Bioresorbable and bioactive composite materials based on polylactide foams filled with and coated by Bioglass® particles for tissue engineering applications

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Poly(DL-lactide) (PDLLA) foams and bioactive glass (Bioglass®) particles were used to form bioresorbable and bioactive composite scaffolds for applications in bone tissue engineering. A thermally induced phase separation process was applied to prepare highly porous PDLLA foams filled with 10 wt % Bioglass® particles. Stable and homogeneous layers of Bioglass® particles on the surface of the PDLLA/Bioglass® composite foams as well as infiltration of Bioglass® particles throughout the porous network were achieved using a slurry-dipping technique. The quality of the bioactive glass coatings was reproducible in terms of thickness and microstructure. *In vitro* studies in simulated body fluid (SBF) were performed to study the formation of hydroxyapatite (HA) on the surface of the PDLLA/Bioglass® composites, as an indication of the bioactivity of the materials. Formation of the HA layer after immersion in SBF was confirmed by X-ray diffraction and Raman spectroscopy measurements. The rate of HA formation in Bioglass®-coated samples was higher than that observed in non-coated samples. SEM analysis showed that the HA layer thickness rapidly increased with increasing time in SBF in the Bioglass®-coated samples. The high bioactivity of the developed composites suggests that the materials are attractive for use as bioactive, resorbable scaffolds in bone tissue engineering.

1. Introduction

Tissue engineering presents an alternative approach to the repair of a damaged tissue, avoiding the need for a permanent implant made of an engineered material. The underlying principle involved is the regeneration of living tissue, where a loss or damage has occurred as a result of injury or disease [1]. A suitable temporary scaffold material exhibiting adequate mechanical and biological properties is required to enable tissue regeneration by exploiting the body's inherent repair mechanisms [2, 3]. Tissue engineering scaffolds required for bone tissue regeneration and repair need to satisfy a variety of criteria, which have been summarized by Hutmacher [4]. Ideally, a scaffold should have the following characteristics: (i) three dimensional and highly porous with an interconnected pore network for cell growth and flow transport of nutrients and metabolic waste, (ii) biocompatible and bioresorbable with a controllable degradation and resorption rate to match cell/tissue growth *in vitro* and/or *in vivo*, (iii) suitable surface chemistry for cell attachment, proliferation, and differentiation and (iv) mechanical properties to match those of the tissues at the site of implantation. It has also been reported, that a suitable scaffold for bone repair should bond to the host tissue without the formation of scar tissue, i.e. it should exhibit some degree of bioactivity and osteoconductivity [5]. Moreover a suitable scaffold should be made from versatile processing techniques that can produce irregular or complex shapes to match those of the defects in the tissue of the patient.

Numerous bioresorbable materials have been investigated as scaffolds for tissue engineering, including naturally occurring [3, 6] and synthetic polymers [7–11]. Many practical advantages arise when using synthetic scaffolds because precise engineering of material composition and micro and macrostructure is possible. This allows adequate control of scaffold

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properties, thus creating optimal conditions for cell survival, proliferation, and subsequent tissue formation [12]. Synthetic bioresorbable polymers have attracted increasing attention for their use as tissue engineering scaffolds in the last ten years, in particular polylactic acid (PLA), polyglycolic acid (PGA) or their copolymers [7–11]. These materials have already demonstrated promising results in clinical use, for example, as resorbable surgical sutures and meshes or in drug delivery systems [11, 13]. Some problems have been encountered regarding the use of these polymers in tissue engineering applications, however, which are related to the release of acidic degradation products leading to inflammatory responses [7, 8, 11–15]. Other limitation of biodegradable polymers is that they lack a bioactive function, i.e. in particular for bone tissue applications, they do not allow for bone tissue apposition or bonding on the polymer surface [16].

Certain ceramic materials, such as hydroxyapatite (HA), tricalcium phosphate (TCP) and selected compositions of silicate and phosphate glasses and glass-ceramics, for example, the commercially available Bioglass®, react with physiological fluids and form tenacious bonds to hard and soft tissues through cellular activity [5]. These materials are therefore known as ''bioactive'' [17].

For applications in bone regeneration and repair a scaffold material should exhibit both bioresorbability and bioactivity. The most efficient way to achieve this combination of properties is to combine resorbable polymers and bioactive materials in a composite scaffold. A further advantage of composites is that their micro and macrostructure can be engineered in such a way that their resorption rate after implantation matches the formation rate of new tissue. Moreover the addition of bioactive ceramic phases to degradable polymer (polyesters) may be used to counteract the acidic release degradation of these polymers by the pH buffering effect of the alkaline dissolution of the glass or ceramic [18, 19]. In addition, bioactive glass or ceramic phases embedded in polymer matrices may provide a means to increase the overall mechanical properties and structural integrity of the scaffold [18–20].

To the authors' knowledge, the first reports on the development of biodegradable polymer composites containing HA or bioactive glasses were published about 15 years ago [21–24]. Early composites developed by Japanese researchers were based on PLA containing up to 50 wt % HA and they were intended for biodegradable bone fillers [21]. Other early developments were based on addition of calcium metaphosphate fibers to PLA and related polymer matrices for bone fixation devices [22, 23]. Currently, various approaches to the development of bioresorbable and bioactive composites for tissue engineering applications are being investigated, including combinations of PLA, PGA and other resorbable polymers with HA, TCP or bioactive glasses and glass-ceramics in different scaffold architectures [13, 18–20, 24–45].

In the present work novel bioactive and bioresorbable composite materials were fabricated using macroporous poly(DL-lactide) (PDLLA) foams filled and coated with bioactive glass (Bioglass®) particles. The *in vitro* response of the composites in contact with simulated body fluid (SBF) was assessed. The materials are primarily intended as scaffolds for bone tissue engineering applications. This study extends related recent research on composite materials based on bioresorbable Bioglass®-coated surgical sutures [13, 35, 37] and meshes [38]. Moreover polymer foams related to those used in this study (but without Bioglass® additions) have been characterized recently, in particular for applications in repairing nerve sections, both in the peripheral and central nervous systems [46, 47].

