J Mater Sci: Mater Med (2007) 18:1537–1542
DOI 10.1007/s10856-007-3057-y

Novel fabrication of a polymer scaffold with a dense bioactive ceramic coating layer

In-Kook Jun · Young-Hag Koh · Su-Hee Lee · Hyoun-Ee Kim

Received: 26 July 2005 / Accepted: 5 June 2006 / Published online: 17 April 2007

Abstract A novel method of coating a polymeric scaffold with a dense ceramic layer was developed. This method exploits the fact that only one of the two interlaced 3-D channels formed in a ceramic dual-scaffold can be infiltrated with a polymer. Firstly, a 3-D graphite network prepared by the rapid prototyping (RP) method was dip-coated with hydroxyapatite (HA) slurry, followed by heat-treatment at 1250°C for 3 h in air. This created an additional 3-D channel through the removal of the graphite network, while preserving the pre-existing 3-D channel. Thereafter, only one channel was infiltrated with a molten poly(ε-caprolactone) (PCL) polymer at 140°C for 12 h, producing a PCL scaffold with a dense, uniform HA coating layer. The sample showed high compressive strength with ductile behavior, due to the nature of the PCL polymer, and an excellent cellular response afforded by the bioactive HA coating layer. The results indicate that this novel technique provides a highly versatile method of coating various polymeric scaffolds with bioactive layers in order to endow them with advanced functionalities.

Introduction

Scaffolds for tissue engineering have attracted a great deal of attention, since their 3-D open pores and biocompatible surfaces are beneficial to the growth of cells and tissue differentiation [1–11]. They are constructed from a variety of materials, including polymers, ceramics, metals, and their composites. It is well known that their surface characteristics, such as their surface chemistry and topography, play a key role in inducing tissue regeneration [12].

To date, a number of biodegradable polymers have been developed for tissue engineering; however none of them are able to bond to bone or simulate the genes in bone cells. Therefore, much effort has been made to modify the surface of polymeric scaffolds in order to promote tissue regeneration. For example, the surface of polymeric materials can be directly modified by various chemical treatment methods, such as the surface hydrolysis of the PGA scaffold under strongly alkaline conditions [13]. Another approach is to coat the surface of the polymeric material with a bioactive apatite material that has good biocompatibility and osteoconductivity, using such techniques as an alternative soaking process or a biomimetic process [14–17]. These methods can significantly enhance the bone tissue regenerative properties of the polymeric material. However, it is believed that tailored coating layers beyond surface modification or apatite coating layer might further enhance their performances.

Therefore, in this study, we propose a new approach of coating polymeric scaffolds with a variety of bioactive materials, such as calcium phosphates and bioglasses that can control their tissue regenerative properties. This method exploits the fact that only one of the two interlaced 3-D channels formed in the ceramic dual-scaffolds recently developed by our research group can be infiltrated with a polymer (Jun et al. submitted). More specifically, the dual-scaffold consists of two interlaced 3-D channels completely separated by thin ceramic networks, as shown in Fig. 1(A). Thus, a polymeric scaffold with a dense bioactive ceramic coating layer can be achieved by infiltrating only channel II with a polymer, as shown in Fig. 1(B).

I.-K. Jun · Y.-H. Koh (✉) · S.-H. Lee · H.-E. Kim
School of Materials Science and Engineering, Seoul National University, Seoul 151-742, Korea
e-mail: kohyh@snu.ac.kr

