

Poly(lactic Acid–Phosphate Glass Composite Foams as Scaffolds for Bone Tissue Engineering

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Abstract: Phosphate glass (PG) of the composition 0.46(CaO)–0.04(Na₂O)–0.5(P₂O₅) was used as filler in poly-L-lactic acid (PLA) foams developed as degradable scaffolds for bone tissue engineering. The effect of PG on PLA was assessed both in bulk and porous composite foams. Composites with various PG content (0, 5, 10, and 20 wt %) were melt-extruded, and either compression-molded or foamed through supercritical CO₂. Dynamic mechanical analysis on the bulk composites showed that incorporating 20 wt % PG resulted in a significant increase in storage modulus. Aging studies in deionized water in terms of weight loss, pH change, and ion release inferred that the degradation was due to PG dissolution, and dependent on the amount of glass in the composites. Foaming was only possible for composites containing 5 and 10 wt % PG, as an increase in PG increased the foam densities; however, the level of porosity was maintained above 75%. PLA-*T_g* in the foams was higher than those obtained for the bulk. Compressive moduli showed no significant reinforcement with glass incorporation in either expansion direction, indicating no anisotropy. Biocompatibility showed that proliferation of human fetal bone cells was more rapid for PLA compared to PLA-PG foams. However, the proliferation rate of PLA-PG foams were similar to those obtained for foams of PLA with either hydroxyapatite or β-tricalcium phosphate. © 2006 Wiley Periodicals, Inc. *J Biomed Mater Res Part B: Appl Biomater* 80B: 322–331, 2007

Keywords: phosphate-based glass; bone tissue engineering; poly-L-lactic acid (PLA); scaffolds; foams

INTRODUCTION

Extensive research has been undertaken in the last few decades geared toward the development of bioresorbable polymers, such as aliphatic polyesters, for their potential application as biomedical materials.¹ Poly(α -hydroxyl acids) are well known biodegradable materials that have been tailored for applications, such as sutures, pins, screws, drug delivery systems, and scaffolds, for bone tissue engineering.^{2–6} In the development of scaffolds for bone tissue engineering, it is recognized that these should be porous, biocompatible, bioactive, easy to manufacture, and with appropriate mechanical properties.^{7,8} Studies involving polylactic acid (PLA) and

poly(DL-lactic-co-glycolic acid) have shown that the presence of these biodegradable scaffolds can facilitate the formation of bone mineral when implanted *in vivo*.^{5,6}

Polymeric foams alone, however, may not have the capacity to withstand higher loads in *load-bearing* applications.⁴ For this reason, the incorporation of an inorganic phase into the polymeric matrix has been developed to overcome these limitations.^{7,9,10} Ideally, the addition of ceramics and glasses into the PLA matrix should improve not only the mechanical properties of the material, but also its bioactivity and biological behavior. For this reason, bioactive fibers or particles of calcium phosphates have been incorporated as fillers.^{11–14} Factors such as the constitution of the filler, content, and solubility can influence the aforementioned properties. However, not all calcium phosphate materials are ideal in this, e.g., hydroxyapatite (HA) or bioactive silicate-based glasses are not readily soluble and, therefore, do not reflect the required degradation properties.^{15,16} Providing a biodegradable composite material where all its constituent parts

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meet the degradation criteria would prove a significant step in tissue regeneration. For this purpose alone, the incorporation of degradable phosphate-based glasses (PG) as the inorganic phase of the composite materials may be appropriate. PGs having a ternary system (P_2O_5 —CaO— Na_2O) have been previously investigated in considerable depth with relation to chemistry, degradation, biocompatibility, and cytotoxicity.^{17–24} Furthermore, the addition of PG into polymers such as PLA may further enhance the complex nature of the degradation of the matrix.^{25,26} The degradation of PLA has been shown to be affected by a number of factors, such as chemical structure, crystallinity, molecular weight, processing conditions, shape, and size.^{27,28}

This study reports on the use of degradable PG particles as fillers in PLA composite foams for potential applications in bone tissue engineering. Previous studies carried out on the ternary glass formulation 0.46(CaO)—0.04(Na_2O)—0.5(P_2O_5) alone have shown that this glass composition maintains cell attachment, differentiation, and limited proliferation of osteoblasts and fibroblasts.²⁹ Here, the effect of incorporating particulates of this glass formulation into the PLA matrix was assessed in both the bulk properties of the composites produced through compression molding, and on the foamed PLA-PG composites. Supercritical CO_2 ($ScCO_2$) was used as the foaming agent, where it acts as a solvent used to control the porosity and cellular morphology of the scaffolds. $ScCO_2$ is safely removed out of the polymer when the pressure is released in order to create the desired pore morphology through gas diffusion.^{30,31} The effect of PG as filler in PLA scaffolds was investigated in terms of processability, morphology, aging, mechanical behavior, and biocompatibility with human primary bone cells.

