydrophilic polymers. During the infiltration process, PCL smoothed the surface of the BCP scaffold. As a result of this process, cell attachment and cell spreading ability decreased slightly but not to a significant extent (Fig. 8). Conversely, from the viewpoint of increasing compressive strength, the infiltration process is important.

4. Conclusions

A novel method combining the sponge replica method and PCL infiltration was developed to increase mechanical properties of scaffolds. PCL was infiltrated into the hollow space, which was formed after the burning-out of the PU sponge, through micropores that formed after burning-out of PMMA. To optimize the microstructure, various amounts of PMMA were used in the BCP slurry. At 30 vol% PMMA, the hollow space was completely filled with PCL, and it exhibited approximately 2 MPa of compressive strength. Following infiltration of PCL, the porosity of BP30 slightly decreased to 86% from 88%, which was the porosity of the BCP scaffold. From a bioanalytical point of view, the BCP scaffolds and PCL-BCP scaffolds are biocompatible and exhibit no cytotoxicity. Although the BCP scaffolds provide better cell growth conditions than the PCL-BCP scaffolds, cell attachment and spreading ability on both samples was similar. These results indicate that the bio-polymer infiltration process is a promising method for augmenting properties of scaffolds produced by the sponge replica method for tissue engineering applications.

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