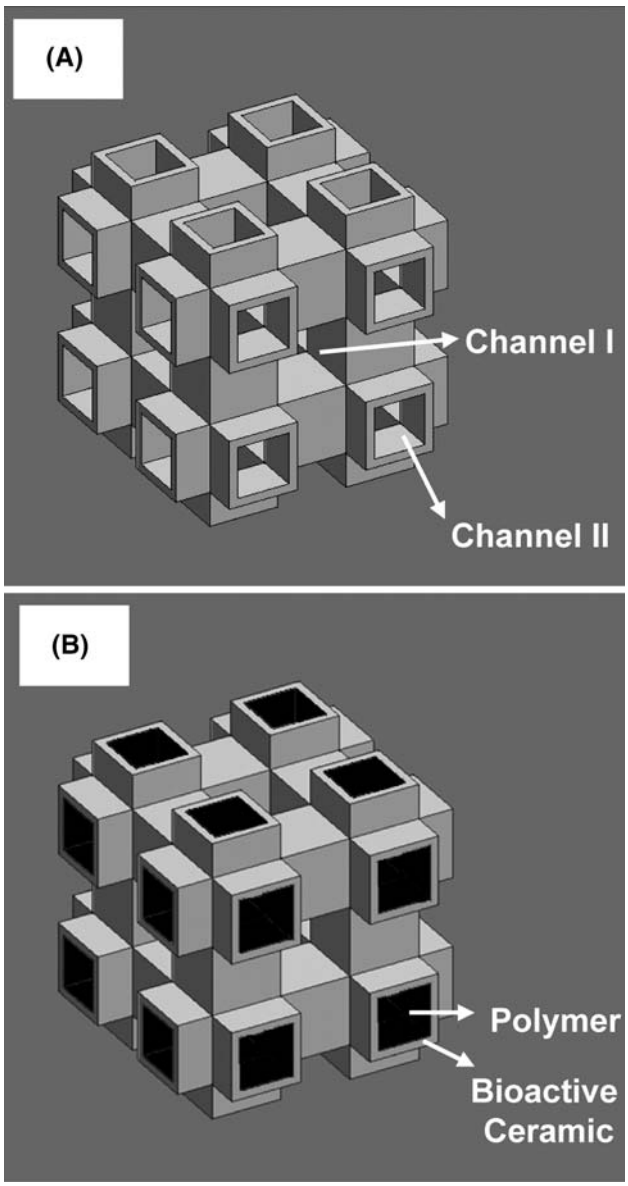

Fig. 1 Schematics illustrating (**A**) the ceramic dual-scaffold that has two interlaced 3-D channels (channels I and II) and (**B**) the polymer scaffold with the ceramic coating layer after infiltrating a polymer into channel II formed in the HA dual-scaffold

We selected a hydroxyapatite (HA: $Ca_{10}(PO_4)_6(OH)_2$) as the bioactive coating layer because of its good biocompatibility and osteoconductivity [18], and a poly(ε-caprolactone) (PCL) as the biodegradable polymer because of its good biocompatibility and mechanical properties [19, 20]. More specifically, the HA dual-scaffold was fabricated by dip-coating a 3-D graphite network by the rapid prototyping (RP) method followed by heat-treatment at 1250 °C for 3 h in air. This produced an additional 3-D channel surrounded by a dense HA network through the removal of the graphite network, while preserving the pre-existing channel. Thereafter, only one channel was infiltrated with molten PCL at 140 °C for 12 h. The processability of the fabricated sample was characterized using several analytical tools. Compressive strength tests were conducted for the samples, in order to evaluate their structural integrities. In addition, the cellular responses to the samples were characterized to evaluate the effect of the bioactive HA coating layer. The unique features of this novel approach are discussed in terms of its possible use in the field of tissue engineering involving the use of scaffolds with advanced functionalities.

Materials and methods

HA dual-scaffold fabrication

The fabrication of the HA dual-scaffold using a combination of the RP method and dip-coating process is described in detail in our previous report (Jun et al. submitted). Briefly, a 3-D graphite network was prepared by the RP method using a mini-CNC machine (Modela; Roland DGA Corp., Hamamatsu, Japan) in accordance with a predetermined CAD design, and then dip-coated with an HA slurry comprising HA powders (Alfa Aesar Co., Milwaukee, WI), polyvinylbutyl binder and triethyl phosphate (TEP; $(C_2H_5)_3PO_4$, Aldrich, USA) dispersant, followed by heat-treatment at 1250°C for 3 h in air. This produced an additional 3-D channel through the removal of the graphite network, while preserving the pre-existing channel.