MATERIALS AND METHODS

Development of PLA and PLA-PG Composites

PG Production. A glass was prepared with the following composition (in molar fraction): 0.46(CaO)—0.04(Na_2O)—0.5(P_2O_5). This was carried out by thoroughly mixing three starting reagents in the following weights: NaH_2PO_4 , 4.8 g; P_2O_5 , 32.66 g; $CaCO_3$, 23 g, and placing them into a platinum crucible. The crucible was placed in a carbolite furnace preheated to 700°C. Heating at this temperature ensures that excess gas produced (CO_2 and water vapor) due to the chemical reaction is expelled. After 10–15 min the temperature of the furnace was increased to 1100°C for 1 h, where the glass forms a melt. The crucible was then taken out of the furnace, and the glass melt was quickly poured onto a flat steel plate. This enabled the glass to go through a process of rapid quenching to form a solid amorphous glass of very high viscosity. The glass was then ground using a ball and agate mill to a suitable particle size for sieving. The particles were then passed through sieves of aperture sizes ranging from 1000 to 32 μm . Figure 1 shows a scanning electron micrograph of PG, giving an indication of the particle size range and morphology.

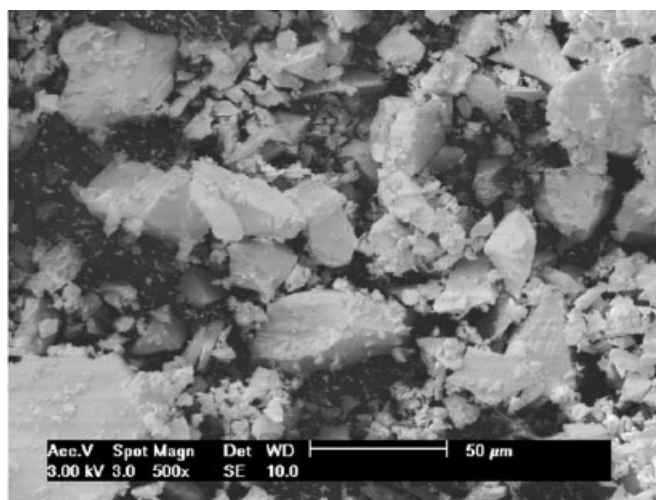


Figure 1. SEM image showing the size distribution of PG particles used as reinforcing agents in bulk and foamed PLA composites.

Composite Manufacturing: Melt Extrusion and Compression Molding. A commercial bioresorbable polymer, PLA ($IV = 1.6$ dL/g, $T_m = 187.7^\circ C$, $\rho = 1.25$ g/cm³) (Boehringer Ingelheim, Germany) was used without further purification. PLA pellets and 0, 5, 10, or 20 wt % PG particles were mixed in the dry state, and dried overnight under vacuum at 105°C before processing. A microcompounder (Micro 5 Compounder, DSM, The Netherlands) with two conical corotating screws, of small capacity (5 cm³), was used to prepare the PLA-PG composites. This was carried out at 205°C, with a screw rotation speed of 100 rpm and a residence time of 4 min, under a flow of nitrogen to limit polymer degradation. Extruded composite rods were further dried again at 105°C under vacuum, before being ready for either foaming or compression molding for bulk composite analysis. The latter was carried out using a Fontjine Holland Table Press (The Netherlands) by applying 20 MPa at 195°C for 10 min.

Composite Foaming. The foaming equipment was composed of a custom-made high pressure chamber (Autoclave France, France) and a computerized acquisition data system. The technique has been previously described in detail.³¹ Briefly, extruded PLA-PG composite rods were put into cylindrical open molds (diameter, 35 mm) and loaded in the pressure vessel. Pressure and temperature were increased up to a saturation pressure, P_{sat} (150–250 bar), and 195°C, respectively, to obtain a saturated polymer- CO_2 solution. Foam expansion was then induced by sudden gas release: because of supersaturation, gas nuclei were created, which grew further by the gas diffusing out of the polymer. Porous structures were stabilized by an increase in viscosity, resulting from a decrease in temperature and CO_2 concentration, where polymer solidification finally fixed the cellular morphology. Initial depressurization rate dP/dt (2.5–5.5 bar/s), controlled by a back-pressure regulator (ER3000, Tescom, USA), and maximum cooling rate dT/dt (2–6°C/s) are significant parameters that influence pore expansion and stabilization.