2. Experimental

2.1. Materials

Purasorb® poly(DL-lactide) (PDLLA) with inherent viscosity of 1.52 dl/g was obtained from Purac Biochem (Goerinchen, The Netherlands). The bioactive material used was a melt-derived bioactive glass powder (Bioglass® grade 45S5, US Biomaterials Co., Alachua, FL, USA). The powder had a mean particle size $< 5\,\mu m$. The composition of the bioactive glass used was (in weight percentage): 45% SiO_2, 24.5% Na_2O, 24.5% CaO and 6% P_2O_5, which is the original composition of the first bioactive glass developed by Hench and coworkers in 1971 [48]. Dimethylcarbonate (DMC) of 99% purity was purchased from Sigma Aldrich. Polymers and solvents were used without further purification.

2.2. Fabrication of Bioglass®-filled foams

The preparation of the Bioglass®-filled poly(DL-lactide) (PDLLA) foams, referred to here as composite foams, followed a thermally induced phase separation process, also termed freeze-drying, which has been described in detail elsewhere [10]. The process was conveniently modified for the incorporation of Bioglass® particles, as described next. The polymer was dissolved in DMC to produce a polymer weight to solvent volume ratio of 5%. The mixture was stirred overnight to obtain a homogeneous polymer solution. A given amount of Bioglass® powder, calculated to result in a final proportion of 10 wt % of Bioglass® in the composite, was added into the polymer solution, resulting in a homogeneous PDLLA/Bioglass® mixture. The mixture was then transferred into a 150 mL lyophilization flask and sonicated for 15 min in order to improve the dispersion of the Bioglass® into the polymer solution. The flask was then rapidly immersed into liquid nitrogen and was maintained at $-196\,^{\circ}C$ for 2 h. The frozen mixture was then transferred into an ethylenglycol bath at $-10\,^{\circ}C$ and connected to a vacuum pump (10^{-2} Torr). The solvent was sublimated at $-10\,^{\circ}C$ for 48 h, and then at $0\,^{\circ}C$ for additional 48 h. The sample was completely dried at room temperature in a vacuum oven until reaching a constant weight.

2.3. Fabrication of Bioglass®-coated composite foams

Bioglass® coatings were made using a slurry-dipping technique, similar to that proposed recently to coat surgical sutures and polymeric meshes with Bioglass®

particles [37, 38]. The coating technique for foams similar to those used here has been optimized in previous studies [49]. Briefly, the technique involves preparation of a stable slurry with 42 wt % of Bioglass® particles in distilled and deionized water, which is continuously stirred magnetically. Pre-treatment of the PDLLA foams with ethanol was carried out following a procedure originally described by Mikos *et al*. [50]. It was shown in previous studies that better results in terms of degree of infiltration and coating homogeneity are achieved by the ethanol pre-treatment procedure because the high hydrophobicity of the PDLLA foam can be decreased [49]. In the present experiments, ethanol-treated PDLLA foams were lowered into the slurry using tweezers and left in immersion for 5 min. Following immersion, the samples were carefully withdrawn to avoid damage at a withdrawal velocity of $\sim 5\,\mathrm{cm\,s^{-1}}$. The samples were subsequently dried on glass plates at room temperature in a humid-controled atmosphere.

2.4. Characterization and *in vitro* studies

PDLLA/Bioglass® composite samples were characterized by using scanning electron microscopy (SEM) (Jeol JSM-T220), in order to assess the quality of the Bioglass® coating and degree of infiltration. Dried samples were carefully cut using a calliper, gold-coated and observed at an accelerating voltage of 20–25 kV.

In vitro studies were carried out using the simulated body fluid (SBF) composition and following standard procedures described by researchers at the University of Kyoto (Japan) [51]. Dry Bioglass®-coated and uncoated composite foams were immersed in 75 mL of SBF in clean conical flasks, which had previously been washed using hydrochloric acid and deionized water. The conical flasks containing the foams were placed inside an orbital shaker (New Brunswick Scientific, C24 Incubator Shaker), which rotated at 175 rpm at a controlled temperature of 37 °C. All samples were left in immersion in SBF for varying time periods of 7, 14, 21 and 28 days. The SBF was changed every seven days as cation concentration decreased during the course of *in vitro* studies, as a result of the changes in the chemistry of the samples, as discussed below. After immersion in SBF the microstructure of the samples surface was observed using SEM. X-ray diffraction (XRD) analysis (Philips PW 1700 Series, Cu Kα radiation, 40 kV) was used to verify whether or not crystalline HA had been formed on the surfaces of samples treated in SBF. With this aim, Raman spectroscopy also was conducted on SBF-treated samples. Raman spectroscopy has been shown to be extremely sensitive in detecting the formation of crystalline HA on bioactive surfaces [52]. These measurements were carried out using a Renishaw 1000 Raman microspectrometer. For excitation, a diode laser was used at 830 nm wavelength and 100 mW power. The exposure time was 10 s and 10 scans were accumulated in order to improve signal-to-noise ratio. The spectral resolution was $1\,\mathrm{cm^{-1}}$.