PCL scaffold fabrication

In the case of the HA dual-scaffold, the outermost surface of the HA coated graphite network was ground to reveal the two interlaced channels, designed to direct the growth of cells. However, in this study, the outermost surface was preserved without any damage, in order to completely isolate the additional channel formed inside of the HA dual-scaffold. This allowed only one channel to be infiltrated with molten poly(ε-caprolactone) (PCL;-$[(CH_2)_5COO]_n$-, MW = 80,000, Sigma-Aldrich, Milwaukee, WI) at 140 °C for 12 h, producing a PCL scaffold with a dense bioactive HA coating layer.

Characterization

The processability of the fabricated PCL scaffold with its HA coating was characterized using several analytical tools. The porosities of the HA dual-scaffold and PCL scaffold were calculated by measuring their dimensions and weights. The macro-and microstructures of the samples were examined with optical microscopy (PMG3, Olympus, Tokyo, Japan) and scanning electron microscopy (SEM, JSM-6330, JEOL Technics, Tokyo, Japan).

For the compressive strength tests, the PCL scaffolds with the HA coating layer were loaded at a crosshead speed of 10 mm/min using a screw driven load frame (Instron 5565, Instron Corp., Canton, MA) equipped with a 5 kN load cell. In addition, the compressive strengths of the HA dual-scaffolds were measured. During the compressive strength tests, the stress and strain responses of the samples were monitored. More than five samples were tested to obtain the average value along with its standard deviation.

The cellular response to the PCL scaffold with the HA coating layer was evaluated using osteoblast-like MG63 cells. Osteoblast-like MG63 cells were 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 sample, and cultured for 1 day in an incubator humidified with 5% CO_2/95% air at 37 °C. The morphologies of the proliferated cells on the sample 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

Characterization of 3-D graphite network

A HA dual-scaffold was prepared by coating a 3-D graphite network with HA slurry followed by heat-treatment at 1250 °C for 3 h in air. In the case of the ceramic dual-scaffold that utilizes two channels for the growth of cells, the HA coating layer on the outermost surface of the graphite network needs to be ground to expose the two channels before applying the heat-treatment. However, in order to infiltrate only one channel in the dual-scaffold, it is preferable to preserve the outermost HA layer. The fabricated HA dual-scaffold exhibited good shape tolerance without any noticeable defects being generated, such as distortion or cracking, as shown in Fig. 2(A, B). In this case, only the pre-existing channel is visible, because the additional channel formed after the removal of the graphite network was completely covered with dense HA networks. In the plan view, periodic cylindrical channels are visible (Fig. 2B). The formation of this controlled pore structure was attributed to the unique advantages of the RP method. The two channels (channels I and II) are visible after the grinding of the outermost surface of the sample, as shown in Fig. 2(C). These two channels were completely separated by a HA network, even though some pores were visible in this HA network. This allowed only one channel, in this case channel II, to be filled with molten PCL polymer, while preserving channel I. The measured porosity and thickness of the HA network of the sample were ~92% and ~12 μm, respectively. If necessary, these values can be controlled by adjusting the number of replication cycles during the dip-coating process.

Characterization of 3-D PCL scaffold with HA coating layer

A PCL scaffold with a dense HA coating layer was fabricated by infiltrating PCL polymer into channel II at 150 °C

