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Porous poly(α-hydroxyacid)/Bioglass® composite scaffolds for bone tissue engineering. I: preparation and in vitro characterisation

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Abstract

Highly porous composites scaffolds of poly-D,L-lactide (PDLLA) and poly(lactide-co-glycolide) (PLGA) containing different amounts (10, 25 and 50 wt%) of bioactive glass (45S5 Bioglass®) were prepared by thermally induced solid–liquid phase separation (TIPS) and subsequent solvent sublimation. The addition of increasing amounts of Bioglass® into the polymer foams decreased the pore volume. Conversely, the mechanical properties of the polymer materials were improved. The composites were incubated in phosphate buffer saline at 37°C to study the in vitro degradation of the polymer by measurement of water absorption, weight loss as well as changes in the average molecular weight of the polymer and in the pH of the incubation medium as a function of the incubation time. The addition of Bioglass® to polymer foams increased the water absorption and weight loss compared to neat polymer foams. However, the polymer molecular weight, determined by size exclusion chromatography, was found to decrease more rapidly and to a larger extent in absence of Bioglass®. The presence of the bioactive filler was therefore found to delay the degradation rate of the polymer as compared to the neat polymer foams. Formation of hydroxyapatite on the surface of composites, as an indication of their bioactivity, was recorded by EDXA, X-ray diffractometry and confirmed by Raman spectroscopy.
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1. Introduction

In the past few years, increasing attention has been paid to composites made of polymers and ceramics for application in tissue engineering [1–3]. Composite scaffolds may prove necessary for reconstruction of multi-tissue organs, tissues interfaces, and structural tissue including bone, cartilage, tendons, ligaments and muscles. Ceramics including dense and porous hydroxyapatite (HA), tricalcium phosphate (TCP) ceramics and bioactive glasses and glass–ceramics have been combined with a large number of polymers including natural collagen [4], chitosan [5], non-biodegradable poly(ethylene) [6], poly(methyl methacrylate) [7,8], polylsulfone [9], and biodegradable poly(α-hydroxyacids) [10–16].

Bioresorbable poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(lactic acid-co-glycolic acid) (PLGA) copolymers are very attractive for scaffolds for tissue engineering [17,18]. The combination of such polymers with a bioactive component takes advantage of the osteoconducting properties (bioactivity) of HA and bioactive glasses and of their strengthening effect on polymer matrices. The composite is expected to have superior mechanical properties than the neat (unreinforced) polymer and to improve structural integrity and flexibility over brittle glasses and ceramics for eventual load-bearing applications.

Composite fabrication research has focused on developing polymer/ceramic blends, precipitating ceramic onto polymer templates and coating polymers onto ceramics or ceramic onto polymers. Porous and nonporous implants have been made from biodegradable polyesters and ceramics, especially HA. Melt-extrusion

or compression-molding of PLA and bioactive glass or HA particles was used for the preparation of non-porous bone fixation devices [15]. Compatible polymer processing techniques including combined solvent-casting and salt-leaching [14,19], phase separation and freeze-drying [13] and immersion-precipitation [20] have been used for the preparation of highly porous PLLA/HA and PLGA/HA scaffolds. Each processing method has advantages that suit different tissue engineering applications. In situ apatite formation can also be induced by a biomimetic process in which polymer foams were incubated into a simulated body fluid [21]. In recent studies [22,23], particles of 45S5 Bioglass®, a commercially available bioactive glass powder (US Biomaterials), have been used to produce bioactive coatings on commercially available sutures (Vicryl®) and on PDLLA foams. More recently, we have described the preparation, characterisation and in vitro degradation of porous composites made of high molecular weight PDLLA and Bioglass® by phase separation and freeze-drying [24]. Preliminary results about the bioactivity of the composites in phosphate buffer saline (PBS) have been also presented [24,25].

In the present study, Bioglass® particles were combined with two different amorphous poly(α-hydroxyacids), a low molecular weight poly(D,L-lactide) and a poly(D,L-lactide-co-glycolide) copolymer. The present work thus complements and expands the previous research [23,25–28]. The commercial bioactive glass 45S5 Bioglass® was chosen in this study as the bioactive phase because it has the greatest bioactivity index (it is reported as a Class A bioactive material as opposed to HA, Class B) and can simulate osteoblast function faster than HA [29].

2. Experimental

2.1. Materials

Purasorb® poly(D,L-lactide) (PDLLA) with inherent viscosity of 0.39 dl/g was purchased from Purac biochem (Goerinchem, The Netherlands). Poly(lactide-co-glycolide) (PLGA) copolymers with a 75:25 LA:GA molar ratio and an intrinsic viscosity of 0.6 dl/g was provided by Boehringer-Ingelheim (Resomer RG 756). These polymers were used without further purification. Dimethylcarbonate (DMC, 99% in purity) was obtained from Sigma Aldrich. 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 wt%): 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 co-workers in 1971 [30].

2.2. Preparation of polymer/Bioglass® composites

Polymer/Bioglass® porous composites were prepared by freeze-drying as previously described [24]. Briefly, the polymer was dissolved in dimethylcarbonate to produce a polymer weight to solvent volume ratio of 5% (w/v). The mixture was stirred overnight to obtain a homogeneous polymer solution. A given amount of Bioglass® powder was added into the polymer solution. The mixture was transferred into a lyophilisation flask and sonicated for 15 min in order to improve the dispersion of the bioactive glass particles into the polymer solution. The flask was then immersed into liquid nitrogen and maintained at $-196^{\circ}C$ for 2 h. The frozen mixture was then transferred into an ethyleneglycol 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 48 h. The sample was completely dried at room temperature in a vacuum oven until reaching a constant weight. Two series of composite scaffolds made of PDLLA and PLGA were prepared as described above by adding different amounts of the Bioglass® (10, 25 and 50 wt%) in the mixture as described. For the sake of comparison, neat polymer foams were prepared without Bioglass®. Each composite sample was prepared in duplicate.

