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Macrochanneled poly (ε-caprolactone)/ hydroxyapatite scaffold by combination of bi-axial machining and lamination

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Abstract A combination of bi-axial machining and lamination was used to fabricate macrochanneled poly (ε-caprolactone) (PCL)/hydroxyapatite (HA) scaffolds. Thermoplastic PCL/HA sheets with a thickness of 1 mm, consisting of a 40 wt% PCL polymer and 60 wt% HA particles, were bi-axially machined. The thermoplastic PCL/HA exhibited an excellent surface finish with negligible tearing of the PCL polymer and pull-out of the HA particles. The bi-axially machined sheets were laminated with a solvent to give permanent bonding between the lamina. This novel process produced three-directionally connected macrochannels in the dense PCL/HA body. The macrochanneled PCL/HA scaffold exhibited excellent ductility and reasonably high strength. In addition, good cellular responses were observed due to the osteoconductive HA particles.

1. Introduction

Porous hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) has received a lot of attention for use as a bone graft material, because of its excellent resorbable and osteoconductive properties, which result from its crystallographic and chemical similarity with various calcified tissues in vertebrates [1, 2]. Nevertheless, it has not been extensively utilized, because of its brittleness and insufficient toughness [3, 4]. Therefore, more recently, efforts have been made to develop hybrid composites, in which ceramic particles are embedded in a biodegradable polymer matrix [5–9]. These hybrid composites offer improved biocompatibility and hard tissue integration, in that ceramic fillers allow for the increased initial flash spread of serum proteins, as compared to the more hydrophobic polymer surface. Among the available biodegradable polymers, poly(ε-caprolactone) (PCL) polymer is a particularly promising material, because it undergoes auto-catalyzed bulk hydrolysis without producing any toxic by-products and possesses rubbery characteristics [10, 11].

There are many manufacturing processes which can be employed for producing porous scaffolds, including the conventional techniques [7–9] and solid freeform fabrication (SFF) [12–16]. The SFF approach is of particular interest, as it allow the creation of a green ceramic object in a layer-by-layer building sequence, resulting in precise control over the pore configuration. Another simple approach involves the computer numeric controlled (CNC)-machining of green bodies, which is the most cost-effective way [17].

Therefore, in this study, we used a combination of bi-axial machining and lamination process to fabricate macrochanneled PCL/HA scaffolds with a controlled pore configuration, in order for it to be endowed with the properties of noncatastrophic failure and reasonable biodegradability. Firstly, a thermoplastic PCL/HA sheet, in which 60 wt% HA particles were dispersed in a PCL polymer matrix, was prepared by the extrusion process. The resulting sheets were then bi-axially machined and laminated using a solvent, creating 3-directionally connected macrochannels in a dense PCL/HA body. This novel process was evaluated in terms of the machinability of the PCL/HA composite, solvent lamination and control over the pore configuration. The stress versus strain responses under compression of the macrochanneled PCL/HA scaffolds were monitored.

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2. Materials and methods

2.1. Sample preparation

Schematic illustrations of experimental procedure to fabricate macrochanneled PCL/HA scaffolds by a combination of bi-axial machining and lamination are illustrated in Fig. 1. Commercially available poly(ε-caprolactone) pellets (PCL) [-[$(CH_2)_5COO]_n$-, MW = 80,000, Sigma-Aldrich, Milwaukee, WI] were used as the biopolymer matrix and hydroxyapatite (HA) powder [$Ca_{10}(PO_4)_6(OH)_2$, Alfa Aesar Co., Ward Hill, MA, Milwaukee, WI], calcined at 900°C for 1 h in air, was used as the ceramic filler. A thermoplastic PCL/HA composite with a thickness of 1 mm was prepared by blending 60 wt% HA with molten 40 wt% PCL polymer at 90°C for 1 h using a heated shear mixer (Jeongsung Inc., Seoul, Korea) (Fig. 1 (A)). Once compounded, the thermoplastic compound was warm-pressed at 100°C using a 24-mm cylindrical mold with an applied load of 30 MPa, and then extruded through a 24 × 1 mm orifice using a piston extruder (Jung-min Ind. Co., Seoul, Korea) (Fig. 1(B)). This produced a continuous thermoplastic PCL/HA sheet with a thickness of 1 mm.