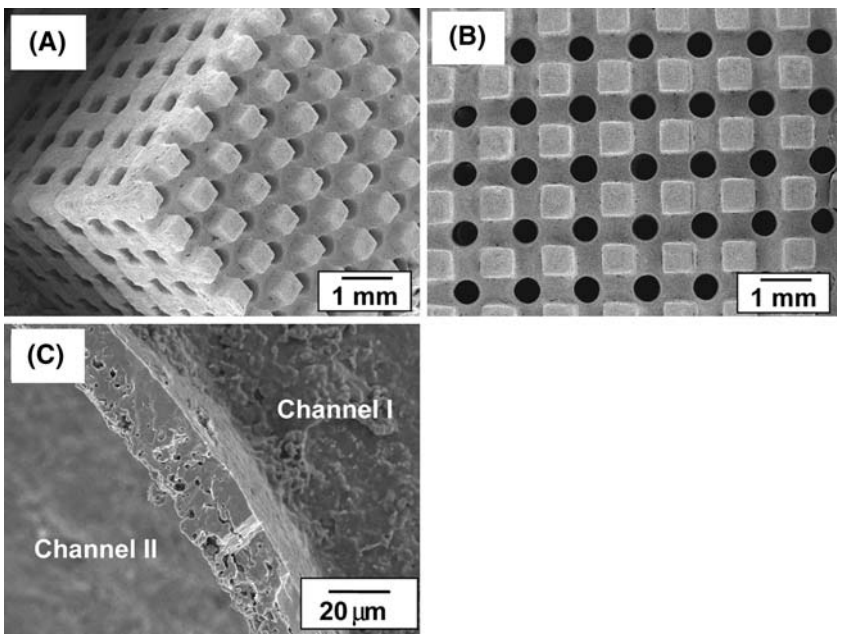

Fig. 2 SEM micrographs of the fabricated HA dual-scaffold showing (**A**) a 3-D image at low magnification, (**B**) periodic channels in the plan view, and (**C**) two channels separated by the HA network after grinding the outermost surface of the sample

for 12 h, as shown in Fig. 3(A, B). We empirically determined the temperature and time required for complete PCL infiltration, based on its degradation temperature of 350 °C and viscosity. The physiochemical properties of the PCL after infiltration did not change significantly. At low magnification, the PCL scaffold showed excellent shape tolerance without any noticeable defects (Fig. 3A). In the plan view, the construction of periodic channels in the PCL scaffold is visible (Fig. 3B). The average pore size for the square channel was ~511 μm. Little variation was observed in the sizes of the channels and the networks, due to the advantages of the RP method, which allows the pore structure of the dual-scaffold to be precisely controlled.

The formation of a dense HA coating layer on the PCL network is shown in Fig. 4(A). The HA coating layer was in good contact with the PCL network, without any delamination being observed. Good densification of the HA coating layer was observed on its free surface that was exposed to the channel, as shown in Fig. 4(B). These results imply that the PCL network maintains the applied load, while the bioactive HA layer effectively directs the growth of cells.

