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Fabrication of hydroxyapatite-poly(ε-caprolactone) scaffolds by a combination of the extrusion and bi-axial lamination processes

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Abstract Hydroxyapatite (HA)/poly(ε-caprolactone) (PCL) composite scaffolds were fabricated using a combination of the extrusion and bi-axial lamination processes. Firstly, HA/PCL composites with various HA contents (0, 50, 60, 70 wt%) were prepared by mixing the HA powders and the molten PCL at 100 °C and then extruded through an orifice with dimensions of 600 × 600 μm to produce HA/PCL composite fibers. Isobutyl methacrylate (IBMA) polymer fiber was also prepared in a similar manner for use as a fugitive material. The 3-D scaffold was then produced by the bi-axial lamination of the HA/PCL and IBMA fibers, followed by solvent leaching to remove the IBMA. It was observed that the HA/PCL composites had a superior elastic modulus and biological properties, as compared to the pure PCL. The fabricated HA/PCL scaffold showed a controlled pore structure (porosity of ~49% and pore size of ~512 μm) and excellent welding between the HA/PCL fibers, as well as a high compressive strength of ~7.8 MPa.

Introduction

Porous biomaterials have attracted a great deal of attention for use as tissue engineering scaffolds, since their three-dimensional (3-D) open pore and biocompatible surfaces provide ideal conditions for cell growth and tissue differentiation [1–4]. Among these biomaterials, hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is regarded as one of the most promising materials, owing to its excellent resorbable and osteoconductive properties [5, 6]. Nevertheless, it has not been extensively utilized, because of its brittleness and insufficient toughness [7, 8].

Recently, much effort has been made to develop hybrid ceramic/polymer composites, in which bioactive ceramic particles are embedded in a biodegradable polymer matrix [9–13]. These hybrid composites were found to possess improved mechanical and biological properties, compared to those of the individual components. For example, poly(ε-caprolactone) (PCL)/apatite composites have been found to have good potential for use as hard tissue regeneratives, in that the PCL matrix protects them against non-brittle failure and the apatite enhances the cellular response [14, 15].

There are many manufacturing processes which can be employed for producing porous scaffolds [11–13, 16–18]. The solid freeform fabrication (SFF) approach is of particular interest, as it allows the creation of an object in a layer-by-layer building sequence, allowing for precise control over the pore configuration [4]. For example, fused deposition modeling (FDM), in which a thermoplastic filament is directly deposited through a fine orifice in a 3-D manner, is one of the most widely used methods [17, 18]. However, few attempts have been made to fabricate ceramic/polymer composite scaffolds having a relatively high ceramic content, presumably due to the limitation associated with the welding between the filaments [18].

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Therefore, in this study, we attempted to fabricate HA/PCL scaffolds with a high HA content and a controlled pore structure using a combination of the extrusion and bi-axial lamination processes. Firstly, the HA/PCL composite fiber and fugitive IBMA fiber were produced by the extrusion method. These two kinds of fibers were then aligned and bi-axially laminated by warm-pressing, ensuring excellent welding between the HA/PCL fibers. Porous HA/PCL scaffolds were obtained after removing the 3-D IBMA structure via the solvent leaching technique. The processability of the present method is discussed. The mechanical and biological properties of the porous scaffolds were evaluated, and their feasibility for use as bone scaffolds was addressed.

Materials and methods

A flow chart of the fabrication route using the combination of the extrusion and bi-axial lamination processes is shown in Fig. 1. Commercially available $Ca_{10}(PO_4)_6(OH)_2$ (HA: Alfa Aesar Co., Ward Hill, MA, USA) powder and poly(ε-caprolactone)(PCL; -$[(CH_2)_5COO]_n$-, Mw = 80,000, Sigma-Aldrich, USA) were used as the ceramic and biodegradable polymer materials, respectively. In addition, isobutyl methacrylate (IBMA; Rohm and Haas, PA, USA) was used as a fugitive material that could be removed by the solvent leaching technique.