The thermoplastic PCL/HA sheets were bi-axially machined using a mini-CNC machine (Modela, Roland DGA Corp., Japan) in accordance with a predetermined CAD design to create periodic channels (Fig. 1(C)). Milling was conducted at 6500 rpm using a carbide-endmill with a diameter of 500 μm. Firstly, a thermoplastic PCL/HA sheet with a thickness of 1 mm was machined to produce an array of macrochannels with dimensions of 12 × 0.5 × 0.5 mm, separated by PCL/HA frameworks with the same dimensions, resulting in a porosity of 50 vol%. Thereafter, the remaining half of the sheet was rotated at 90° and machined again in the same way, in order to produce macrochannels perpendicular to the previously built macrochannels. The resulting sheets were laminated by applying dichloromethane (DCM; Sigma-Aldrich, Milwaukee, WI) as a solvent to produce permanent bonding between the lamina (Fig. 1(D)).

2.2. Characterizations

This novel hybrid bi-axial machining and lamination process was evaluated in terms of the machinability of the thermoplastic PCL/HA compound and the solvent lamination, using optical microscopy (PMG3, Olympus, Tokyo, Japan) and scanning electron microscopy (SEM, JSM-6330, JEOL Technics, Tokyo, Japan). The stress versus strain responses under compression of the macrochannel PCL/HA scaffolds were monitored using an Instron (Instron 5565, Instron Corp., Canton, MA) at a crosshead speed of 0.05 mm/min. Ten samples were tested for reproducibility.

2.3. *In vitro* cellular assay

Also, the cellular responses (proliferation and ALP activity) to the scaffold were evaluated, following a well-established procedure for assessment of biomaterials [19]. The human ostosarcoma (HOS) cell line was used after being cultured in flasks containing Dulbecco's modified Eagle's medium (DMEM, Life Technologies Inc., MD, USA) supplemented

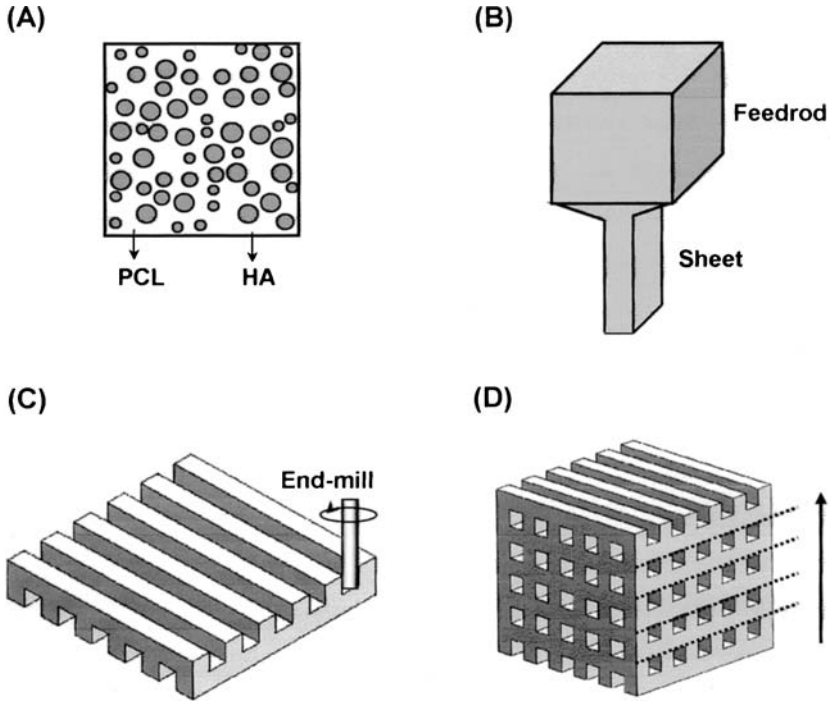

Fig. 1 Schematic illustrations showing the processing route used to fabricate macrochanneled PCL/HA scaffolds by a combination of bi-axial machining and lamination; (A) prepare thermoplastic PCL/HA composite, (B) extrude the feedrod into the sheet, (C) machine the sheet bi-axially, and (D) laminate the sheets to form a scaffold.