3. Results and discussion

3.1. Materials characterization

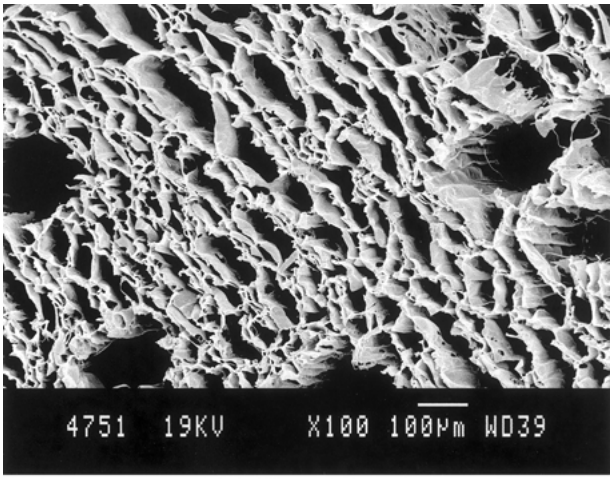
The morphology of a Bioglass®-filled PDLLA foam prepared by thermal-induced separation and subsequent freeze-drying is shown in the SEM micrographs in Fig. 1(a), (b). The foam exhibits a high porosity $> 90\%$, comprising two distinct pores sizes: (i) macropores of $\geq 100\,\mu\mathrm{m}$ average diameter, and (ii) micropores with an average diameter of 20–30 μm, which form an interconnected network. The tubular macropores were highly oriented as a result of the unidirectional cooling process. This kind of morphology is typically formed after freeze-drying of poly (α-hydroxyacids) [10], including polylactide and copolymers of ε-caprolactone with lactide or glycolide in solvents such as dioxane and dimethyl carbonate. To the authors' knowledge this is the first experimental study reporting on the fabrication of highly porous PDLLA foams containing additions of glass particles by the freeze-drying technique. It is interesting to point out that addition of 10 wt % Bioglass® particles of mean size $< 5\,\mu\mathrm{m}$ had no effects on the development of a well-defined tubular and interconnected porous structure in the foam. The foams produced here are thus comparable in terms of porosity and porous structure to those fabricated by the same technique but without addition of glass particles, which have been described elsewhere [53, 54].

The high-magnification SEM image (Fig. 1(b)) shows the agglomeration of Bioglass® particles on the surface of the fractured walls of the foams. The presence of Bioglass® on the pores boundaries is required to encourage bone-tissue growth into the porous scaffold, i.e. providing the scaffold osteoconductive properties, as discussed below.

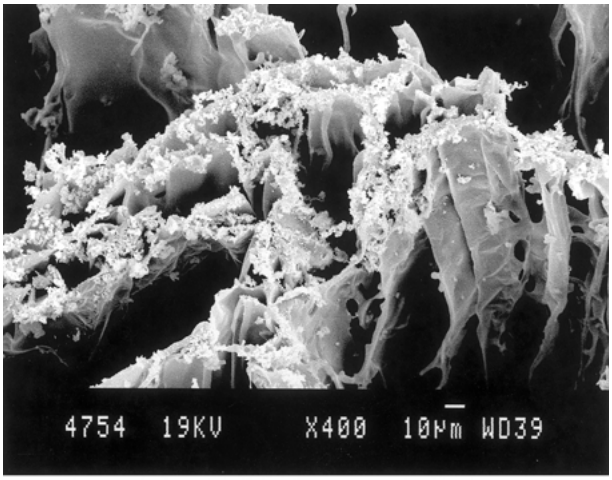
Fig. 2(a)–(c) show SEM micrographs of Bioglass®-coated composite foams produced by slurry-dipping at different magnifications. After drying, the particles were strongly adhered to the polymer substrate as neither peeling-off of Bioglass® particles nor macro-delamination of the Bioglass® coating were observed. At low magnifications (Fig. 2(a)), it can be observed that Bioglass® particles homogeneously cover the outer surfaces of the foam. Using an immersion time of 5 min, the Bioglass® particles have also deeply infiltrated the pores of the foam and glass particles are deposited along the internal surfaces of the porous structure, as Fig. 2(b) shows. The experimental technique also allowed for obtaining thick coatings characterized by a highly packing density of Bioglass® particles, as shown in Fig. 2(c). The microstructure of the Bioglass® coating was highly reproducible and its typical features, e.g. porosity, were the result of the narrow size distribution of the Bioglass® particles used.

3.2. *In vitro* studies in SBF

The response of Bioglass®-filled PDLLA foams without and with Bioglass® coating in contact with SBF was analyzed qualitatively using SEM, XRD analyses and Raman spectroscopy. The SEM micrographs in Fig. 3(a)–(c) show the gradual development of a HA layer on the surface of the coated composite samples as a result of

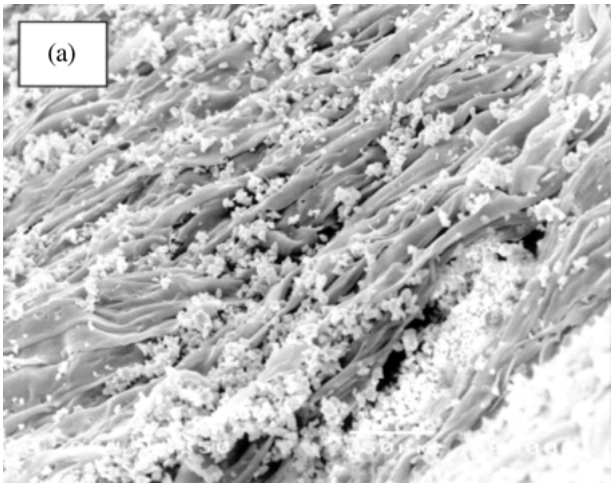


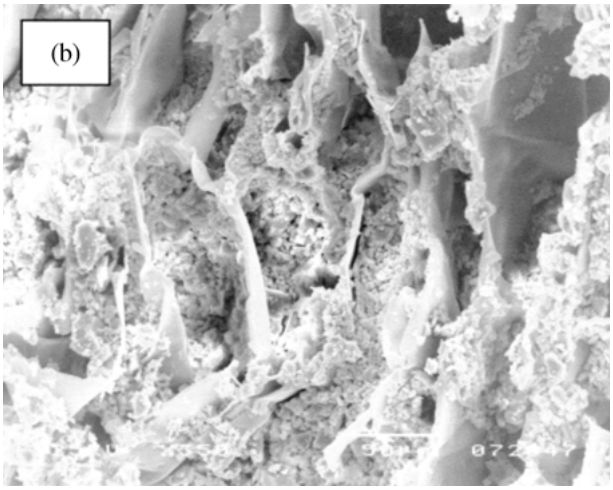
(a)



(b)

Figure 1 SEM micrographs showing typical cross sections of Bioglass®-filled PDLLA foams at low (a) and high (b) magnification.