Firstly, HA-PCL composites with various HA contents, ranging from 0 to 70 wt% in the composite, were prepared by mixing the HA powders and the molten PCL at 100 °C for 1 h using a high shear mixer (Jeongsung Inc, Seoul, Korea). Once compounded, the HA-PCL composite was extruded through an orifice with dimensions of 600 × 600 μm at 70 °C using a piston extruder (Jung-min Ind. Co., Seoul, Korea) (Fig. 1A). Similarly, IBMA fiber with dimensions of 600 × 600 μm was also prepared by the extrusion process operated at 110 °C.

The two types of fibers (HA-PCL and IBMA) were set out alternately and warm-pressed at 80 °C with an applied load of 10 MPa to produce aligned fiber sheets, in which the IBMA fibers were adjacent to the HA-PCL fibers (Fig. 1B). The sheets were then cut and bi-axially stacked into a 24 × 24 mm square mold, followed by warm-pressing at 90 °C with an applied load of 20 MPa to form a 3-dimensionally interconnected structure (Fig. 1C). Thereafter, the prepared samples

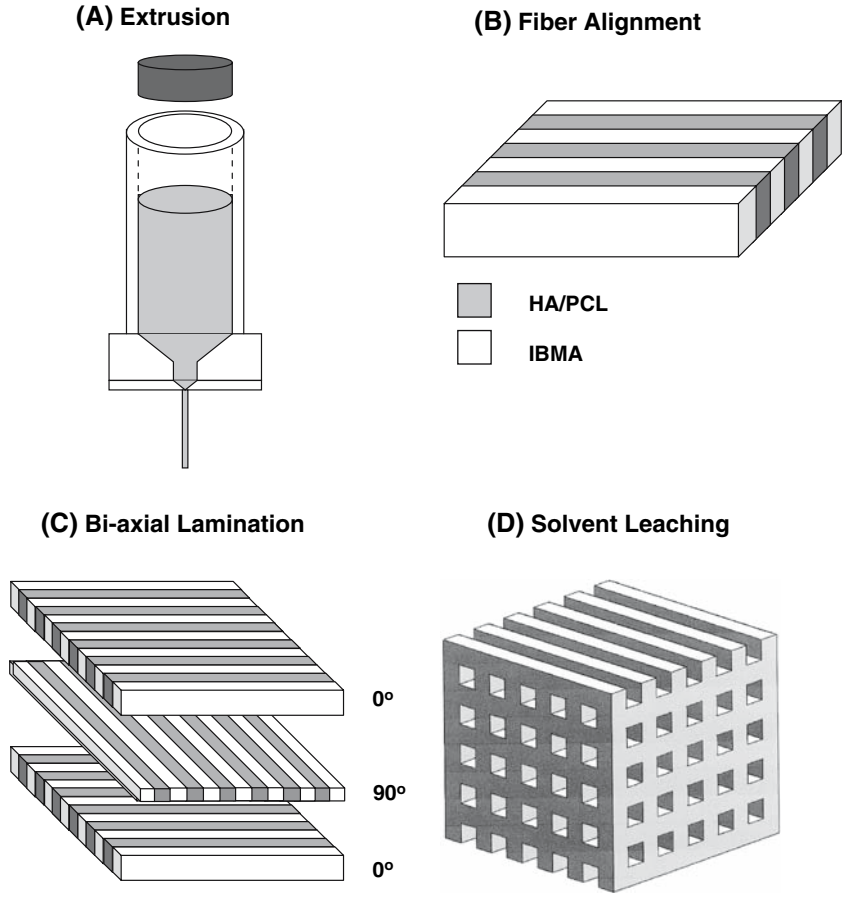

Fig. 1 Flow chart of the combination of the extrusion and bi-axial lamination processes used for the fabrication of the HA-PCL scaffolds: (**A**) extrusion to prepare the HA/PCL and IBMA polymers, (**B**) fiber alignment, (**C**) bi-axial lamination, and (**D**) solvent leaching to remove the IBMA used as a fugitive material

were immersed in a 25 wt% acetone: 75 wt% ethanol mixture with magnetic stirring for 24 h at room temperature to completely remove the IBMA fibers (Fig. 1D). Using this method, we were able to produce HA/PCL scaffolds with a tightly controlled pore structure.