2.3. Characterisation

The apparent density of the foams (ρ_a) was measured by mercury pycnometry as reported previously [24]. A sample of weight W_s was placed in a pycnometer, which was completely filled with mercury and weighted to obtain W_{sl}. ρ_a was calculated according to

$$\rho_a = \frac{W_s}{W_1 - W_{sl} + W_s} \times \rho_{Hg}, \tag{1}$$

where W_1 is the weight of the pycnometer filled with mercury, and ρ_{Hg} is the density of mercury (13.5 g/cm^3). Pore volume (V_p) was calculated according to

$$V_p = \frac{1}{\rho_a} - \frac{1}{\rho_{sk}}, \tag{2}$$

where ρ_{sk} is the skeletal density of polymer/Bioglass® composite as measured by Helium pycnometry using a AccuPyc 1330 pycnometer (Micrometrics Co.). Four specimens of each composite were used for the density measurements and the results were averaged.

The pore architecture of polymer foams and polymer/Bioglass® composites was examined with scanning electron microscopy (SEM) (Jeol JSM-840A). Samples were coated with platinum for 120 s under a current of 30 mA before examination under an accelerating voltage of 20 kV.

The compressive mechanical properties of the foams were measured with a rheometer (Ares, Rheometric

Scientific). Cubic specimens with a side length of 5 mm were compressed with a crosshead speed of 0.5 mm/min. The compressive modulus was determined from the initial linear region of the stress–strain curve. At least five specimens were tested for each sample, and the averages and standard deviations were determined.

2.4. In vitro degradation study

For degradation experiments, samples of neat polymer and composites with dimensions of 13 mm × 3–4 mm height (diameter × thickness) and weighing $\cong 50$ mg (W_o) were cut with a core-borer. The samples were sterilised by UV exposure under a laminar flow hood for 10 min on each side and placed in sterile Falcon tube containing 50 ml of pre-filtered (0.22 μm porosity) phosphate buffer saline (PBS: 0.13 M, NaCl: 0.9%, NaN$_3$: 0.02%, pH: 7.4). The samples were incubated under slow tangential agitation at 37°C and allowed to degrade. The pH of the buffer was monitored during the experiment. At each time point, 3–4 samples of each scaffold composition were removed from the buffer, and weighted wet (W_a) after surface wiping. They were abundantly rinsed with deionised distilled water (ddH$_2$O) obtained from Milli-Q Plus, Ultra-pure water systems (Millipore) in order to remove the soluble inorganic salt, and weighed after freeze-drying (W_t).

Water absorption (WA%) and weight loss (WL%) were calculated according to Eqs. (3) and (4), respectively:

$$WA = \frac{(W_a - W_o)}{W_o} 100\% \qquad (3)$$

$$WL = \frac{(W_o - W_t)}{W_o} 100\% \qquad (4)$$

Three to four samples of each composition were measured and the results averaged. The results are presented as the mean $\pm$ standard deviation. The pH of the medium was recorded at each time point. Number and weight average molecular weight (M_n and M_w, respectively) and polydispersity (M_w/M_n) were determined by size exclusion chromatography (SEC, Helwett-Packard HP-1090 equipped with three Ultrastyragel columns from 10^2 to 10^5 Å). Tetrahydrofuran was used as an eluent (flow rate: 1 ml/min) and calibration was performed using monodisperse polystyrene standards (Polymer Laboratories Ltd., Shropshire, UK).

2.5. Surface analysis

Information on the elementary composition of Bioglass® particles at the surface of the composite scaffolds was obtained using environmental scanning electron microscopy (ESEM) (Philips FEG XL-30) combined with energy dispersive X-ray analysis (EDXA). EDXA was carried out to determine the Ca/P ratio of the Bioglass® particles prior and during in vitro degradation and to monitor HA formation at the composite surface. The mean Ca/P ratios were determined from five separate measurements in different areas of the composite samples.

After incubation in PBS for several time periods, selected neat polymer and composite were analysed using X-ray diffraction analysis (Philips PW 1700, Cu kα radiation at 40 kV and 40 mA). Raman spectroscopy was also conducted on as-fabricated and PBS-treated samples. These measurements were carried out using a Renishaw 1000 Raman micro-spectrometer. For excitation, a diode laser was used at 830 nm wavelength and 300 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 cm^{-1}.

3. Results

3.1. Porosity and morphology

Highly porous PDLLA/Bioglass® and PLGA/Bioglass® composite foams have been prepared by solid–liquid phase separation and subsequent sublimation of the solvent. The apparent density of the foams increases with the Bioglass® content in the two series of composites (Table 1). In parallel, the pore volume decreases with increasing Bioglass® content. The pore volumes of the PDLLA/Bioglass® range between 11.8 and 8.4 cm^3/g which is slightly higher than those of the PLGA foams prepared from the same Bioglass® content (from 11.2 to 7.1 cm^3/g). Such pore volumes correspond to high porosities (>90%) whatever the polymer and Bioglass® content.