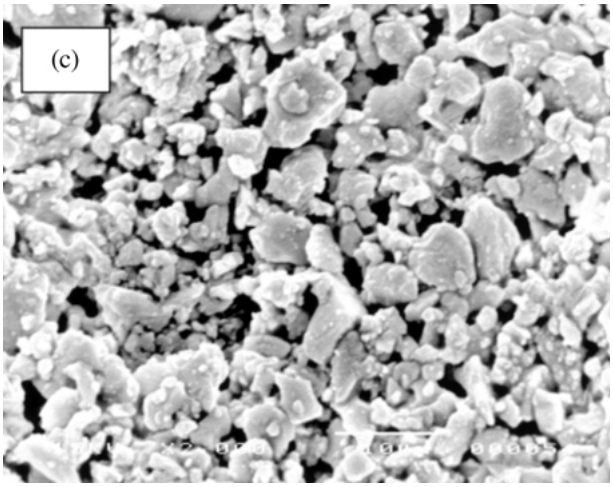


Figure 2 SEM micrographs showing the typical microstructure of PDLLA foams coated by Bioglass® particles produced by slurry-dipping. The homogeneous coating and efficient infiltration of Bioglass® particles can be observed at (a) low and (b) high magnifications. The micrograph in (c) shows the uniform Bioglass® coating microstructure.

increasing days of immersion in SBF, i.e. after 7, 21 and 28 days.

The results were compared to those of uncoated PDLLA composite foam samples, which were also immersed in SBF for varying times. Fig. 4(a), (b) show SEM micrographs of an uncoated composite sample after 28 days of immersion in SBF at two different magnifications. It is apparent that HA crystals have also formed as a result of contact of Bioglass® particles incorporated in the foams with SBF. However the HA phase remains discretely distributed on the surface of the foam and localized in the areas where Bioglass® particles have been in direct contact with SBF. No continuous layer formation, as in the case of the coated samples (see Fig. 3(c)), is observed. In fact, in non-coated foams, the stage of HA formation after 28 days immersion in SBF is similar to the stage reached by the coated samples after just 7 days, as a direct comparison of Figs. 3(a) and 4(a) reveals.

The crystallinity of the structure formed on the surface of the foams was investigated using XRD. For the Bioglass®-coated composite samples XRD analyses confirmed that crystalline HA formed on the composite surface after 7 days immersion in SBF, as Fig. 5 shows. Since the analyzed samples varied in size and morphology, a quantitative analysis revealing the amount of HA formed in dependence of immersion time in SBF could not be carried out. Nevertheless, the *in vitro* studies were successful in confirming the high ability for hydroxyapatite (HA) formation on the surfaces of Bioglass®-coated samples, which is a measure of the significant biaoctivity of the material. The formation of HA crystals on the surface of the Bioglass®-coated