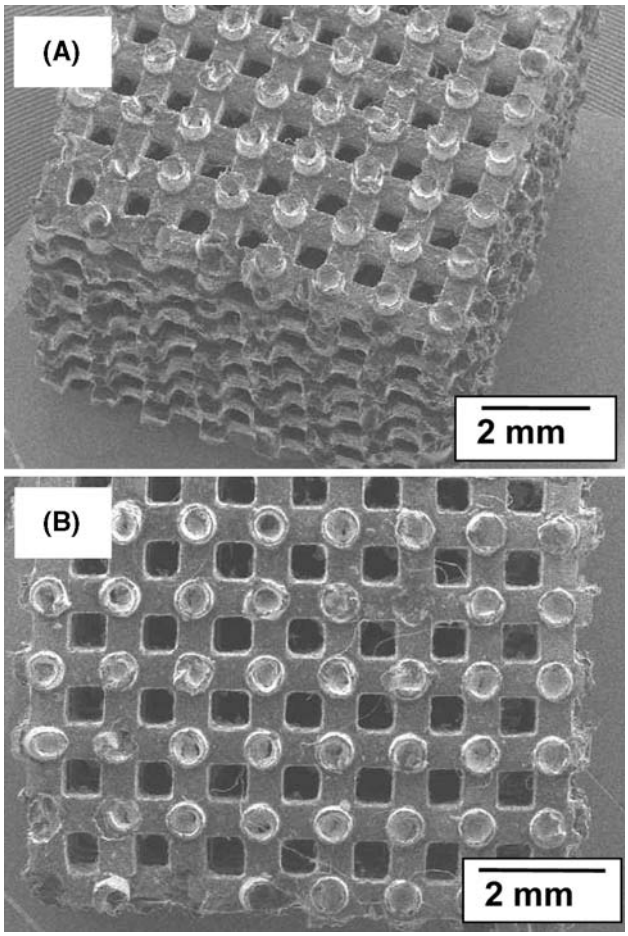

Fig. 3 SEM micrographs of the fabricated PCL scaffold with the HA coating layer after infiltrating a PCL polymer into the 3-D channel in the HA dual-scaffold; (**A**) 3-D image at low magnification and (**B**) periodic channels in the plan view

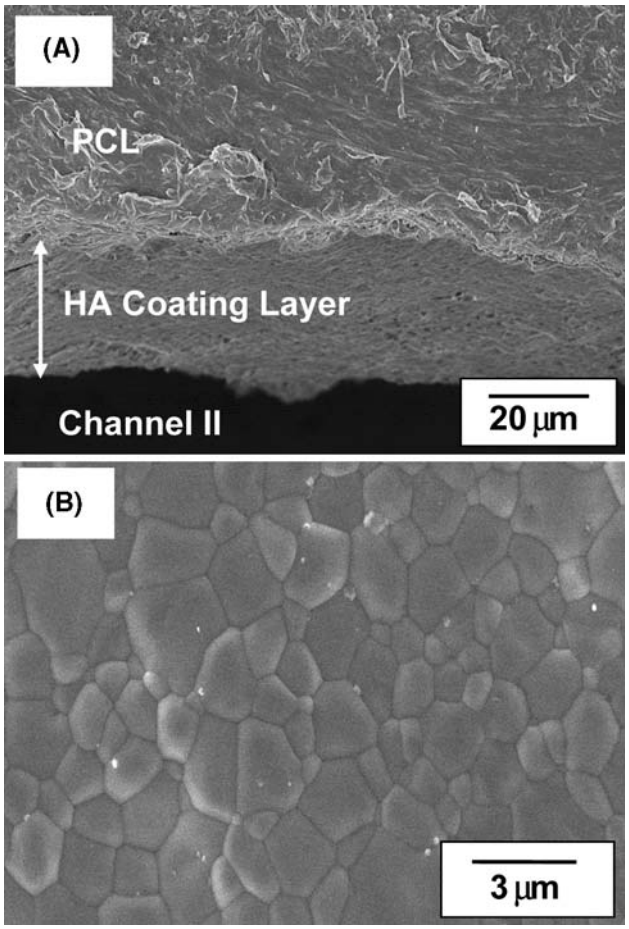

Fig. 4 SEM micrograph of the fabricated PCL scaffold illustrating (**A**) the HA coating layer on the PCL network and (**B**) the microstructural evolution on the surface of the HA coating layer

Compressive strength

In order to evaluate the mechanical properties of the PCL scaffold with the HA coating layer, compressive strength tests were conducted. The typical stress versus strain responses during the compression of the HA dual-scaffold and the PCL scaffold are shown in Fig. 5(A, B), respectively. The HA dual-scaffold showed typical brittle fracture, that is, the stresses increased linearly with the elastic response and then dropped rapidly due to fast fracture (Fig. 5A). The measured compressive strength was as high as 0.63 ± 0.08 MPa. On the other hand, the PCL scaffold exhibited the typical stress versus strain response of a ductile polymer, as shown in Fig. 5(B). In other words, the stress versus strain response of the sample shows initial elastic deformation (I), followed by a plateau of roughly