Table 1
Density and porosity of PLGA/Bioglass® composite foams prepared in this study

Composition	Apparent density (g/cm^3)	Pore volume (cm^3/g)
PDLLA/Bioglass®: 100/0	0.080 ± 0.004	11.8 ± 07
PDLLA/Bioglass®: 100/10	0.101 ± 0.003	9.1 ± 0.3
PDLLA/Bioglass®: 100/25	0.094 ± 0.003	9.8 ± 0.4
PDLLA/Bioglass®: 100/50	0.110 ± 0.003	8.4 ± 0.3
PLGA/Bioglass®: 100/0	0.083 ± 0.004	11.2 ± 0.5
PLGA/Bioglass®: 100/10	0.091 ± 0.001	10.1 ± 0.2
PLGA/Bioglass®: 100/25	0.092 ± 0.002	10.2 ± 0.3
PLGA/Bioglass®: 100/50	0.123 ± 0.002	7.1 ± 0.7

A typical SEM micrograph of the PDLLA/Bioglass® composite foam prepared from a 100/50 weight ratio shows the continuous structure of interconnected pores with a preferential orientation (Fig. 1a) and ranging from 10 up to about 100 μm in diameter. The walls of the pores are covered by Bioglass® particles, either isolated or assembled to form aggregates with ⩽ 10 μm in size, and uniformly distributed in the PDLLA matrix (Fig. 1b and c). The morphology of the PDLLA/Bioglass® composite is different from neat PDLLA foam (Fig. 1d) prepared by the same process. Such PDLLA foams are characterized by a highly anisotropic tubular morphology with an internal ladder-like sub-structure. These features are typical of polymer foams prepared by thermally induced phase separation using freeze-drying in solvents such as dioxane and dimethyl-carbonate [31,32]. The pores are parallel to the heat transfer direction during the solvent crystallisation.

The effect of Bioglass® content on the structure of the polymer/Bioglass® foams has been investigated by varying the Bioglass® content while maintaining the polymer concentration constant. For a 10 wt% of Bioglass® content, the macroporous structure was similar to that of pure polymer foams, indicating that at low content, the solid Bioglass® particles do not perturb the solvent crystallisation to a large extent. The pores are still preferentially oriented along the cooling direction. Bioglass® particles are less uniformly distributed with a higher particle density at the upper part of the foam. Composites with higher Bioglass® content exhibit a more irregular pore structure, the pore anisotropy being less visible. Bioglass® particles can be easily identified on the polymer matrix. The homogeneity of the particles dispersion was found to be
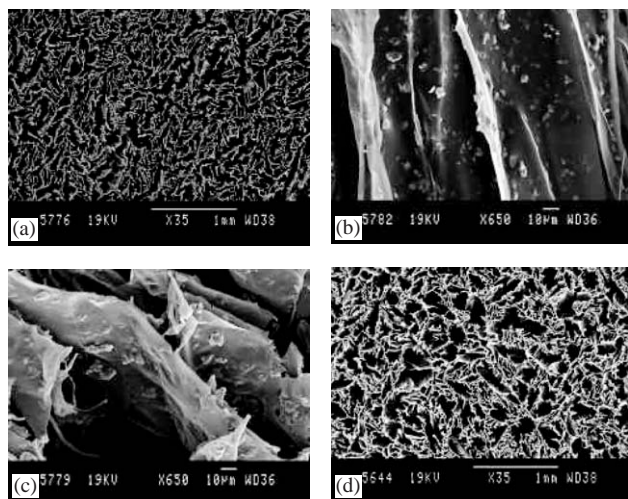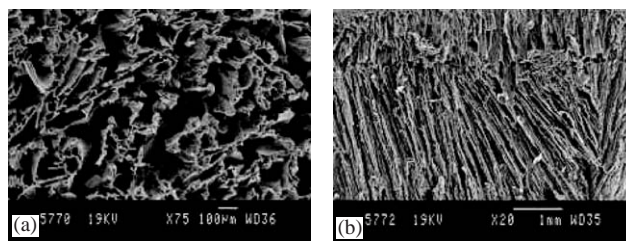

Fig. 2. SEM micrographs PLGA/Bioglass® composite foams (50 wt%). View of transverse (a) and longitudinal (b) section at low magnification.
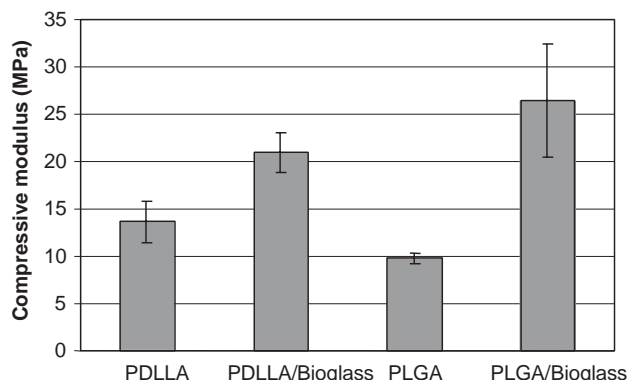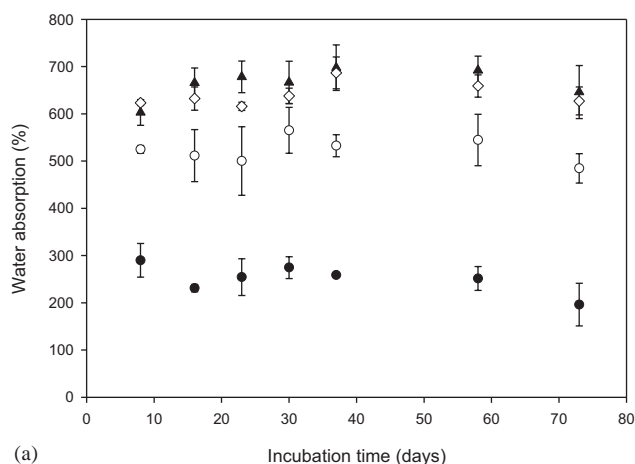