Methods of Analysis

Characterization of the Bulk PLA-PG Composites. Dynamic mechanical analysis (DMA) was carried out on the compression-molded polymer and composite samples, using a Perkin–Elmer DMA (DMA-7e, Perkin–Elmer Instruments), under a three-point-bend measuring system. Rectangular samples ($n = 3$) of $\sim 1 \times 3 \times 24 \text{ mm}^3$ were tested through a temperature range of 25 to 180°C at a heating rate of 4°C min^{-1} . Two test controls were applied, a static tension control of 120% (i.e., ratio of 1.2, static to dynamic stress) and a dynamic strain control of 0.02%. The storage (E') and loss (E'') moduli as well as the mechanical loss tangent ($\tan \delta$) were measured as a function of temperature. The polymer glass transition temperature (T_g) was measured using the peaks obtained in both E'' and $\tan \delta$.

Differential scanning calorimetry (DSC) was also used to confirm T_g of the PLA matrix. This was carried out using a DSC (Pyris Diamond DSC, Perkin–Elmer Instruments). Two repeat samples ($\sim 5 \text{ mg}$) were taken through heat/cool/heat ramps from 0 to 200°C at a rate of 20°C min^{-1} . T_g values were calculated from the point of inflection on the T_g curve.

The surfaces of the bulk composite were investigated by elemental analysis through EDAX. Samples were mounted on aluminium stubs and coated using a Polaron CC7650 carbon coater. The specimen surfaces were then observed using a Cambridge stereoscan S90B scanning electron microscope (SEM), and the elemental analysis was carried out using the EDAX system that was fitted to the SEM.

An aging study of the polymer and composites was carried out in ultra-pure distilled water, which involved undertaking a sample weight loss, pH change, and ion-release measurements of the solution through ion chromatography (IC). Three repeat specimens from each of the materials were cut to an area of $\sim 10 \times 10 \text{ mm}^2$. Prior to commencing this study, each specimen was dried, weighed, and individually placed into separate plastic vials containing pH-7 adjusted water and incubated at 37°C. The aging solution was replaced at 6, 24, 48, 72, 144, 192, and 240 h, and the specimens were weighed after the excess surface fluid was removed. The pH of the solution was simultaneously measured (Jenway 3045 ion analyser with pH combination electrode), and IC was carried out for cation and anion release measurements, using a Dionex ICS 1000 and 2500 IC system. For cation (Na^+ and Ca^{2+})-release study, the solutions were filtered in order to remove the larger phosphate ions and were taken over a retention time of 12 min, in which the peaks for Na^+ and Ca^{2+} were expected to give conductivity readings at 4.5 and 10.5 min, respectively. For anion release, the measurements were taken over a retention time of 11 min, in which a peak for PO_4^{3-} was expected to give a conductivity reading of 2.9 min. The concentration values that are illustrated in parts per million (ppm) were extrapolated against the peak areas ($\mu\text{S min}$) from calibration curves.

Characterization of PLA-PG Composite Foams. The apparent density of the composite foams was measured to

calculate the expansion due to gas foaming. This was carried out by weighing a sample of measured volume. The morphological properties of the composite foams were also characterized using SEM. Samples were cut in the foam core with a razor blade and gold-coated, to avoid charge accumulation and prevent sample damage. A Philips XL30 SEM (Holland) was used to observe the specimens under an accelerating voltage of 3 kV, using secondary electrons for simple topographic observation.

DSC was also carried out to investigate the effect of foaming and PG inclusion on the PLA- T_g . The test conditions were as described earlier for the compression-molded samples.

The compressive mechanical properties of the composite foams were evaluated on specimens of measured density, and prepared by paying attention to obtaining parallel surfaces, perpendicular to the testing direction. Measurements were carried out on three repeat specimens, using a Universal Testing Machine (UTS Test System, Germany), at a cross-head speed of 0.5 mm min^{-1} . The elastic modulus (E) was evaluated from the initial linear elastic part of the stress-strain curve.