The fabricated HA/PCL composites and scaffolds were characterized using several analytical tools. The macro- and microstructures of the samples were examined using scanning electron microscopy (SEM, JSM-6330, JEOL Technics, Tokyo, Japan). The mechanical properties, such as the tensile strengths of the PCL-HA fibers and the compressive strength of the PCL/HA scaffolds, were examined using an Instron universal testing machine (Instron 5565, Instron Corp., Canton, MA) equipped with a 5 kN load cell at a crosshead speed of 0.05 mm/min. During the tests, the stress versus strain responses were recorded.

The biological responses (proliferation and ALP activity) to the HA/PCL composites were evaluated using a well-established procedure for the assessment of biomaterials [19]. The human osteosarcoma (HOS) cell line was used after being cultured in flasks containing Dulbecco's modified Eagle's medium (DMEM, Life Technologies Inc., MD, USA) supplemented with 10% fetal bovine serum (FBS, Life Technologies Inc., MD, USA). The cells were then plated at a density of 1×10^4 cells/ml on a 24-well plate containing the HA/PCL composites, and cultured for 5 days in an incubator humidified with 5% CO_2/95% air at 37 °C. The morphologies of the proliferated cells on the HA/PCL composites were observed with SEM after fixation with glutaraldehyde (2.5%), dehydration with graded ethanols (70, 90, and 100%), and critical point drying in CO_2.

Results and discussion

For the fabrication of the HA/PCL scaffolds, we used a combination of the extrusion and bi-axial lamination processes, since this method employs a warm-pressing technique which ensures strong welding between the HA/PCL fibers. Firstly, the HA/PCL composites with various HA contents (0, 50, 60, 70 wt%) were prepared by mixing the HA powders and molten PCL polymer at 100 °C using a high shear-mixer, whereupon the HA powders were dispersed uniformly though the PCL matrix over the whole range of compositions studied (up to 70 wt%).

Tensile strength tests were performed on the HA/PCL composite fibers, which were the building units for producing the 3-D porous scaffold. The typical stress versus strain responses of the PCL and the HA/PCL composite with an HA content of 50 wt% during the tensile strength tests are shown in Figs. 2A and B, respectively. The pure PCL displayed a rubbery characteristic with extensive elongation at peak (~28%, 17 MPa), as shown in Fig. 2A. On the other hand, the HA/PCL composite exhibited brittle fracture with a tensile strength of 11 MPa and less elongation (~6%), as shown in Fig. 2B.

The tensile strength and elastic modulus of the HA/PCL composites were strongly influenced by the HA content, as shown in Fig. 3. The tensile strength of the fibers decreased with increasing HA content, while the elastic modulus was remarkably increased. Compared to the pure PCL, the HA/PCL composites showed a much higher elastic modulus, ranging from 201~244 MPa. These results indicate that the combination of the ductile polymer and hard ceramic filler

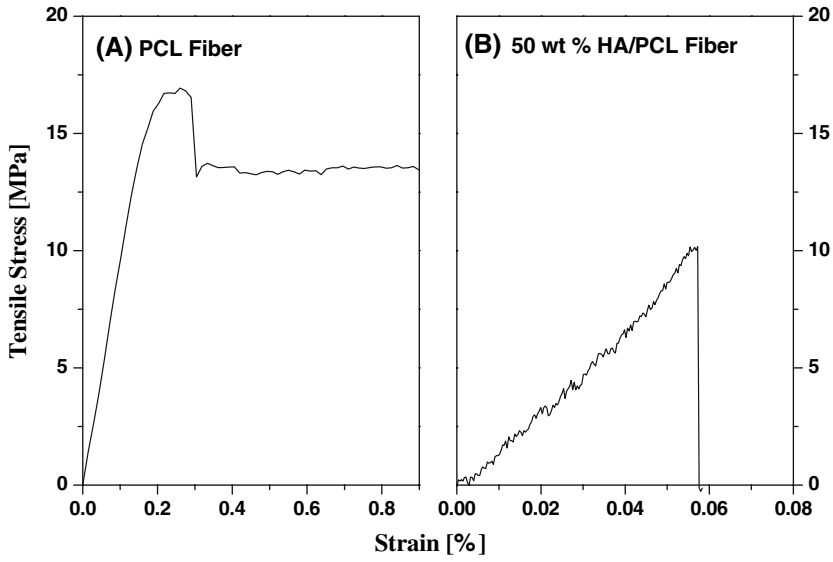

Fig. 2 Typical stress versus strain responses of (**A**) the PCL and (**B**) the HA-PCL composite with an HA content of 50 wt% during the tensile strength tests