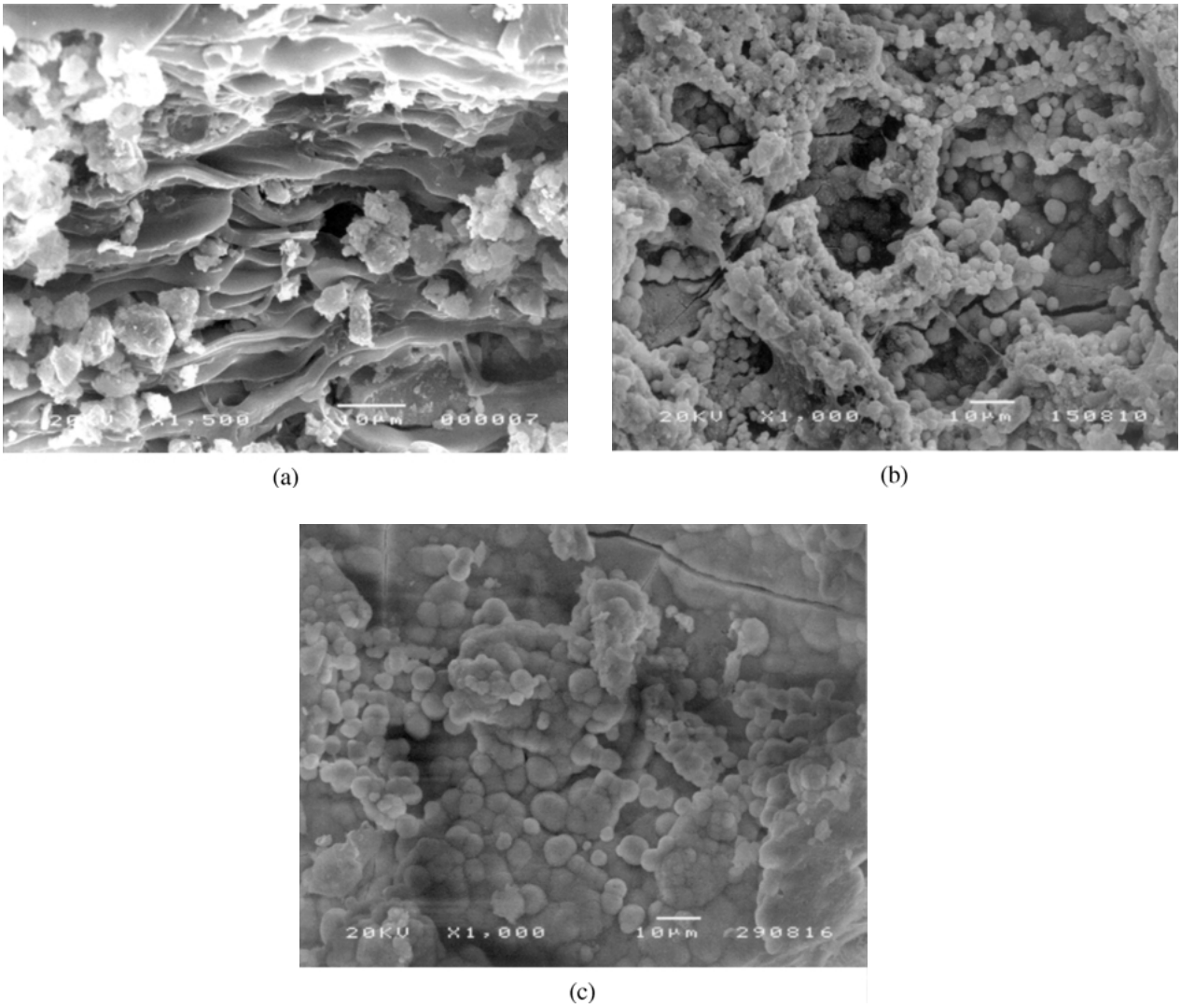


Figure 3 SEM micrographs showing surfaces of Bioglass®-coated PDLLA foams after degradation in contact with SBF for: (a) 7, (b) 21 and (c) 28 days. The micrographs reveal progressive formation of HA crystals and development of a surface HA layer.

samples after immersion in SBF was also confirmed by the Raman spectroscopy measurements. Fig. 6 presents the Raman spectra of Bioglass®-coated composite foams after having been soaked in SBF for 7 and 14 days. The spectrum for a sample in the as-fabricated condition is also shown.

The formation of the hydroxyapatite layer on the surface of the PDLLA foams is indicated by the strong peak at 964 cm^{-1}, which corresponds to the symmetric stretching vibration of P–O in PO_4^{3-} tetrahedra belonging to hydroxyapatite crystals [55, 56]. The small peak at 1075 cm^{-1} also belongs to PO_4^{3-} tetrahedra and

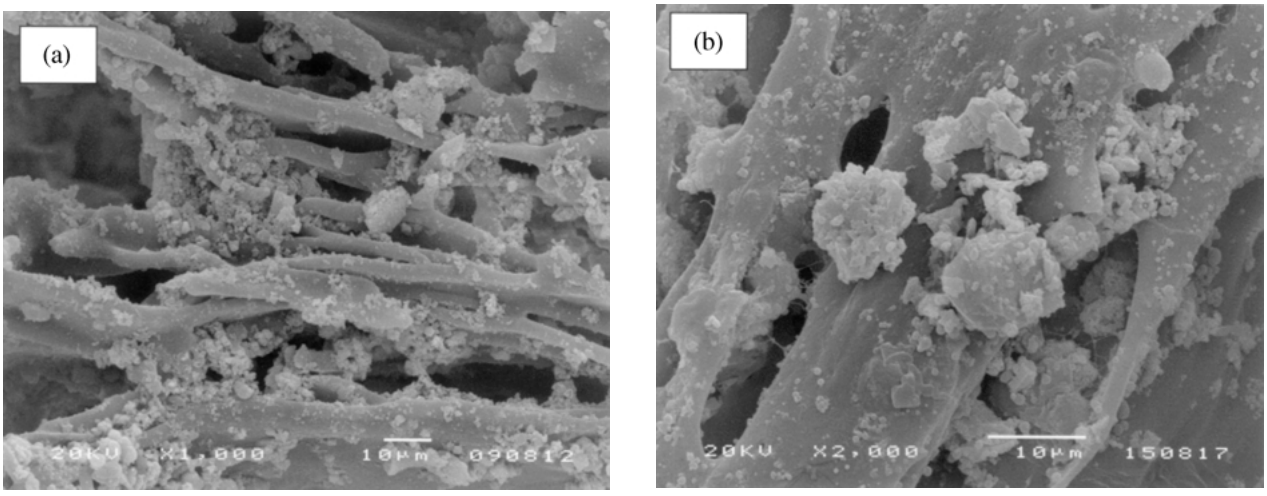


Figure 4 SEM micrographs of an uncoated PDLLA foam after 28 days immersion in SBF showing limited formation of HA particles, at low (a) and high (b) magnifications.