Biocompatibility evaluations were carried out on the scaffolds in terms of a proliferation study, using human primary osteoblasts. These cells were obtained from the Laboratory of Orthopedic Research (EPFL-LRO, Lausanne). Research bank of bone cells comprises four *fetal* donors (one female and three male) at the end of March 2004. Care was taken to obtain healthy bone samples. Biopsies were obtained in accordance with the Ethics Committee of the University Hospital in Lausanne (Ethical Protocol 51/01). Foams were sterilized overnight in 70% ethanol solution and 30-min UV exposure. Fetal bone cells were cultured in Dulbecco's modified Eagle medium (DMEM) and 10% FCS, at 37°C, in a 5% CO_2 humidified atmosphere. To favor cell differentiation, the culture medium, refreshed every second day, was implemented with osteogenic factors (ascorbic acid, 50 $\mu\text{g/mL}$; β -glycerophosphate, 1 mM ; dexamethasone, 10 nM). The proliferation study involved seeding the cells onto scaffolds in a 48-well plate at a density of 20,000 cells/well. For every time point, three samples of each material were tested. Every second day, samples were removed from culture medium, put into 100 μL of DMEM, and 20 μL of CellTiter dye (Catalys Promega). After 1-h incubation, samples were removed and light absorbance was measured at 490 nm by a Multilabel Counter (Wallac Victor2). Cell numbers were obtained by comparison to a standard curve. Cell proliferation was also observed under SEM, after cell fixation and dehydration.

Statistical Analysis

Statistical analysis was performed to test the significance in the difference between the two mean values by using the Student's *t*-test, which was used to determine *p*-values at a significance level of 0.05.

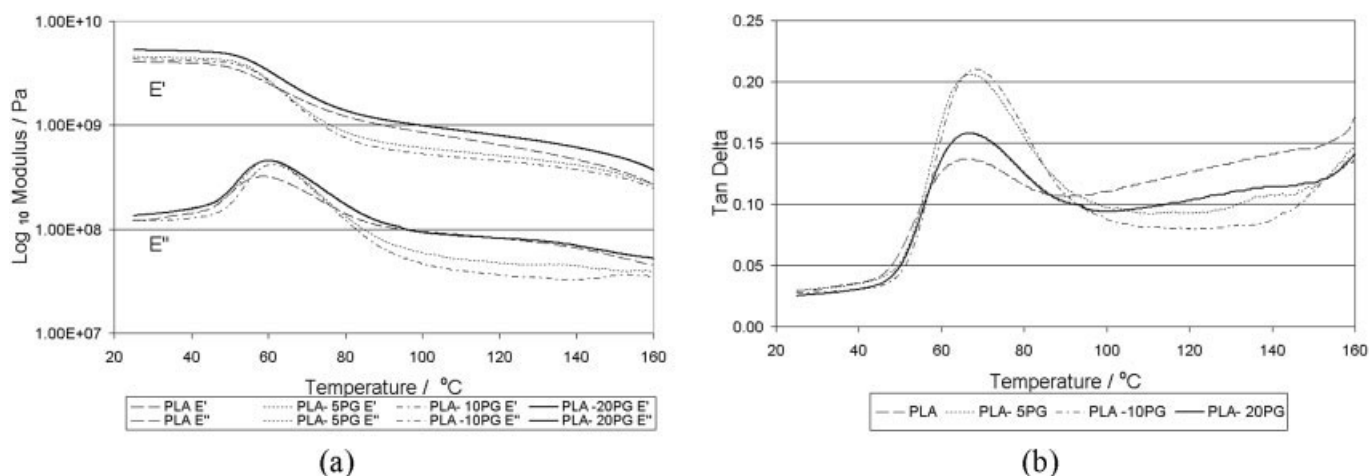


Figure 2. (a) Storage and loss moduli versus temperature, (b) $\tan \delta$ versus temperature for representative samples of compression-molded PLA-PG composites.

RESULTS AND DISCUSSION

Bulk PLA-PG Composites

Thermal and Mechanical Analysis. Figure 2(a,b) shows the dynamic mechanical properties of a representative sample from the compression-molded PLA and PLA-PG composites as a function of temperature. Table I compares the mean E' values at 25 and 37°C as well as the $PLA-T_g$, as measured through the peaks obtained in E'' and $\tan \delta$, respectively. The PLA-20PG composite gave statistically significant ($p < 0.05$) higher modulus values at both temperatures in comparison to the other PLA-PG composites, while there were no statistical significant differences ($p > 0.05$) in E' for all materials between the two temperature points. T_g appeared to be higher when using the $\tan \delta$ peak compared to the peak obtained for E'' . All three PLA-PG composites exhibited a slight increase in T_g values compared to PLA alone. This effect is exemplified through the broader slope of the transition region from the storage modulus curves and accompanied by peak widening from the loss modulus curves in Figure 2(a) and $\tan \delta$ in Figure 2(b). A similar increase in T_g has been observed in HA or tricalcium phosphate (TCP)-incorporated PLA composites, which attributed to the polymer/filler surface interactions.^{12,13} This slight increase in the matrix T_g in the molded composites, demonstrated via DMA, was also shown in the first heating ramp through DSC mea-

surements as given in Table II, where PLA-PG composites exhibited a slight increase in T_g compared to PLA. However, by removing all thermal history through a second heating ramp, the differences in T_g values for PLA and PLA-PG composites were not statistically significant ($p > 0.05$), indicating that the slight changes in T_g observed through DSC (1st heat run) and DMA were influenced by processing parameters of extrusion and compression molding.