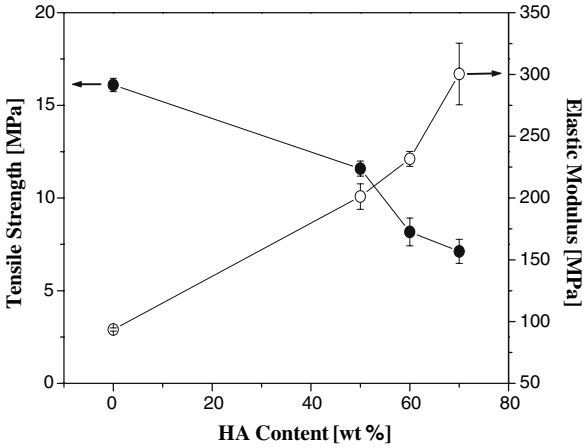

Fig. 3 Tensile strength and elastic modulus of the HA-PCL composites as a function of the HA content

can mitigate the rubbery or brittle characteristics of the scaffold derived from the pure PCL [17] and pure HA [7], respectively, thereby allowing the mechanical properties of the scaffold to be improved.

The observation of the failed surfaces of the HA/PCL composites with SEM revealed that the failure mostly resulted from the HA agglomerates, as shown in Figs. 4A–C. The sample with an HA content of 50 wt% still showed extensive deformation, owing to the trait of the ductile PCL polymer (Fig. 4A). With increasing HA content, the degree of deformation was mitigated (Figs. 4B and C). These observations were well matched with the strain to failure measurement obtained during the tensile strength test, which indicated that the strain to failure was decreased by increasing HA content.

The cellular responses to the HA-PCL composites were measured using human osteoblast-like cells. The typical cell growth morphology on the HA/PCL composites after culturing for 5 days are shown in Figs. 5A–D. Regardless of the HA content, the cells attached and grew well on the surfaces of the HA-PCL composites. However, the cells proliferated significantly higher on the HA-PCL composites than on the pure PCL, as summarized in Table 1, suggesting that the cell viability was improved on the HA-PCL composites. In particular, the proliferation level increased abruptly at an HA content of 60 wt% in the composite, presumably due to the variation in the distribution of HA within the PCL matrix.

Based on these investigations, we employed the 60 wt% HA/40 wt% PCL composite fiber as the building unit to produce the 3-D scaffold. IBMA fiber was also prepared using the same extrusion process for use as a fugitive material. The HA/PCL scaffold was built by bi-axially laminating the aligned fiber sheets, followed by solvent leaching to remove the IBMA, as shown in Fig. 6A. Controlled straight pore channels were formed with three-dimensional connectivity within the dense HA/PCL body, as shown in Fig. 6B. The measured porosity was 49 vol% and the pore size was ~512 ± 29 μm. Only a small variation was observed in the sizes of the pores and HA/PCL frameworks, such that the pore configuration in the scaffold was able to be precisely controlled. Furthermore, the interconnection