Fig. 3. Compressive modulus of PDLLA and PLGA foams and 100/50 PDLLA/Bioglass® and PLGA/Bioglass® composite foams.

improved at high Bioglass® contents. A qualitative good adhesion was found between the polymer matrix and Bioglass® particles; however, it is quite a problem to obtain quantitative information about the interfacial bonding strength between polymer and Bioglass®.
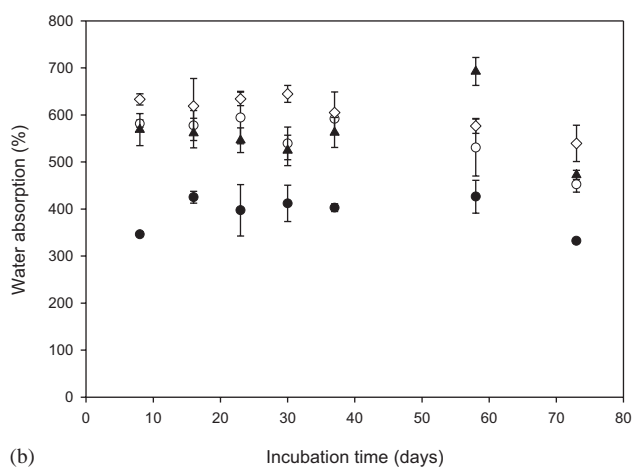
Composites foams can be prepared from different poly(α-hydroxyacids) using the same procedure as described above. Fig. 2 shows the morphology of PLGA/Bioglass® foam produced form a 100/50 polymer/Bioglass® mixture. The microstructure of the PLGA/Bioglass® foam is similar to that of PDLLA/Bioglass® for the same polymer/Bioglass® weight ratio. Fig. 3 shows that the compressive modulus of the composites is significantly improved by the Bioglass®. The compression modulus of both PDLLA/Bioglass® and PLGA/Bioglass® composites foams (100/50) is significantly higher than that of the neat polymer foams, the positive effect being more pronounced for the PLGA foams. Such an improvement of the mechanical properties has also been observed for PLLA/HA composites, however to a smaller extent [33] and more recently for similar composites prepared from a 50/50 mixture of PLGA with 75:25 and 50:50 LA:GA molar ratio [25].

3.2. In vitro degradation

As shown in Fig. 4a, the ability of the foams to absorb water during incubation in PBS increases with the Bioglass® content. The PDLLA/Bioglass® composites

Fig. 1. SEM micrographs of PDLLA/Bioglass® composite foams (50 wt%): (a) view of transverse section at low magnification; (b,c) Bioglass® particles on the walls of the polymer matrix and (d) neat PDLLA foams.
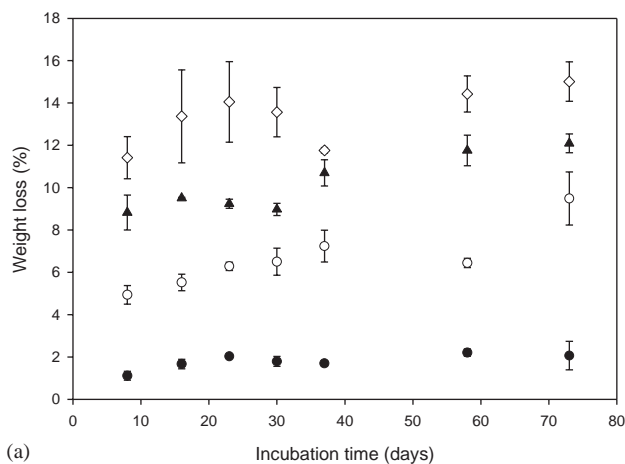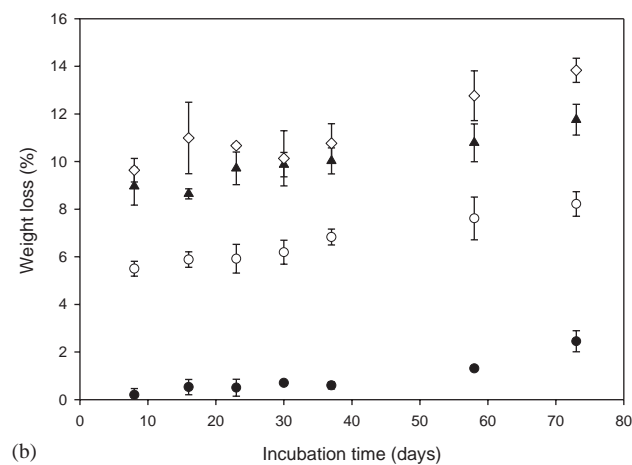
Fig. 4. Water absorption versus incubation time in PBS for the PDLLA/Bioglass® (a) and PLGA/Bioglass® (b) composite foams with 0 wt% (●), 5 wt% (○), 10 wt% (▲) and 40 wt% (◇) of Bioglass®.

Fig. 5. Weight loss versus incubation time for the PDLLA/Bioglass® (a) and PLGA/ Bioglass® (b) composite foams with 0 wt % (●), 5 wt % (○), 10 wt % (▲) and 40 wt % (◇) of Bioglass®.

absorbed a large amount of water ($\geqslant 500$ wt% as compared to their initial weight) during the first 2 weeks of incubation. Between day 16 and day 58, water absorption (WA) reached a plateau value whatever the Bioglass® content and then started to slightly decrease at the end of the incubation period. WA at the end of the incubation period was around 500 wt% in composites containing 10 wt% of Bioglass® and slightly higher than 600 wt% in the 100/50 and 100/25 PDLLA/ Bioglass® composites. The percentage of WA in all composites was largely higher than in the neat PDLLA foam which is <300 wt% during the whole incubation period.