Elemental Analysis. The elemental analysis of the surfaces of all four materials is illustrated in Figure 3, which are presented as a weight percentage of calcium and phosphorous as a function of glass inclusion. As expected, the PLA exhibited no trace of these elements. In contrast, as the amount of PG increased in the composites, there was an increase in the presence of calcium and phosphorous. As sodium is only added at 4 mol % in the glass, the EDAX system is not sensitive enough in detecting sodium X-ray photon energies; therefore, there is no data present for sodium. These results represent the percentage of each element on the surface, which appears to be less than in the bulk, and may be due to a polymer skin effect at the surface.

Aging Study. Figure 4(a,b) shows the aging behavior of these materials in water in terms of percentage weight loss and pH change as a function of immersion time, respectively.

TABLE I. DMA Data of Compression-Molded Composites in Terms of the Mean Storage Modulus (E'), at 25 and 37°C, and Glass Transition Temperature (T_g) As Defined by Peaks in Loss Modulus and $\tan \delta$

Material	Storage Modulus (GPa)		Glass Transition Temperature (°C)	
	At 25°C	At 37°C	Loss Modulus	$\tan \delta$
PLA	4.4 ± 0.3	4.3 ± 0.3	58.8 ± 0.4	66.4 ± 0.2
PLA-5PG	4.4 ± 0.2	4.3 ± 0.2	60.5 ± 0.2	67.4 ± 0.4
PLA-10PG	4.4 ± 0.1	4.3 ± 0.1	61.2 ± 0.1	68.4 ± 0.3
PLA-20PG	5.2 ± 0.3	5.0 ± 0.3	59.5 ± 0.2	66.7 ± 0.7

TABLE II. DSC Data of Compression-Molded and Foamed PLA-PG Composites Comparing the PLA- T_g Taken from the First and Second Heating Ramps

Material	T_g for Molded Composites ($^{\circ}\text{C}$)		T_g for Foamed Composites ($^{\circ}\text{C}$)	
	1st Heat	2nd Heat	1st Heat	2nd Heat
PLA	60.3 \pm 0.5	62.5 \pm 0.2	68.2 \pm 3.0	62.2 \pm 0.1
PLA-5G	62.2 \pm 0.1	62.1 \pm 0.2	69.1 \pm 6.1	62.3 \pm 0.2
PLA-10G	61.7 \pm 0.2	62.4 \pm 0.1	70.6 \pm 0.1	61.9 \pm 0.5
PLA-20G	62.1 \pm 1.0	63.0 \pm 0.6	–	–

For the first 48 h of the aging process, all four compositions displayed a weight gain [Figure 4(a)]. This was attributed to the water absorption into the materials. After this time point, all composites displayed a weight loss where the rate was dependent on the initial amount of glass present in the composites, whereas PLA showed no significant change in weight throughout this study. This time point represents an event in which an ion-diffusion process occurs, resulting in substantial ion release from the glass on the surface, therefore, leading to a significant breakdown in the glass. There was little change in the pH of the solution where the PLA was placed, and it remained relatively neutral [Figure 4(b)]. In contrast, the pH of the solutions for the composites underwent significant reduction, particularly after 48 h. The magnitude of this reduction was dependent on the amount of PG in the composites, with the PLA-20PG composite demonstrating the most rapid rate of reduction in pH. The drop also correlated well with the weight loss behavior observed in various materials.