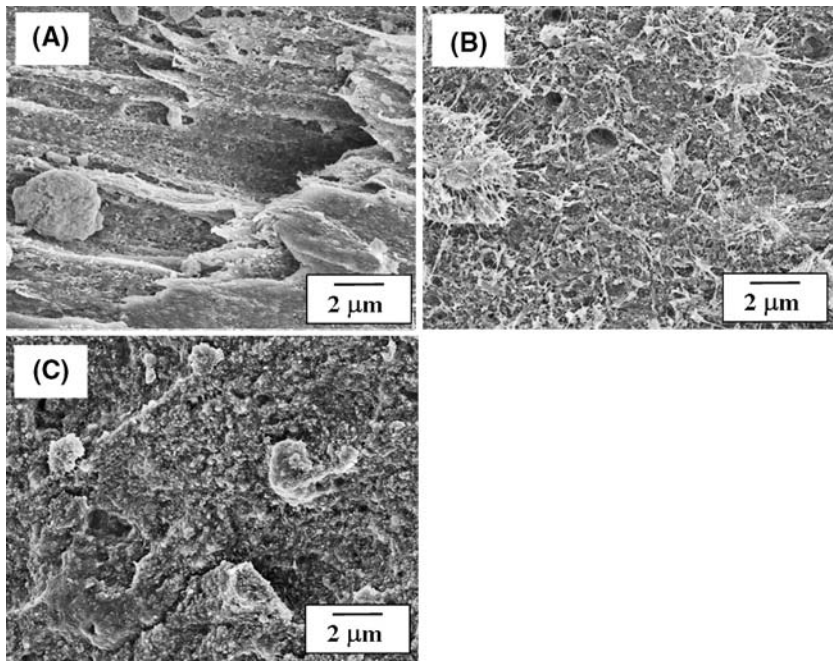

Fig. 4 Fracture surfaces of the HA-PCL composites after the tensile strength tests with various HA contents: (**A**) 50 wt%, (**B**) 60 wt%, and (**C**) 70 wt%

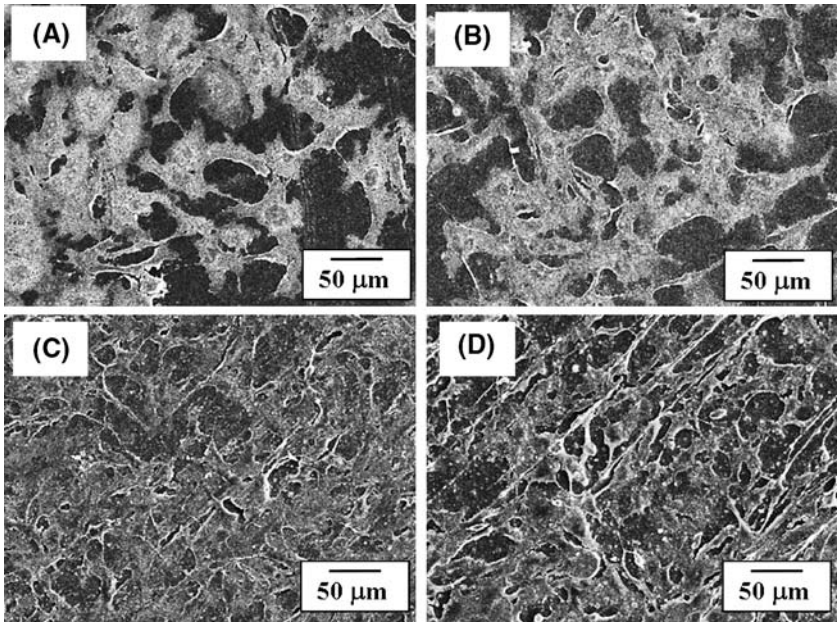

Fig. 5 Typical SEM micrographs showing the HOS cells on the HA-PCL composites with various HA contents: (**A**) 0 wt%, (**B**) 50 wt%, (**C**) 60 wt%, and (D) 70 wt%

Table 1 Cell proliferation on the HA-PCL composites after culturing for 5 days

HA content [wt%]	0	50	60	70
Cell number [$\times 10^3/cm^2$]	98 ± 6	107 ± 3.4	122 ± 3.4	121 ± 2.7

size was almost the same as the pore size, providing a framework for bone growth into the matrix of the implant, and thus allowing the prosthesis to be anchored to the surrounding bone.

The present method allowed for sufficient pressure to be applied during the bi-axial lamination process conducted at a temperature of 80 °C, which is higher than the melting temperature of the PCL polymer, so as to produce a monolithic composite material comprised of HA/PCL and IBMA fibers, which accordingly allowed strong welding between the HA/PCL fibers. Only a trace of the interface was visible, as shown in Fig. 6C. Using a filament with a high ceramic loading as a building unit in the FDM process often results in insufficiently strong welding between the filaments, which is liable to deteriorate the strength of the scaffold [20]. Even though we did not measure the adhesion strength, it is believed that the present method ensures stronger welding as compare to that produced by other processes [20, 21].