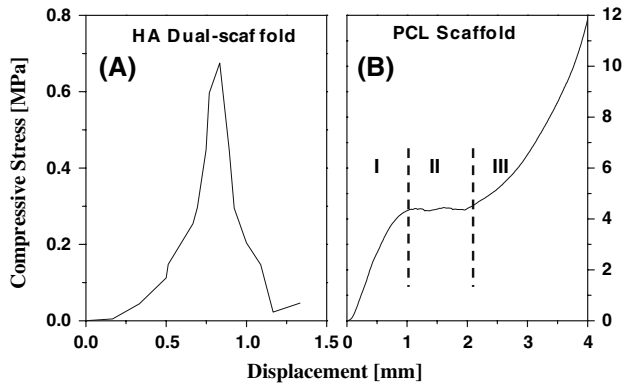

Fig. 5 Typical stress versus strain responses of (**A**) the HA dual-scaffold and (**B**) the PCL scaffold with an HA coating layer

constant stress (II), which ultimately gives way to a region of steeply rising stress (III). The measured yield stress was as high as 4.5 ± 0.2 MPa, which is comparable with that of other scaffolds fabricated by fused deposition modeling [21, 22]. This result indicates that the PCL polymer does not degrade during infiltration at 140 °C for 12 h.

Cellular responses

The cellular response to the PCL scaffold with the HA coating layer was evaluated using osteoblast-like MG63 cells, as shown in Fig. 6. As expected, enhanced cell attachment was observed on the surface of the HA coating layer. More specifically, numerous cells proliferated favorably on the surface of the HA coating, and the cell membranes were in intimate contact with the HA coating and well flattened on the surface, due to the osteoconductive nature of the HA material. These observations indicate that the HA coating on the PCL scaffold deposited using the approach described herein can effectively direct the growth of cells.

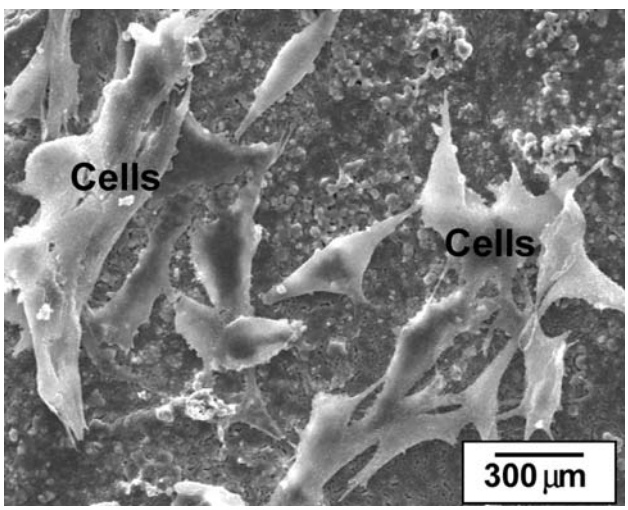

Fig. 6 SEM micrograph illustrating the cell attachments on the surfaces of the PCL scaffold with the bioactive HA coating layer

Dual-scaffold for tissue engineering

This paper describes a new way of coating a polymeric scaffold with a tailored bioactive ceramic layer using a ceramic dual-scaffold. As an exemplar, we fabricated a bioactive HA coated PCL scaffold, in which the polymer network served as the loading-bearing part, while the bioactive HA layer served to induce tissue regeneration. This approach allows a broad range of polymers and bioactive materials to be selected, beyond the PCL-HA system described in this study. For example, a sol-gel derived or melt-derived bioglass that is biocompatible, bioactive, osteoconductive, and even osteoproductive, can be used as the resorbable part [23, 24]. In addition, a variety of biodegradable polymers and polymer-ceramic composites can be used as the load-bearing part. This approach should find very useful applications in the field of tissue engineering, allowing for the formation of scaffolds with advanced properties.