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 24-well plate, containing macrochanneled PCL/HA scaffolds, 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 macrochanneled PCL/HA scaffolds were observed with SEM after fixation glutaraldehyde (2.5%), dehydration with graded ethanol (70, 90, and 100%), and critical point drying in CO_2.

3. Results and discussion

A calcined powder was used to facilitate the blending process with molten PCL. After calcination, the specific surface area was notably reduced from 63 to 16 m^2/g due to the growth of particles by partial sintering, thereby improving shear-mixing behavior of the calcined HA powder with PCL polymer [18]. The 60 wt% of HA content was chosen so as to achieve high biodegradation rate and high elastic modulus. A typical SEM image of a PCL/HA composite is shown in Fig. 2, illustrating well dispersed HA particles in PCL matrix without noticeable agglomerations.

The typical stress versus strain responses of a PCL/HA composite in tensile test is shown in Fig. 3. For comparison, a pure PCL was also tested. A PCL-HA composite displayed a brittle fracture with a tensile strength of 8.2 MPa and less elongation (∼3%), while a pure PCL displayed a rubbery strain versus stress response with extensive elongation at peak (∼27%, 16.1 MPa). On the other hand, the elastic modulus of the PCL-HA composite was remarkably increased by a factor of 2.5 compared to that of the pure PCL. Such combination of ductile polymer and hard filler can mitigate the rubbery or brittle characteristics of the scaffold, as in the case of pure PCL and pure HA, respectively.

Figure 4(A) shows an as-machined thermoplastic PCL/HA sheet with dimensions of 12 × 12 × 1 mm

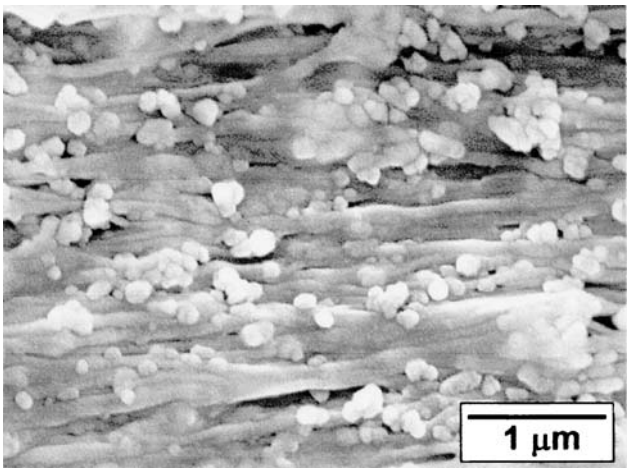

Fig. 2 SEM micrograph of a thermoplastic PCL-HA composite illustrating well dispersed HA particles in PCL matrix.

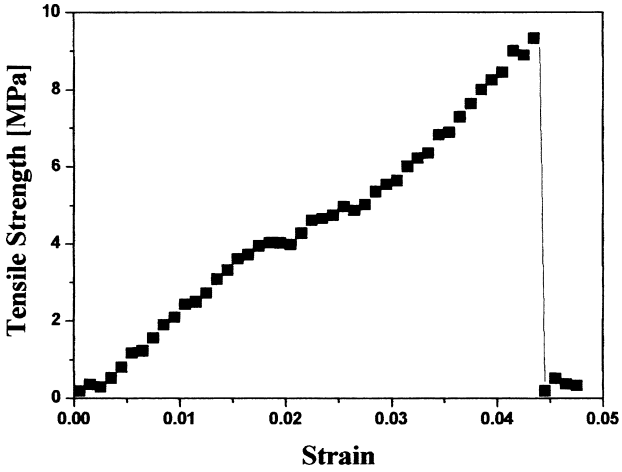

Fig. 3 The typical stress versus strain curves of a PCL-HA composite in tensile test.