In order to verify the mechanical stability of the HA/PCL scaffold, compressive strength tests were

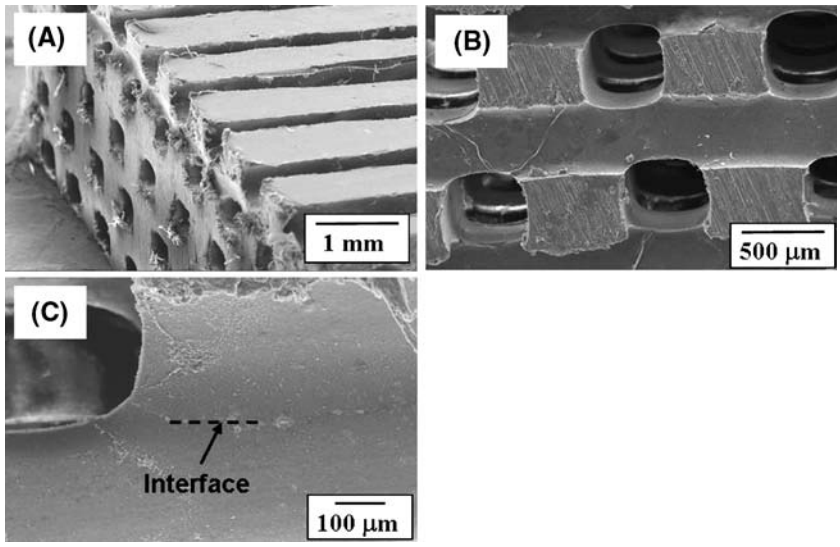

Fig. 6 SEM micrographs of the fabricated HA/PCL scaffold showing (**A**) the 3-D porous structure, (**B**) the tightly controlled pore architecture, and (**C**) good welding between the HA/PCL fibers

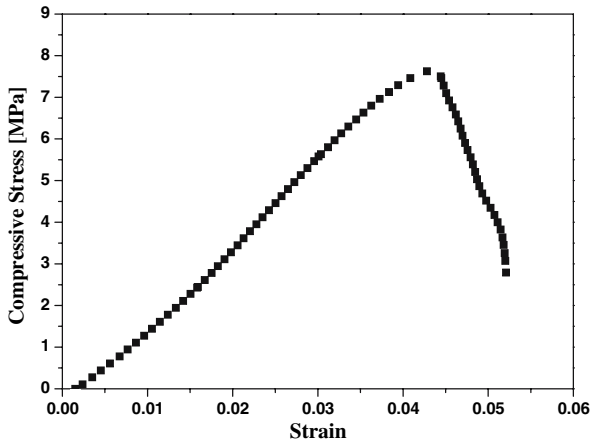

Fig. 7 Typical stress versus strain response under compression of the HA/PCL scaffold

conducted. The typical stress versus strain response is shown in Fig. 7. The compressive stress increased linearly with increasing elastic response and reached a maximum value of 7.6 MPa, before rapidly decreasing due to the failure of the scaffold. The average compressive strength of the scaffold was as high as 7.8 MPa, as summarized in Table 2. These results indicate that HA/PCL scaffolds could offer sufficiently high mechanical strength, as well as the controlled pore structures required for their use in implants [16].

The combination of the extrusion and bi-axial lamination processes was found to provide a facile approach to the fabrication of ceramic/polymer scaffolds with a well-controlled pore configuration. One of the most striking features of this method is that it allows sufficient pressure to be applied to ensure excellent welding between polymer fibers having a high ceramic loading. In addition, the porosity can be varied simply by adjusting the dimensions of the two types of fibers used for the scaffolding material and fugitive material, respectively, while keeping the pore size constant. Also, a variety of ceramic/polymer composites can be used as the scaffolding material. However, this approach may be limited to a simpler pore configuration and final structure, as compared to the SFF techniques that allow scaffolds to be built automatically [4].