Conclusion

A novel method of coating a polymeric scaffold with a dense ceramic layer for the purpose of tissue engineering was developed using a ceramic dual-scaffold. More specifically, a hydroxyapatite (HA) dual-scaffold was fabricated by dip-coating the surface of a 3-D graphite network prepared by the RP method, followed by heat-treatment at 1250 °C for 3 h in air. This produced two interlaced 3-D channels with dense HA networks, by removing the graphite network and preserving the pre-existing channel. Thereafter, only one channel formed in the HA dual-scaffold was infiltrated with a molten PCL polymer at 140 °C for 12 h, producing a PCL scaffold with a dense, uniform HA coating layer. The sample exhibited a high-yield strength (4.5 ± 0.2 MPa) with ductile behavior and excellent cellular response, owing to the bioactive HA coating layer. These results indicate that the approach described herein using a ceramic dual-scaffold is very versatile, allowing polymeric scaffolds to be coated with bioactive coating layers in order to endow them with advanced functionalities.

Acknowledgements This work was supported by a grant from the Korea Health 21 R&D Project, the ministry of Health & Welfare, Republic of Korea (02-PJ3-PG6EV11-0002).

References

1. L. G. GRIFFITH and G. NAUGHTON, *Science* **295** (2002) 1009
2. L. L. HENCH and J. M. POLAK, *Science* **295** (2002) 1014
3. V. MAQUET and R. JEROME, *Mater. Sci. Forum* **250** (1997) 15
4. J. X. LU, B. FLAUTRE, K. ANSELME, P. HARDOIUN, A. GALLUR, M. DESCHAMPS and B. THIERRY, *J. Mater. Sci.: Mater. Med.* **10** (1999) 111
5. K. F. LEONG, C. M. CHEAH and K. CHUA, *Biomaterials* **24** (2003) 2363
6. P. X. MA, R. Y. ZHANG, G. Z. XIAO and R. FRANCESCHI, *J. Biomed. Mater. Res.* **54** (2001) 284
7. L. L. HENCH, *J. Am. Ceram. Soc.* **81** (1998) 1705
8. N. PASSUTI, G. DACULSI, J. M. ROGEZ, S. MARTIN and J. V. BAINVEL, *Clin. Orthop. Relat. Res.* **248** (1989) 169
9. N. O. ENGIN and A. C. TAS, *J. Eur. Ceram. Soc.* **19** (1999) 2569
10. T. M. G. CHU, D. G. ORTON, S. J. HOLLISTER, S. E. FEINBERG and J. W. HALLORAN, *Biomaterials* **23** (2002) 1283
11. I. R. GIBSON and W. BONFIELD, *J. Biomed. Mater. Res.* **59** (2002) 697
12. B. D. BOYAN, T. W. HUMMERT, D. D. DEAN and Z. SCHWARTZ, *Biomaterials* **17** (1996) 137
13. J. M. GAO, L. NIKLASON and R. LANGER, *J. Biomed. Mater. Res.* **42** (1998) 417
14. T. TAGUCHI, A. KISHIDA and M. AKASHI, *Chem. Lett.* **8** (1998) 711
15. T. KOKUBO, H. KUSHITANI, S. SAKKA, T. KITSUGI and T. YAMAMURO, *J. Biomed. Mater. Res.* **24** (1990) 723
16. R. Y. ZHANG and P. X. MA, *J. Biomed. Mater. Res.* **45** (1999) 285
17. A. OYANE, M. UCHIDA, C. CHOONG, J. TRIFFITT, J. JONES and A. ITO, *Biomaterials* **26** (2005) 2407
18. I. R. GIBSON and W. BONFIELD, *J. Biomed. Mater. Res.* **59** (2002) 697
19. S. A. ALI, S. P. ZHONG, P. J. DOHERTY and D. F. WILLIMAS, *Biomaterials* **14** (1993) 648
20. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529
21. D. W. HUTMACHER, T. SCHANTZ, I. ZEIN, K. W. NG, S. H. TEOH and K. C. TAN, *J. Biomed. Mater. Res.* **55** (2001) 203
22. I. ZEIN, D. W. HUTMACHER, K. C. TAN and S. H. TEOH, *Biomaterials* **23** (2002) 1169
23. L. L. HENCH, *Curr. Opin. Sol. St.* **M2** (1997) 604
24. P. SEPULVEDA, J. R. JONES and L. L. HENCH, *J. Biomed. Mater. Res.* **61** (2002) 301