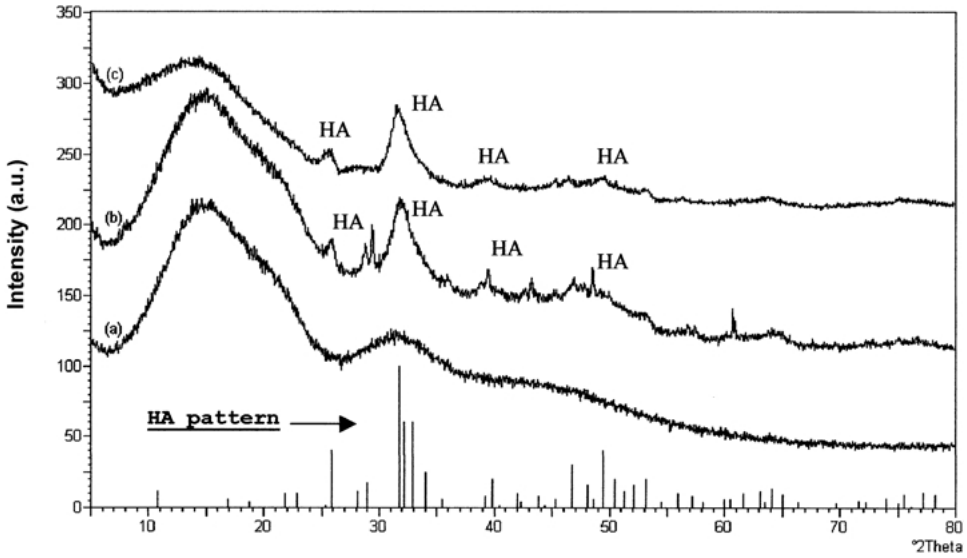


Figure 5 XRD patterns of Bioglass®-coated PDLLA foams: in (a) as-fabricated state, and after immersion in SBF for (b) 28 and (c) 7 days. The XRD patterns demonstrate the development of crystalline HA with increasing time of immersion in SBF. Note that the intensity is given in arbitrary units, i.e. the relative height of the peaks does not correlate with the relative amount of HA present in the different samples.

corresponds to P–O asymmetric stretching. A qualitative estimation of the growth in thickness of the hydroxyapatite layer can be obtained by considering the ratio of the 964 cm^{-1} peak and the CH_3 deformation peak at 1462 cm^{-1} or C=O stretching at 1780 cm^{-1}, which correspond to PDLLA. This ratio indicates that the hydroxyapatite layer becomes thicker when the PDLLA/Bioglass® composite sample is immersed in SBF for longer times, in agreement with the SEM results (Fig. 3).

As Fig. 3(a)–(c) show, the structure and morphology of the HA layer changed during immersion in SBF. Small HA crystals deposited after the first week of immersion, which developed into a continuous HA layer formed by coalescence of large crystals after the third and fourth week of immersion in SBF. The HA layer formed on the Bioglass®-coated composite surfaces was relatively thick, compared to other studies on bioactive and resorbable composites reported in the literature, where HA was used as the bioactive phase (see, for example, Ma *et al*. [27]). In the present composites, the thickness of the HA layer formed on Bioglass®-coated surfaces was increased manifold (of the order of 10 μm after 21 days immersion in SBF), an indication of the enhanced bioactivity of these materials. It is well-known that Bioglass® has a higher index of bioactivity than HA, which makes it a more suitable material for applications in bone reconstruction [17]. Moreover Bioglass®, being a class A bioactive material (as opposed to HA, which is class B), has shown strong bond also to soft tissues [57]. The application potential of the Bioglass®-coated composite foams fabricated here, therefore, could compass both hard and soft tissue regeneration and repair.

For the non-coated composites, however, the incorporation of Bioglass® particles into the polymer matrix resulted in a lesser degree of formation of HA. The behavior of these composites in SBF was comparable to that reported in the literature, where bioactive glasses in the form of particles or fibers have been incorporated into solid biodegradable polymer matrices [18, 42, 43]. It has been shown, for example, that incorporation of 10 wt % Bioglass® particles into a dense biodegradable starch-based polymer led to formation of apatite crystals only after 14 days immersion in SBF [42], in close agreement with the present results. Although the bioactivity of the PDLLA material seems to be little affected by addition of 10 wt % Bioglass® particles, the stiff particles used as filler can, however, be conveniently used to enhance the mechanical properties of the porous constructs. In a parallel study [58], the addition of higher concentration of Bioglass® particles (up to 40 wt %) is being investigated both in terms of its effect on bioactivity and mechanical properties of PDLLA foams.

Another motivation for fabricating composites both filled and coated by bioactive glass particles concerns the positive effect that the Bioglass® dissolution may have on the degradation of the polymer. This has not been yet quantitatively studied for the present composites. However, it can be speculated that the bioactive glass coating acts as a protective hydrolysis barrier affecting both the extent and rate of degradation of the polymer substrate [37, 38]. The rapid exchange of protons in water for alkali in the glass provide a pH buffering effect at the polymer surface, therefore acceleration of polymer degradation will not occur due to small pH changes during bioactive glass dissolution. Recent results on *in vivo* and *in vitro* degradation of bulk composites based on PDLLA containing bioactive glass particles seem to confirm this approach [18].

It is therefore envisaged that using Bioglass® both as filler and coating of porous resorbable polymer scaffolds will add to the possibilities of tailoring the mechanical properties and the rate of resorption of the composite scaffolds to that of the required application. Further logical steps to the optimization of scaffolds for tissue engineering should focus on tailoring the microstructure of the composite foams for determined applications,

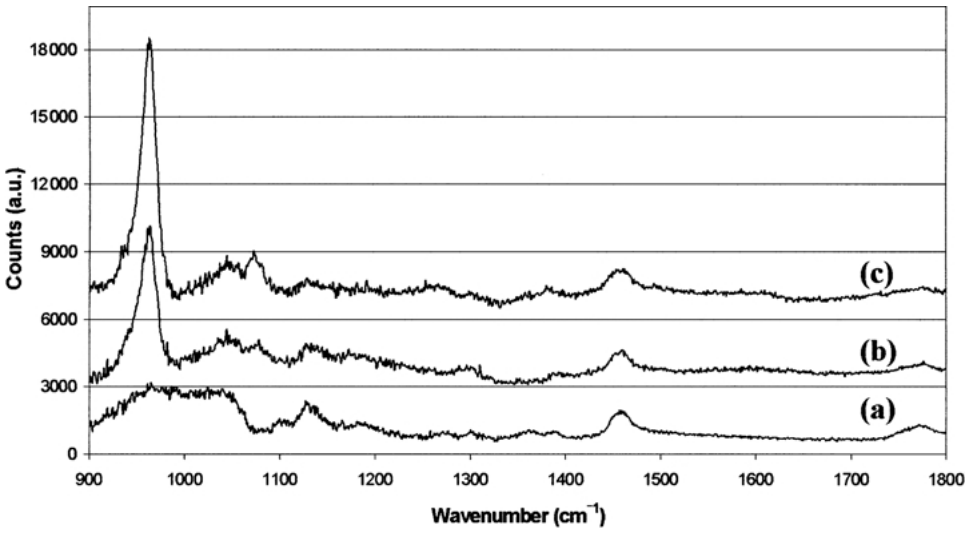


Figure 6 Raman spectra of Bioglass®-coated PDLLA foams: (a) before exposure, (b) after 7 days in SBF, (c) after 14 days. The strong peak at 964 cm^{-1} corresponds to the symmetric stretching vibration of P–O in PO_4^{3-} tetrahedra in HA crystals.

including the development of graded porosity and graded bioactive Bioglass® coatings, this being the focus of current developments.