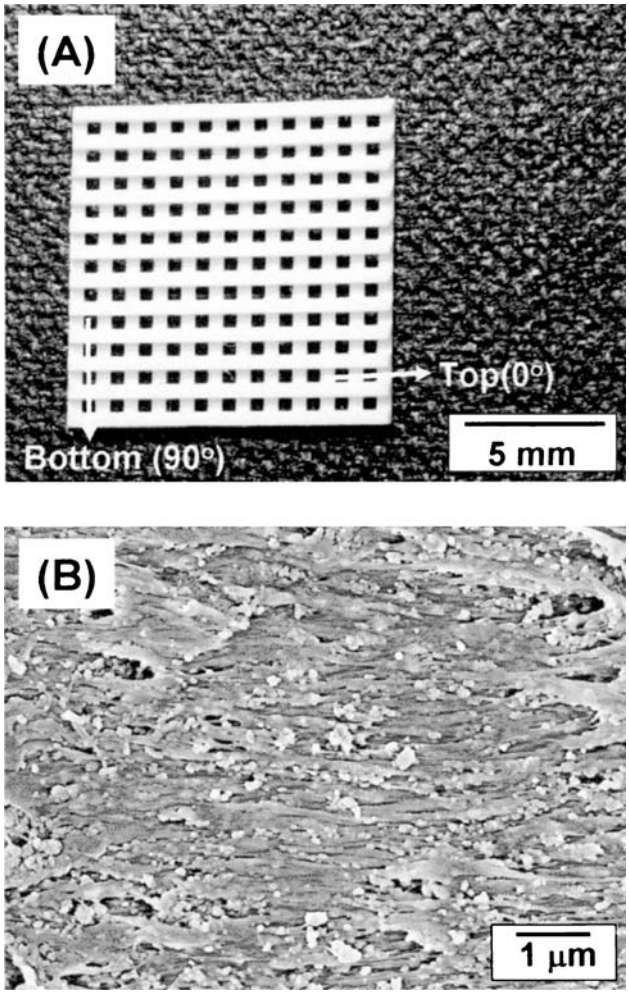

Fig. 4 (A) Optical micrograph illustrating a bi-axially machined thermoplastic PCL/HA sheet and (B) SEM micrograph illustrating a good surface finish after machining.

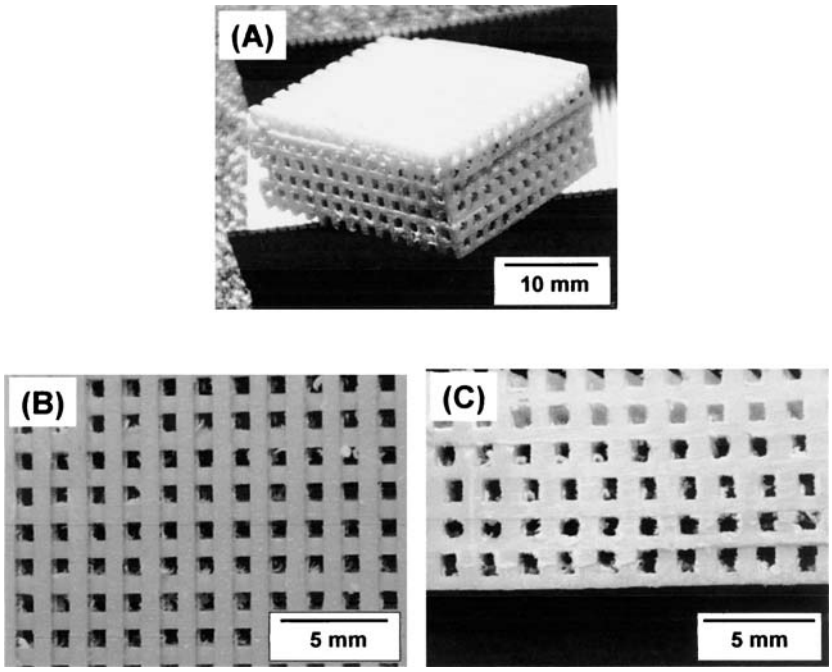

Fig. 5 Optical micrographs illustrating (A) a 3-directionally macrochanneled PCL-HA scaffold, (B) straight macrochannels in the plan view, and (C) periodic macrochannels in the side view.