Fig. 4b shows that WA also increased very rapidly in the PLGA/Bioglass® composites for 1 week of incubation. The composites adsorbed a very large amount of water ($\sim 600\%$) in the early incubation time before reaching a plateau value, the amount of WA being quite unchanged between day 16 and day 58. A slight decrease of WA was observed up to 58 days of incubation for all composites as for the PDLLA ones. WA of the neat

PLGA foams was significantly lower than in the composites but slightly higher than in the neat PDLLA foams. This confirms the more hydrophilicity of PLGA as compared to PDLLA. The weight loss (WL) data for Bioglass®-filled PDLLA and PLGA foams are summarised in Fig. 5. According to Fig. 5a, the WL increased with the Bioglass® content at each time point. There is a burst in weight loss for all PDLLA composites occurring during the first 2 weeks of incubation, then WL tends to stabilise to reach final values around 8, 11, and 14 wt% in the composites containing 10, 25 and 50 wt% of Bioglass®, respectively. The pure PDLLA foams showed a very slight weight loss ($\leqslant 2\%$) during all the incubation period. The patterns of WL for the PLGA composites were similar to those of PDLLA. In the PLGA composites, the weight loss increased over the entire incubation period and proportionally to the Bioglass® content. There was a burst in WL during the first week of incubation, up to which WL increased slightly. WL at the end of the incubation period was around 9, 12 and 15% for the
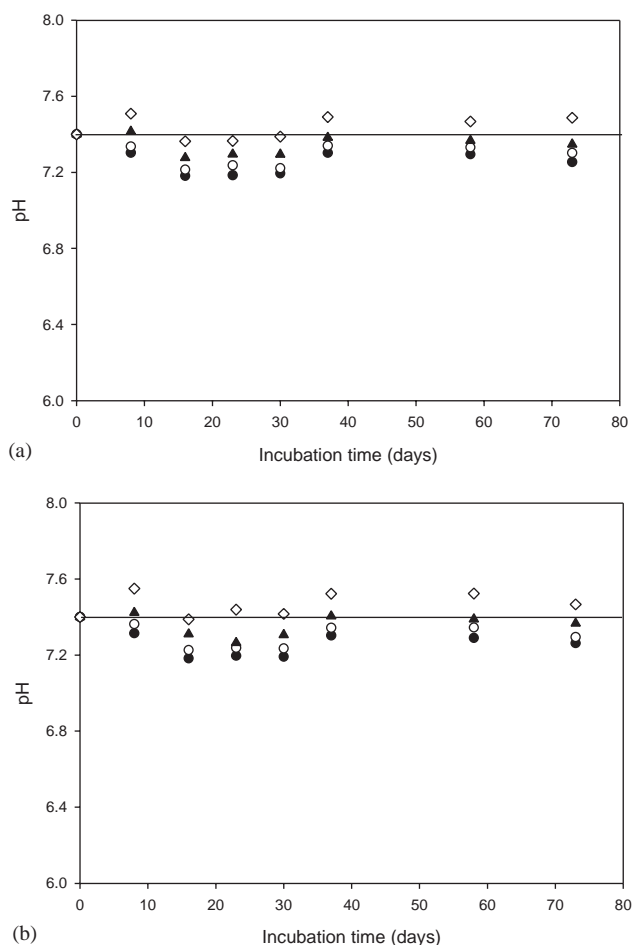
Fig. 6. Changes in pH of the incubation medium versus incubation time for PDLLA/Bioglass[®] (a) and PLGA/Bioglass[®], (b) composite foams with 0 wt % (●), 5 wt % (○), 10 wt % (▲), 10% and 40% (◇) of Bioglass[®].

Fig. 7. Changes in M_w versus incubation time for PDLLA/Bioglass[®] (a) and PLGA/Bioglass[®] (b) composite foams with 0 wt % (●), 5 wt % (○), 10 wt % (▲), 10% and 40% (◇) of Bioglass[®].

PLGA composites containing 10, 25 and 50% of Bioglass[®], respectively. These values are slightly higher than those observed in the PDLLA composites, all the other conditions being the same. As for the PDLLA, the weight of the PLGA foams did not significantly change during the first 37 days of incubation and reached only 2 wt% at the end of the degradation period (78 days).

The pH variation patterns of the media containing the different series of composites foams are shown in Fig. 6. Fig. 6a and b show that the pH of the incubation medium was lower than the initial value (7.4) for the neat PDLLA and PLGA foams and for all composites, except those containing 50 wt% of Bioglass[®]. The pH of the medium containing the 100/50 composites was ⩾7.4 over all the degradation time.