4. Conclusions

Bioactive and bioresorbable composites were developed based on PDLLA foams and Bioglass® particles. The preparation of PDLLA foams filled with 10 wt% Bioglass® particles followed a thermally induced phase separation process. The foam porosity was $> 90\%$. An aqueous slurry-dipping technique was used to coat and infiltrate the PDLLA composite foams with Bioglass® particles. The technique led to stable and uniform glass coatings as well as adequate infiltration of Bioglass® into the porous network of the foams. The PDLLA foam/Bioglass® composites were tested by means of *in vitro* studies in simulated body fluid (SBF) to determine their potential as tissue engineering scaffolds. In Bioglass®-coated foams, it was found that HA particles formed after only 7 days in SBF, which further grew to form a thick and uniform HA layer on the scaffold with increasing time in SBF (up to 28 days investigated). In the non-coated (but Bioglass®-filled) samples the formation of HA was less pronounced and slower than in Bioglass®-coated samples. Bioglass®-coated samples, prepared by a combination of freeze-drying and slurry-dipping techniques, are therefore preferred in terms of bioactive potential. The present work may open a new way for the development of porous bioresorbable scaffolds of high bioactivity for tissue engineering. Future research should focus on tailoring novel microstructures for determined applications, including the development of composites with higher Bioglass® filler content, as well as foams of graded porosity and graded Bioglass® coating microstructures.

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References

1. C. A. VACANTI and J. P. VACANTI, in ''Principles of Tissue Engineering'', edited by R. P. Lanza, R. Langer and W. L. Chick (R. G. Landes Company, Texas, USA, 1997) p. 619.
2. M. KELLOMÄKI, H. NIIRANEN, K. PUUMANEN, N. ASHAMMAKHI, T. WARIS and P. TÖRMÄLÄ, *Biomaterials* **21** (2000) 2495.
3. E. PIŞKIN, *Mater. Sci. Forum* **250** (1997) 1.
4. D. W. HUTMACHER, *Biomaterials* **21** (2000) 2529.
5. J. R. JONES and L. L. HENCH, *Mater. Sci. Tech.* **17** (2001) 891.
6. Y. ZHANG and M. ZHANG, *J. Biomed. Mater. Res.* **55** (2001) 304.
7. V. MAQUET and R. JEROME, *Mater. Sci. Forum* **250** (1997) 15.
8. A. G. MIKOS and J. S. TEMENOFF, *EJB Electronic J. Biotechnol.* **2**(3) (2000) 1.
9. C. M. AGRAWAL, K. A. ATHANASIOU and J. D. HECKMAN, *Mater. Sci. Forum* **250** (1997) 115.
10. (a) C. SCHUGENS, V. MAQUET, C. GRANDFILS, R. JEROME and P. TEYSSIE, *Polymer* **37** (1996) 1027. (b) C. SCHUGENS, V. MAQUET, C. GRANDFILS, R. JEROME and P. TEYSSIE, *J. Biomed. Mater. Res.* **30** (1996) 449.
11. L. G. GRIFFITH, *Acta Mater.* **48** (2000) 263.
12. S. K. ASHIKU, M. A. RANDOLPH and C. A. VACANTI, *Mater. Sci. Forum* **250** (1997) 129.
13. A. STAMBOULIS and L. L. HENCH, *Key Eng. Mater.* **192–195** (2001) 729.
14. S. HURRELL and R. E. CAMERON, *J. Mater. Sci.: Mater. Med.* **12** (2001) 811.
15. C. M. AGRAWAL and R. B. RAY, *J. Biomed. Mater. Res.* **55** (2001) 141.
16. H. SCHLIEPHAKE, F. W. NEUKAM, D. HUTMACHER and J. BECKER, *J. Oral. Maxillofac. Surg.* **52** (1994) 57.
17. L. L. HENCH, *J. Amer. Ceram. Soc.* **81** (1998) 1705.