after bi-axial machining. Periodic straight macrochannels with dimensions of 12 × 0.5 × 0.5 mm were formed in the top layer at 0° orientation and in the bottom layer at 90° orientation, creating 3-directionally connected macrochannels in the PCL/HA sheet. Also, an excellent surface finish was obtained without chipping and cracking (Fig. 4(A)). At a scale corresponding to the grain size, negligible tearing of the PCL polymer and slight grain pullout of the HA particles were observed, as shown in Fig. 4(B).

In the process of machining the thermoplastic compound, the surface roughness is strongly dependent on the manner in which the material is removed, as well as on its green strength. The PCL polymer is generally removed in a ductile manner during machining, because the local temperature in the machining zone is greater than the T_g(-60°C) of the PCL polymer [19]. Moreover, the incorporation of the hard HA particles into the PCL polymer can remarkably increase the stiffness of the PCL/HA composite, mitigating the generation of defects such as curled chips and tearing, which are often produced in the case where pure PCL is used. Therefore, the excellent machinability of the thermoplastic PCL/HA composite was attributed to the increased stiffness obtained by incorporating the HA particles, while still enabling the material to be removed in a ductile manner.

A macrochanneled PCL/HA scaffold was built by laminating the bi-axially machined sheets using a solvent, as shown in Fig. 5(A). Controlled straight macrochannels with square geometry were formed with three-directional connectivity within the dense PCL/HA body, as shown in Fig. 5(B) (C). The average porosity was 48 vol% and the average macrochannel size was ∼511 μm, as described in Table 1. A small variation was observed in the sizes of the macrochannels and PCL/HA frameworks, allowing the pore configuration in the scaffold to be precisely controlled. Furthermore, the interconnection size was almost the same as the macrochannel 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. Also, the locations of macrochannels were well controlled, offering the good periodicity.

In solvent welding, a solvent is applied which can temporarily dissolve the PCL polymer at room temperature. When this occurs, the polymer chains are free to move in the liquid and can entangle with other similarly dissolved chains in the other component. Given sufficient time, the solvent will permeate through the polymer and out into the environment, so that chains lose their mobility. This leaves a solid mass of permanently entangled polymer chains with the HA particles, offering excellent interfacial boding

Table 1 Summarized properties of the macrochanneled poly(ε-caprolactone) (PCL)/hydroxyapatite (HA) scaffold.

Samples	Macrochannel Fraction (vol%)	Macrochannel Size (m)	Compressive Strength (MPa)	Compressive Modulus (MPa)
Macrochanneled Scaffold	48 ± 2	511 ± 12	6.8 ± 1.2	220 ± 24

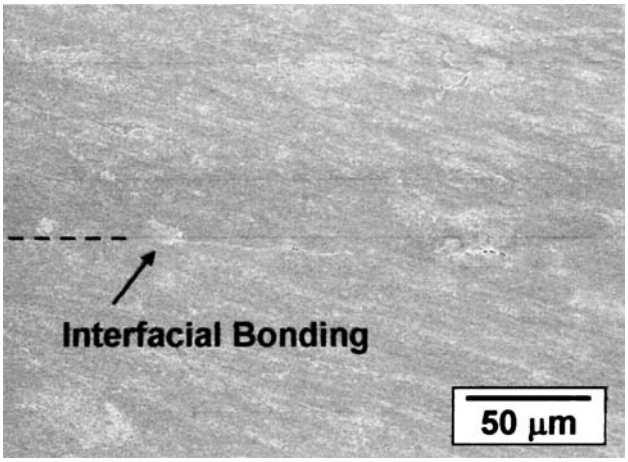

Fig. 6 SEM micrograph illustrating good interfacial bonding between the adjacent PCL/HA frameworks after solvent lamination.