As shown in Fig. 7a and b, the weight-average molecular weight (M_w) of the composite scaffolds made of PDLLA and PLGA decreased with incubation time in PBS, but with a rate that depends on the Bioglass[®] content and polymer composition. In the PDLLA/ Bioglass[®] compo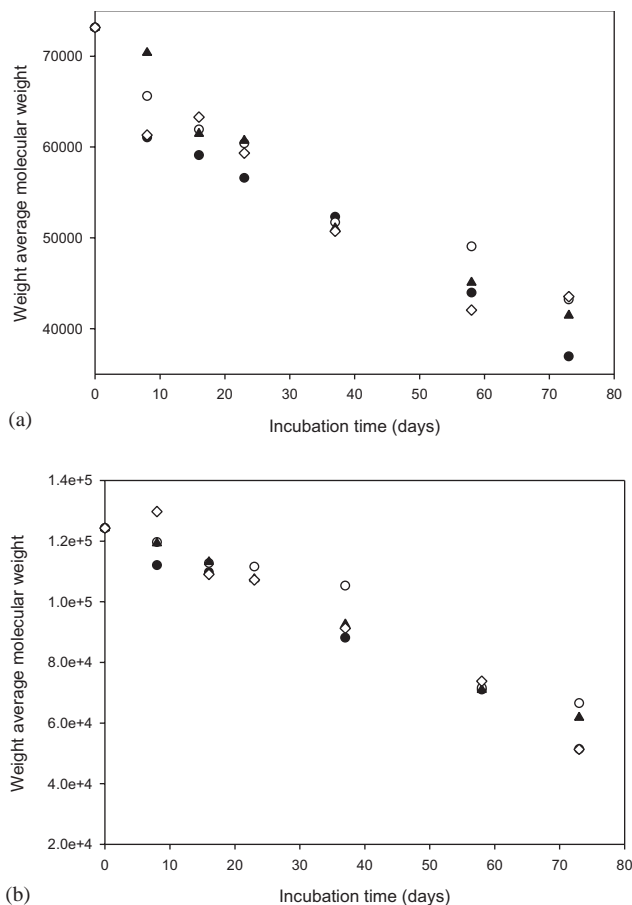sites, the molecular weight of the pure polymer foam decreased more rapidly than that of the composites, particularly during the first 38 days of incubation. Moreover, the polymer M_w at the end of the incubation period was lower in the neat PDLLA foams that in the composites containing Bioglass[®]. In the composites made of PLGA, the kinetics of polymer degradation seems to be less influenced by the presence of Bioglass[®].

3.3. Morphology of the incubated polymer foams and HA formation

Fig. 8 shows the ESEM morphology of porous composites incubated in PBS at 37°C for 4 weeks. Bioglass[®] particles are clearly visible. EDX measurements on these particles was carried out on different regions of the same samples to measure the Ca/P ratio. The results reported in Table 2 are average values obtained from 4–5 measurements on different areas. A difference in the thickness of the external ceramic layer is thought to be responsible for the variation in the intensity of the Ca and P peaks. EDX spectra (not shown here) show the peaks characteristics of the
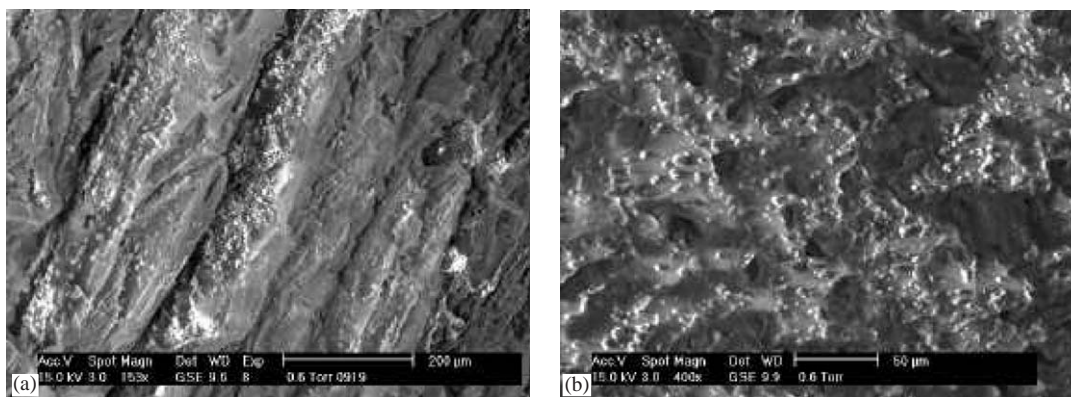
Fig. 8. ESEM micrographs of PDLLA/Bioglass® (a) and PLGA/Bioglass® (b) composite foam incubated in PBS for 4 weeks.

Table 2
Ca/P ratio for the composite samples incubated in PBS

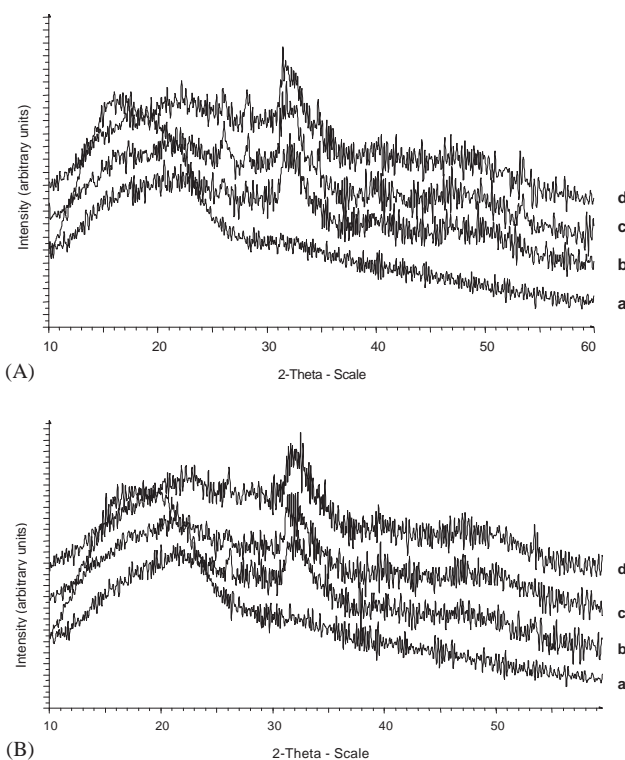| Sample | Incubation time (weeks) | | | |
	0	1	4	8
45S5 Bioglass®	5	—[a]	—[a]	—[a]
100/50 PDLLA/Bioglass®	—[a]	1.6	1.7	1.7
100/50 PLGA/Bioglass®	—[a]	2.7	2	1.4

[a] Not determined.