Figure 5(a–c) shows the ion-release behavior of the composites in terms of sodium (Na^+), calcium (Ca^{2+}), and phosphate (PO_4^{3-}) ions, respectively. These results showed good correlation with weight and pH change as well as the EDAX measurements. First, the rate of release of ions increased relative to glass addition. The glass addition is reflected by elemental analysis, showing the amount of ions present at the surface of each PLA-PG composite. Second, the aging study

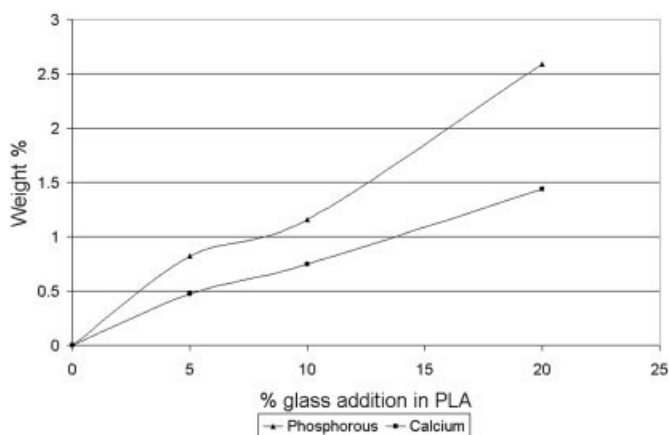


Figure 3. EDAX data showing the weight percentage of calcium and phosphorus at the surface of the bulk composites versus percentage glass addition in PLA.

indicated that the water absorption accounted for the initial weight gain up to 48 h for all materials. As a result, a slower rate of release of Na^+ , Ca^{2+} , and PO_4^{3-} ions in solution for all PLA-PG composites occurred up to this time point. Between 72 and 144 h, the PLA-PG composites exhibit a higher and more linear rate of release of all ions. This indicates that after 48 h the polymer matrix becomes saturated, and the soluble glass particles act as hydrophilic sites within the PLA matrix, where droplets are formed, and therefore allow a more controlled ion-diffusion process to take place. A previous study has found that for a range of ternary PGs the degradation rate and ion release showed a linear relationship with time.³² In all systems the release of PO_4^{3-} was more prominent relative to the cations, indicating a rapid breakdown in the phosphate network of the glass, and increased significantly with glass content. This was attributed to the drop in pH with the formation of phosphoric acid (H_3PO_4).

Longer term aging studies compared percentage weight loss versus percentage glass addition of samples, aged for over 1200 h, and then completely dried. The results indicated that the percentage weight loss of each composite was close to the percentage addition of glass filler (PLA, $-0.01 \pm 0.17\%$; PLA-5PG%, $3.28 \pm 2.80\%$; PLA-10PG, $9.17 \pm 0.14\%$; PLA-20PG, $16.85 \pm 0.77\%$). This confirmed that the linear ion-release behavior shown in this study is typical of ternary PGs, and that the degradation process and subsequent ion release is purely controlled by the glass component in the PLA matrix and not by the PLA matrix itself. This would be expected, since high molecular weight L-PLAs have been shown to degrade slowly, e.g., more than 1 year to degrade.²⁸

Characterization of the Foamed Composites

Morphological Assessment. The effects of foaming parameters have previously been described in detail elsewhere with neat PLA.³¹ Briefly, larger and more interconnected pores were obtained with lower cooling rates, and a higher pore density was a consequence of higher saturation pressure combined with fast depressurizing rates. This study focused on the effect of PG fillers on PLA foams.

Figure 6(a–d) shows the morphological characteristics of these composite foams as observed through SEM. The regularity of foam morphology was highly dependent on glass filler dispersion in the polymer matrix. A polymer-rich zone favored pore growth and expansion compared to a PG-rich zone. An initial homogeneous dispersion of the filler in the