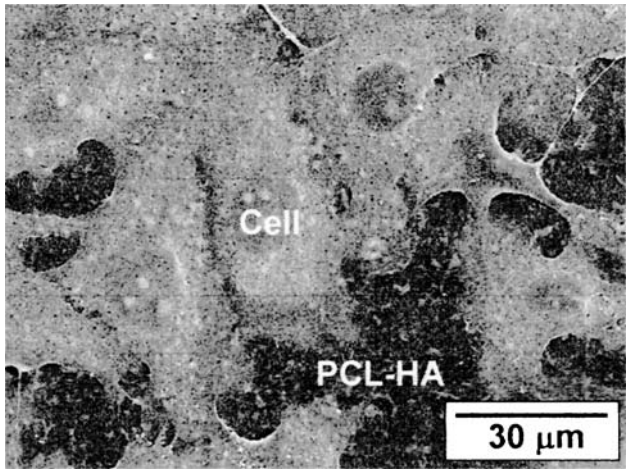

Fig. 8 SEM micrograph illustrating the cell attachment on the surface of the macrochanneled PCL-HA scaffold.

(Fig. 6). Only a trace of the interface marked by an arrow was visible.

The typical stress versus strain response under compression of the macrochanneled PCL/HA scaffold is shown in Fig. 7. The average compressive strength and modulus were 6.8 MPa and 220 MPa, respectively (Table 1), comparable to those of cancellous bone [20]. The compressive strength of the PCL-HS scaffold was much higher than that of the pure PCL scaffold having the similar porosity and geometry, due to reinforcement of hard HA particles in the PCL matrix [13]. Furthermore, noncatastrophic failure was observed, because of the rubbery characteristic of the PCL polymer. These results indicate that PCL/HA scaffolds could offer sufficiently high mechanical strength as well as the noncatastrophic failure required for their use in implants.

The cellular responses to the macrochanneled PCL/HA scaffold were assessed by the proliferation and differentiation behaviors of osteoblastic cells. Figure 8 shows the typical morphologies of the proliferated after culturing five days. At low magnification, lots of cells were observed to proliferate favorably on the surfaces of macrochanneled PCL/HA scaffold (not shown). At high magnification, the cell membranes were in intimate contact with and well flattened on the surface (Fig. 8), due to the presence of osteoconductive HA particles, as well as biocompatible PCL. These observation indicate that the macrochanneled PCL/HA scaffold is a promising candidate as a bone scaffold.

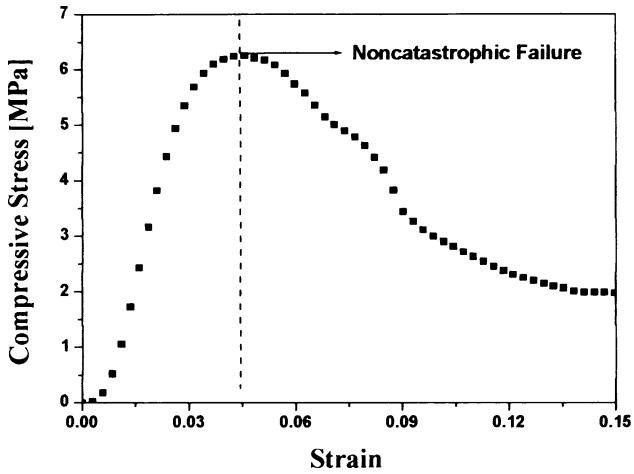

Fig. 7 Stress versus strain response under compression of the macrochanneled PCL-HA scaffold.