Fig. 9. XRD diagrams of PDLLA/Bioglass® (A) and PLGA/Bioglass® (B) composite samples incubated for 8 (b), 16 (c) and 30 (d) days in PBS showing the development of crystalline HA. XRD diagrams for neat PDLLA and PLGA foams are shown for the sake of comparison (a).

mineral filler (Si, Ca, P). Both the polymer and the bioactive filler are contributing to the oxygen peaks while the carbon peak is only due to the polymer component. Table 2 shows that the Ca/P ratio, which is around 5 for the as-received Bioglass® particles, rapidly decreased to 1.6–2.7 after 1 week of incubation for the composites made of PDLLA and PLGA, respectively. This Ca/P ratio decreased further for longer incubation time to reach values around 1.4–1.7, which are similar to that of carbonated HA, thus confirming the transformation of Bioglass® into HA following dissolution mechanisms as reported in the literature [34].

The formation of HA on the surface of polymer/Bioglass® composites was also recorded by both X-ray diffraction and Raman spectroscopy. Fig. 9A and B show XRD diagrams of 100/50 PDLLA/Bioglass® and PLGA/Bioglass® composite samples, respectively, after different incubation times. Well-defined HA peaks can be seen at $2\theta = 32°$ in all composites as soon as 1 week after incubation. Raman spectroscopy was used to confirm the formation of HA on composite surfaces after treatment in PBS and to quantify it. Representative Raman spectra are shown in Fig. 10A and B for PDLLA/Bioglass® and PLGA/Bioglass® composite foams, respectively. The peak at $960 \, cm^{-1}$ corresponds to P–O symmetric stretching in PO_4^{3-} groups in carbonated HA [40] while the $875 \, cm^{-1}$ peak corresponds to C–COO stretching in PLGA and PLA, therefore this ratio represents the amount of HA formed [24]. In Fig. 10A,

the ratio of the 960 and $875 \, cm^{-1}$ peaks heights increases (by factor ~ 2) from 8 to 30 days of immersion, indicating that the amount of HA increases for longer incubation time. This effect is however less pronounced in the PLGA/Bioglass® composites (Fig. 10B).

4. Discussion

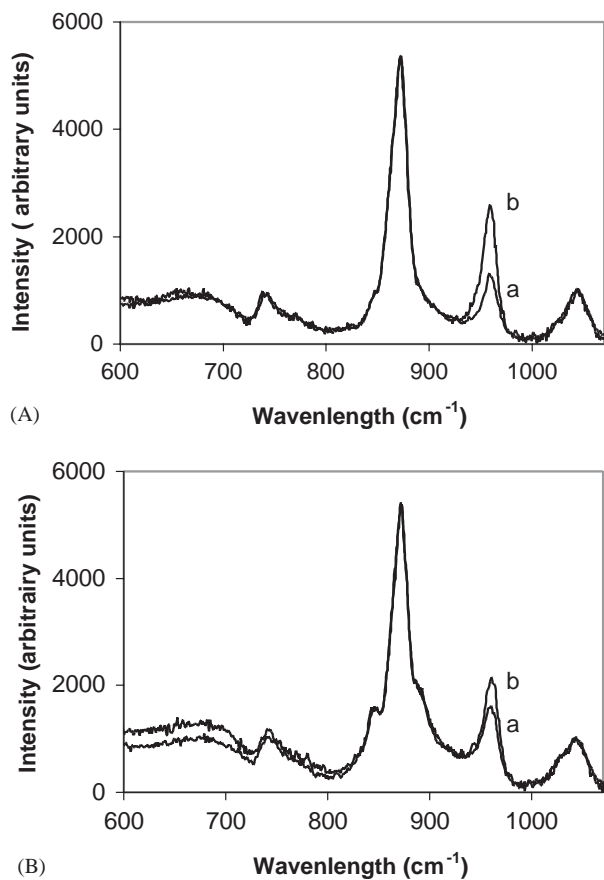We have fabricated composites scaffolds made of biodegradable poly(α-hydroxyacids) and bioactive glass

Fig. 10. Raman spectra for: PDLLA/Bioglass® (A) and PLGA/Bioglass® (B) composite foams before, after 8 (a) and after 30 (b) days of immersion in PBS. Spectra have been normalised according to the polymer peak at $870\,cm^{-1}$.