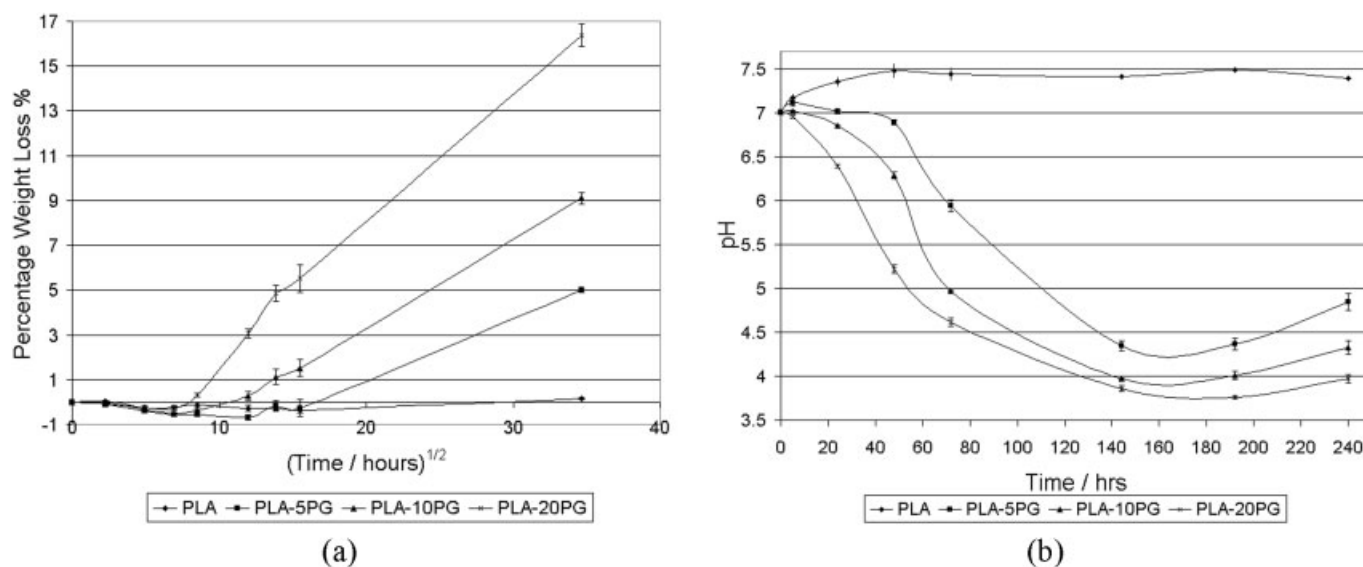


Figure 4. Aging behavior of bulk PLA-PG composites in deionized water (a) Percentage weight loss versus time (b) pH change versus time.

polymer is needed before foaming. Generally with glass fillers, more heterogeneous and less-expanded structures were obtained. For a given set of foaming parameters, foam architecture was dependent on glass weight percentage. The addition of greater amounts of fillers to the matrix increased its viscosity (data not presented), therefore, reducing polymer expansion and leading to more closed pores. With 20% PG, it was difficult to achieve a good dispersion of fillers, therefore leading to foams with a heterogeneous morphology, which were not considered further in the analyses.

Fillers could also serve as nucleation sites, increasing the number of pores created. Fillers were located in the matrix [Figure 6(d)]. Micrographs of edge cross-sections displayed the PG particles embedded in the polymer. This glass particle distribution should favor an efficient reinforcement of the foam, provided fillers were homogeneously dispersed, and did not come in contact with each other. Greater filler content tended to increase foam density, as shown by the apparent density measurements in Table III. Porosity was higher than 75% for all filler concentrations, and 42–49% of the pores (evaluated by image analysis), were of diameters in the range between 200 and 400 μm , which is positive for a bone tissue engineering application.³³

Thermal and Mechanical Analysis. The thermal properties of the foams were assessed with regard to PLA- T_g and are given in Table II. The DSC data obtained through the first heating run, showed a significant increase ($p < 0.05$) in PLA- T_g for all compositions when compared to the T_g of the compression-molded samples. However, when all the thermal history was removed during the second heating run similar T_g values for PLA and PLA-PG composites. This suggested that the apparent increase may have been due to the combined heat and foaming conditions.

As was observed from the DMA data for the PLA-5PG and PLA-10PG bulk composites, the compressive mechanical

properties of the composite foams showed no significant ($p > 0.05$) reinforcing effect of glass fillers at these concentrations (Table III). This lack of reinforcement may be due to a low interfacial mechanical locking between the matrix and filler due to the smooth surface of the glass as compared to other fillers, such as HA or TCP particles [Figure 6(d)]. Measured compressive moduli were also low compared to trabecular bone (~ 300 MPa). Furthermore, no significant anisotropy was observed in which similar modulus values were obtained for samples tested parallel and perpendicularly to the expansion direction. In comparison, by using the same foaming process, along with smaller and more uniform-sized particulate fillers,³⁴ or other foaming techniques, such as thermally induced phase separation, mechanically anisotropic foams could be obtained.⁸

Biocompatibility Assessment. The biocompatibility was evaluated by the direct contact of glass-filled PLA foams with human primary osteoblasts. Fetal bone cells were recently shown to have a high potential, because of their rapid growing rate and their responsiveness to differentiation factors.³⁵ A promising approach for bone tissue engineering was demonstrated by using these fetal bone cells seeded on calcium phosphate-filled PLA foams.³⁶ In this study, proliferation was observed to be initially faster on neat PLA, where cell confluence was reached after 8 days, whereas up to 14 days were needed for PG-filled PLA [Figure 7(a)]. Similar proliferation behaviors were also induced, when foamed composites of PLA-5HA and PLA-5 β -TCP (wt %) were used as comparisons to the PLA-PG composite foams. Proliferation was, however, restricted to construct surfaces after 2 weeks of culture, where cell adhesion was also observed by SEM [Figure 7(b)]. Further work biocompatibility will include more detailed differentiation studies in order to analyze these glass-filled PLA foams.