The combination of hybrid bi-axial machining and lamination process is found to provide a facile approach to fabricate macrochanneled polymer/ceramic scaffolds with a well-controlled pore configuration, by the simple green machining of a thermoplastic polymer/ceramic body. Furthermore, the porosity can be varied simply by adjusting the widths of macrochannels and frameworks, while maintaining the pore size at the same value. Also, various kinds of thermoplastic composite with any kind of complicated shape can be used. However, this approach may be limited to the simpler pore configuration and final structure, when compared to SFF techniques that build scaffolds automatically.

4. Conclusions

Three-directionally macrochanneled PCL/HA scaffolds were fabricated using a combination of bi-axial machining and the lamination process. In this way, thermoplastic PCL/A sheets with a thickness of 1 mm were bi-axially machined followed by solvent lamination. This novel process allowed the pore configuration to be precisely controlled. The fabricated macrochanneled PCL/HA scaffold exhibited a high compressive strength along with excellent ductility. In addition, good cellular responses were observed due to the osteoconductive HA particles. These results indicate that

the combination of bi-axial machining and lamination process provides a facile approach to fabricate macrochanneled polymer/ceramic scaffolds.

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References

1. M. ROY and S. K. LINNEHAN, Nature (London) **247** (1974) 220.
2. C. LAVERNIA and J. M. SCHOENUNG, *Am. Ceram. Soc. Bull.* **70** (1991) 95.
3. T. M. G. CHU, D. G. ORTON, S. J. HOLLISTER, S. E. FEINBERG and J. W. HALLORAN, *Biomaterials* **23** (2002) 1283.
4. Y. H. KOH, H. W. KIM, H. E. KIM and J. W. HALLORAN, *J. Am. Ceram. Soc.* **86** (2003) 2027.
5. 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.
6. Y. SHIKINAMI and M. OKUNO, *Biomaterials*, **20** (1999) 859.
7. R. C. THOMSON, M. J. YASZEMSKI, J. M. POWERS and A. G. MIKOS, *Biomaterials* **19** (1998) 1935.
8. M. C. AZEVEDO, R. L. REIS, B. M. CLAASE, D. W. GRIJPMA and J. FEIJEN, *J. Mater. Sci-Mater. M* **14** (2003) 103.
9. 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.
10. C. G. PITT, F. I. CHASALOW, Y. M. HIBIONADA, D. M. KLIMAS and A. SCHINDLER, *J. Appl. Polym. Sci.* **26** (1981) 3779.
11. S. A. ALI, S. P. ZHONG, P. J. DOHERTY and D. F. WILLIMAS, *Biomaterials* **14** (1993) 648.
12. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
13. I. ZEIN, D. W. HUTMACHER, K. C. TAN K. C and S. H. TEOH, *Biomaterials* **23** (2002) 1169.
14. S. J. KALITA, S. BOSE, H. L. HOSICK and A. BANDYOPADHYAY, *Mat. Sci. Eng. C-Bio. S.* **23** (2003) 611.
15. T. B. F. WOODFIELD, J. MALDA, J. DE WIJN, F. PETERS, J. RIESLE and C. A. VAN BLITTERSWIJK, *Biomaterials* **25** (2004) 4149.
16. B. M. WU, S. W. BORLAND, R. A. GIORDANO, L. G. CIMA, E. M. SACHS and M. J. CIMA, *J. Control. Release* **40** (1996) 77.
17. Y. H. KOH and J. W. HALLORAN, *J. Am. Ceram. Soc.* **87** (2004) 1575.
18. Y. -H. KOH, H. -W. KIM, H. -E. KIM and J. W. HALLORAN, *J. Am. Ceram. Soc.* **85** (2002) 2578.
19. L. J. SUGGS and A. G. MIKOS, "Synthetic Biodegradable Polymers for Medical Applications. In: Mark JE, editor. Physical Properties of Polymers Handbook" (New York: American Institute of Physics, 1996) p. 615.
20. P. H. F. NICHOLSON, X. G. CHENG, G. LOWET, S. BOONEN, M. W J. DAVIE, J. DEQUEKER and G. VAN der PERRE, *Medical Engineering & Physics* **19** (1997) 729.