(45S5 Bioglass®) by freeze-drying which involves a solid–liquid phase separation of polymer solutions in organic solvents and subsequent solvent sublimation. Dimethylcarbonate was used as a solvent because it exhibits high vapour pressure and melting point around $0°C$ which makes it suitable for sublimation. Because of a solubility parameter close to that one of dioxane, this solvent was recently identified as being very convenient as an alternative to dioxane (potentially carcinogen) for freeze-drying of poly(α-hydroxyacids) [35]. This method provides simultaneously versatility because (i) a variety of scaffold designs and materials can be used and (ii) control of the pore size and structure can be achieved by varying polymer concentration and cooling temperature. In this study 5 wt:v% polymer concentration in DMC was chosen because this concentration has proved to ensure consistency to the polymer foams [32]. During the freezing process, crystallisation of the solvent takes place and both the polymer and the Bioglass® particles are expelled from the crystallisation front, forming a polymer–Bioglass®-rich phase and a solvent-rich phase. After solvent sublimation, the polymer/Bioglass® rich

phase forms a dense and continuous skeleton of the foam and the pores are the fingerprints of the sublimated solvent crystals. The temperature gradient along the solidification direction provides orientation to the pore structure. The pore morphology is not deeply affected by the Bioglass® filler particles at least for low content. However, a large number of particles randomly distributed in the polymer solution may change slightly the solvent crystallisation front by impeding crystal growth and by making the crystals of the solvent more irregular. As a result of irregular solvent crystal growth, the pores became more irregular in shape but the pore orientation was mainly dictated by the cooling direction. The higher the Bioglass® content, the higher the apparent density and the lower the pore volume. However, the porosity and pore interconnectivity of the composite foams was still high as compared to that obtained by using other porogenic processes [36]. The mechanical properties of the composite scaffolds can be enhanced by increasing the amount of Bioglass®, especially for amorphous and tough poly(D,L-lactide) and poly(D,L-lactide-co-glycolide). In a recent study, Zhang et al. [20] showed that the elastic modulus of composites made of PLA and bioactive glass (with composition different from that of Bioglass®) by using the immersion-precipitation technique increased with the addition of bioactive glass while the tensile strength of the composite decreased. This is what is usually observed in composite materials, i.e. as a result of the presence of a stiff filler: impact and tensile strengths usually decrease while hardness and stiffness increase. This stiffening effect can be further observed in the storage modulus values [37]. The results of the mechanical test reported here confirmed the increasing trend in composite rigidity as compared to the neat polymer materials. Further studies on dynamical mechanical properties should be performed for a better understanding of the decrease in the polymer segment mobility by the Bioglass® filler, this being the focus of current studies.

The in vitro degradation kinetics of composite samples was determined as a function of the hydrolysis time in PBS in normal physiological conditions (pH 7,4 at $37°C$). Degradation was monitored by water absorption, weight loss and molecular weight changes. At least two parameters have to be taken into consideration for analysing the results, i.e. the composition of the polymer and the amount of Bioglass® incorporated in the composites. Two amorphous polymers were chosen in this study: a PDLLA homopolymer and a PLGA copolymer with a 75:25 LA/GA ratio, the latter being known to degrade faster than the homopolymer. Such biodegradable polyesters degrade with random chain scission by ester hydrolysis in a process autocatalysed by the generation of carboxylic acid end groups [38]. As a result, the degradation of polyester devices is known to

be heterogeneous and divided into a fast degrading centre and a slowly degrading outer layer, which stays intact and retains degradation products until the swelling of the implants or mechanical failure cause it to break, after about 32 weeks in vitro in case of polylactide [39]. In our study, all the polymer and composite foams retained their structural integrity and 3-D morphology until the end of the experiment (i.e. 78 days) which means that the degradation process was still in its early stage. The disruption of the outer layer was not observed and therefore the release of the acidic residues did not occur, which explains why no significant lowering of pH was measured. More interestingly we found that the degradation kinetics is delayed by the presence of Bioglass®, which exert a local buffering effect by the release of alkaline ions. The autocatalytic effect associated with the leakage of acidic products of the polymer degradation accumulated in the internal part of the implant should thus be delayed if not hindered by the presence of the Bioglass® filler. This was clearly demonstrated for longer incubation time in high molecular weight PDLLA/Bioglass® composites [24].

The presence of a bioactive filler in the polymer foams on the pore contours both at the outer surface and in the interior of the composite scaffold may have positive biological effect by encouraging both bone and soft tissue in-growth from the implant/tissue interface to the interior of the scaffold. The bioactivity of the composite scaffolds, determined by the rapid formation of carbonated HA crystals on the sample surfaces during immersion in PBS, was confirmed by electron microscopy, XRD analyses and Raman spectroscopy measurements.

The positive bioactive effect of the composites prepared in this study was qualitatively demonstrated by recording the formation of a Ca/P layer on the surface of the composite samples after incubation in PBS. Both EDXA and XRD techniques confirmed the formation of a crystalline HA layer after a few days of in vitro incubation. The HA formation rate was fast, as compared to other studies in which PLLA foams were immersed into SBF to grow apatite [21]. These results confirms that Bioglass® 45S5 has a higher bioactivity index than HA, which is why it is being increasingly used as substituting material for bone tissue engineering as such or as a filler [10]. Raman spectroscopy appears as a very sensitive tool for collecting qualitative information on the HA formation.

5. Conclusions

This study has shown that freeze-drying followed by solvent sublimation is a valuable process for preparing resorbable polymer/Bioglass® composite foams with controlled pore morphology and homogeneous dispersion of the glass particles in the foam structure. The results of the degradation studies in PBS indicate the possibility to modulate the degradation rate of the composite scaffolds by varying the composition of the foams, i.e. the nature of the polymer and the wt % of Bioglass® added to the polymer matrix. This is relevant for tissue engineering and tissue repair applications knowing that degradation rate of temporary scaffolds must be matched to the rate of formation of new tissue. The rapid formation of HA on composite foams after 7 days of incubation in PBS indicates the high bioactivity of the materials, conferred by the Bioglass® content. The present work opens an innovative way for the development of porous bioresorbable scaffolds of high bioactivity for hard and soft tissue engineering. Future work will focus on investigating the behaviour of osteoblasts and bone progenitor cells cultured on these porous bioactive composites as well as assessing the potential applications of the scaffolds in soft tissue engineering.

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