18. H. NIIRANEN, T. PYHALTO, P. ROKKANEN, T. PAATOLA and P. TORMALA, *Key Eng. Mater.* **192–195** (2001) 721.
19. W. LINHART, F. PETERS, W. LEHMANN, C. SCHWARZ, A. SCHILLING, M. AMLING, J. M. RUEGER and M. EPPLE, *J. Biomed. Mater. Res.* **54** (2001) 162.
20. C. C. P. M. VERHEYEN, J. R. DE WIJN, C. A. VAN BLITTERSWIJK, K. DE GROOT and P. M. ROZING, *ibid.* **27** (1993) 433.
21. S. HIGASHI, T. YAMAMURO, T. NAKAMURA, Y. IKADA, S. -H. HYON and K. JAMSHIDI, *Biomaterials* **7** (1986) 183.
22. R. L. DUNN, R. A. CASPER and B. S. KELLY, in ''Proc. 11th Annual Meeting of the Society for Biomaterials'', San Diego, CA, April 25–28 (1985) p. 213.
23. T. C. LIN, in ''Proc. 12th Annual Meeting of the Society for Biomaterials'', Minneapolis-St. Paul, May 29–June 1 (1986) p. 166.
24. J. E. DEVIN, M. A. ATTAWIA and C. T. LAURENCIN, *J. Biomater. Sci. Polym. Edn.* **7** (1996) 661.
25. C. DURUCAN and P. W. BROWN, *Adv. Eng. Mater.* **3** (2001) 227.
26. C. C. P. M. VERHEYEN, J. R. DE WIJN, C. A. VAN BLITTERSWIJK and K. DE GROOT, *J. Biomed. Mater. Res.* **26** (1992) 1277.
27. P. X. MA, R. ZHANG, G. XIAO and R. FRANCESCHI, *ibid.* **54** (2001) 284.
28. R. C. THOMSON, M. J. YASZEMSKI, J. M. POWERS and A. G. MIKOS, *Biomaterials* **18** (1998) 1935.
29. G. CHEN, T. USHIDA and T. TATEISHI, *Adv. Mater.* **12**(6) (2000) 455.
30. S. N. NAZHAT, M. KELLOMÄKI, P. TÖRMÄLÄ, K. E. TANNER and W. BONFIELD, *J. Biomed. Mater. Res. (Appl. Biomater.)* **58** (2001) 335.
31. N. IGNJATOVIĆ, K. DELIJIĆ, M. VUKCEVIĆ and D. USKOKOVIĆ, *Z. Metallkd.* **92**(2) (2001) 145.
32. X. DENG, J. HAO and M. YUAN, *J. Mater. Sci. Lett.* **20** (2001) 281.
33. H. NIIAREN and P. TORMALA, *J. Mater. Sci. Mater. Med.* **10** (1999) 707.
34. A. A. IGNATIUS, P. AUGAT and L. E. CLAES, *J. Biomater. Sci. Polym. Edn.* **12** (2001) 185.
35. A. R. BOCCACCINI, A. G. STAMBOULIS, J. A. ROETHER and L. L. HENCH, in ''Verbundwerkstoffe und Werkstoffverbunde'', edited by B. Wielage und G. Leonhardt (Wiley-VCH, Weinheim, 2001) p. 420.
36. B. D. BOYAN, G. NIEDERAUER, K. KIESWETTER, N. C. LEATHERBURY and D. C. GREENSPAN, US Pat. 5977 204, November 2 (1999).
37. A. STAMBOULIS, L. L. HENCH and A. R. BOCCACCINI, *J. Mater. Sci.: Mater. Med.* **13** (2002) 843.
38. A. STAMBOULIS, A. R. BOCCACCINI and L. L. HENCH, *Adv. Eng. Mater.* **4** (2002) 105.
39. F. SHARIFI-YAZDI, PhD Thesis, Imperial College, London (2001).
40. E. PIRHONEN, G. GRANDI and P. TORMALA, *Key Eng. Mater.* **192–195** (2001) 725.
41. M. KIKUCHI, Y. KOYAMA, K. TAKAKUDA, H. MIYAIRI and J. TANAKA, *ibid.* **192–195** (2001) 677.
42. I. B. LEONOR, R. A. SOUSA, A. M. CUNHA, Z. P. ZHONG, D. GREENSPAN and R. L. REIS, *ibid.* **192–195** (2001) 705.
43. T. PAATOLA, E. PIRHONEN and P. TORMALA, *ibid.* **192–195** (2001) 717.
44. A. A. IGNATIUS, O. BETZ, P. AUGAT and L. E. CLAES, *J. Biomed. Mater. Res. (Appl. Biomater.)* **58** (2001) 701.
45. M. WANG, J. WENG, J. NI, C. H. GOH and C. X. WANG, *Key Eng. Mater.* **192–195** (2001) 741.
46. V. MAQUET, D. MARTIN, B. MALGRANGE, R. FRANZEN, J. SCHOENEN, G. MOONEN and R. JÉRÔME, *J. Biomed. Mater. Res.* **52** (2000) 639.
47. V. MAQUET, D. MARTIN, F. SCHOLTES, J. SCHOENEN, G. MOONEN and R. JÉRÔME, *Biomaterials* **22** (2001) 1137.
48. L. L. HENCH, R. J. SPLINTER, W. C. ALLEN and T. K. GREENLEE, *J. Biomed. Mater. Res.* **2** (1971) 117.
49. J. A. ROETHER, A. R. BOCCACCINI, L. L. HENCH, V. MAQUET, S. GAUTIER and R. JEROME, *Biomaterials* **23** (2002) 3871.
50. A. G. MIKOS, M. L. LYMAN, L. E. FREED and R. LANGER, *Biomaterials* **15**(1) (1994) 55.
51. http://sung7.kuic.kyoto-u.ac.jp/others/SBF/SBF_E.html
52. I. REHMAN, L. L. HENCH, W. BONFIELD and R. SMITH, *Biomaterials* **15** (1994) 865.
53. V. MAQUET, S. BLACHER, R. PIRARD, J.-P. PIRARD and R. JÉRÔME, *Langmuir* **16** (2000) 10463.
54. S. BLACHER, V. MAQUET, R. PIRARD, J.-P. PIRARD and R. JÉRÔME, *Colloids and Surf. A: Physicochem. Eng. Aspects* **187–188** (2001) 375.
55. K. C. BLAKESLEE and R. A. CONDRATE, *J. Amer. Ceram. Soc.* **54** (1971) 559.
56. G. PENEL, G. LEROY, C. REY and E. BRES, *Calcif. Tissue Int.* **63** (1998) 475.
57. J. WILSON, G. H. PIGGOT, F. J. SCHOEN and L. L. HENCH, *J. Biomed. Mat. Res.* **15** (1981) 805.
58. V. MAQUET, A. R. BOCCACCINI, A. R. BOCCACCINI, L. PRAVATA, I. NOTINGHER, R. JÉRÔME, *J. Biomed. Mater. Research* (in press).

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