Table 2 Summarized properties of the HA/PCL scaffold

HA content [wt%]	Porosity [%]	Pore size [μm]	Compressive strength [MPa]
HA/PCL scaffold	49	512 ± 29	7.8 ± 1.1

Conclusions

HA/PCL composite scaffolds were fabricated using a combination of the extrusion and bi-axial lamination processes. The initial HA/PCL composite fibers and fugitive IBMA fibers were aligned and bi-axially laminated, followed by the removal of the IBMA fiber in a solvent. Compared to the pure PCL fiber, the tensile strength of the HA/PCL fiber was slightly decreased by the addition of the HA, while the elastic modulus increased significantly. The human osteoblast cells proliferated to a significantly greater extent on the HA-PCL composites than on the pure PCL. The fabricated HA/PCL scaffold showed a tightly controlled pore structure with a porosity of 49% and a pore size of 512 ± 29 μm. In addition, excellent welding between the HA/PCL fibers was achieved by applying pressure during the bi-axial lamination process. The scaffold showed a high compressive strength of 7.8 ± 1.1 MPa.

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References

1. L. L. HENCH and J. M. POLAK, *Science* **295** (2002) 1014
2. N. O. ENGIN and A. C TAS, *J. Eur. Ceram. Soc.* **19** (1999) 2569
3. I. R. GIBSON and W. BONFIELD, *J. Biomed. Mater. Res.* **59** (2002) 697
4. K. F. LEONG, C. M. CHEAH and C. K. CHUA, *Biomaterials* **24** (2003) 2363
5. D. M. ROY and S. K. LINNEHAN, *Nature* **247** (1974) 220
6. C. LAVERNIA and J. M. SCHOENUNG, *Am. Ceram. Soc. Bull.* **70** (1991) 95
7. T. M. G. CHU, D. G. ORTON, S. J. HOLLISTER, S. E. FEINBERG and J. W. HALLORAN, *Biomaterials* **23** (2002) 1283
8. Y. H. KOH, H. W. KIM, H. E. KIM and J. W. HALLORAN, *J. Am. Ceram. Soc.* **86** (2003) 2027
9. C. V. M. RODRIGUES, P. SERRICELLA, A. B. R. LINHARES, R. M. GUERDES, R. BOROJEVIC, M. A. ROSSI, M. E. L. DUARTE and M. FARINA, *Biomaterials* **24** (2003) 4987
10. Y. SHIKINAMI and M. OKUNO, *Biomaterials* **20** (1999) 859
11. R. C. THOMSON, M. J. YASZEMSKI, J. M. POWERS and A. G. MIKOS, *Biomaterials* **19** (1998) 1935
12. M. C. AZEVEDO, R. L. REIS, B. M. CLAASE, D. W. GRIJPMA and J. FEIJEN, *J. Mater. Sci-Mater. Med.* **14** (2003) 103
13. G. CIAPETTI, L. AMBROSIO, L. SAVARINO, D. GRANCHI, E. CENNI, N. BALDINI, S. PAGANI, S. GUIZZARDI, F. CAUSA and A. GIUNTI, *Biomaterials* **24** (2003) 3815
14. H. W. KIM, J. C. KNOWLES and H. E. KIM, *Biomaterials* **25** (2004) 1279

15. K. G. MARRA, J. W. SZEM, P. N. KUMTA, P. A. DIMILLA and L. E. WEISS, *J. Biomed. Mater. Res.* **47** (1999) 324
16. D.W. HUTMACHER, *Biomaterials* **21** (2000) 2529
17. I. ZEIN, D. W. HUTMACHER, K. C. TAN and S. H. TEOH, *Biomaterials* **23** (2002) 1169
18. S. J. KALITA, S. BOSE, H. L. HOSICK and A. BANDYOPADHYAY, *Mat. Sci. Eng. C-Bio. S*. **23** (2003) 611
19. L. J. SUGGS and A. G. MIKOS, ''Synthetic biodegradable polymers for medical applications'', Physical Properties of Polymers Handbook, edited by J. E. Mark (American Institute of Physics, 1996), p. 615
20. I. GRIDA and J. R. G. EVANS, *J. Eur. Ceram. Soc*. **23** (2003) 629
21. Y. H. KOH, I. K. JUN and H. E. KIM, *Mater. Lett*. **60** (2006) 1184