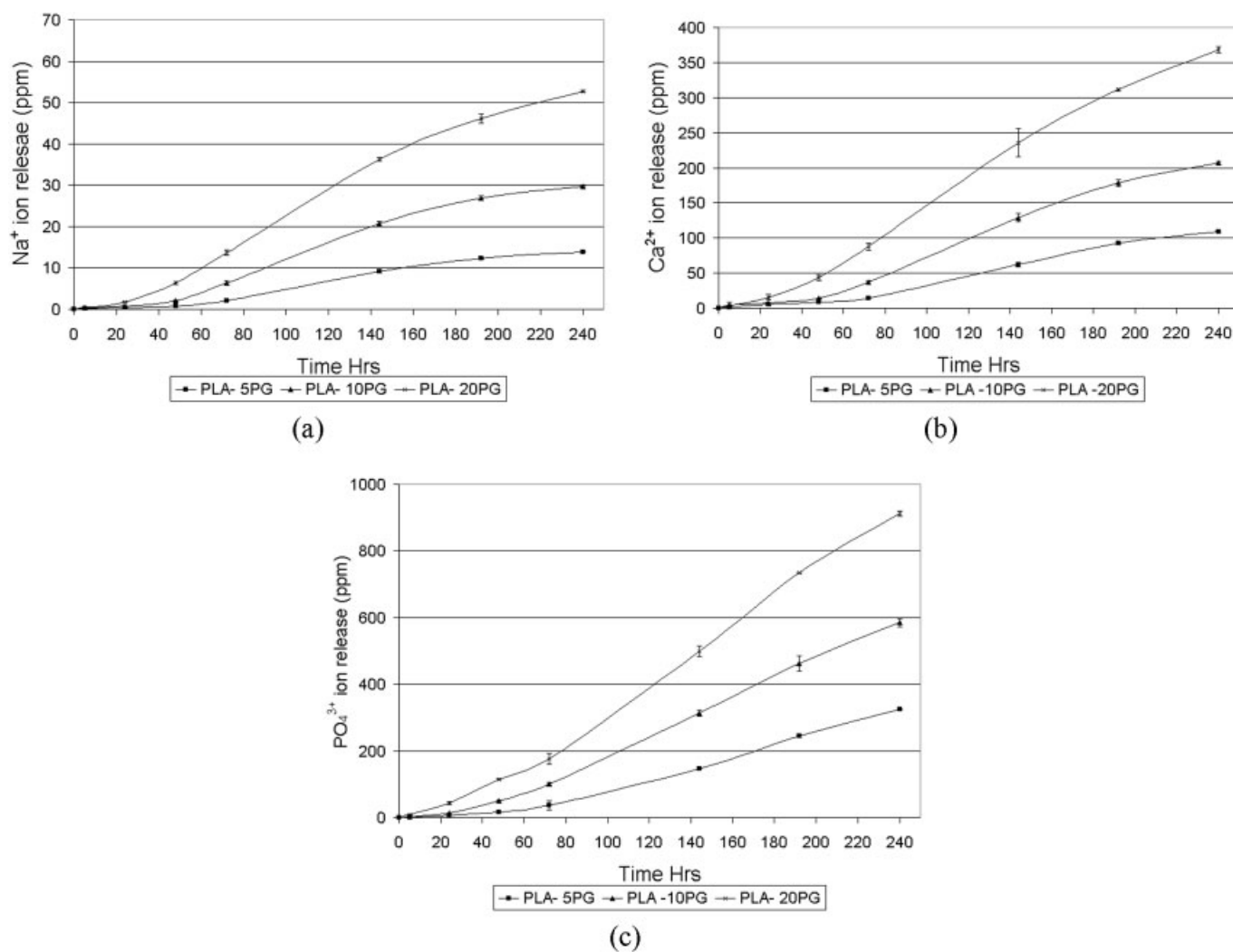


Figure 5. Aging behavior of bulk PLA-PG composites in deionized water in terms of ion-release measurements (a) Cumulative sodium ion release versus time (b) Cumulative calcium ion release versus time (c) Cumulative phosphate ion release versus time.

This study focused on investigating the effect of different percentage inclusions of the same PG composition on the properties of the composite foams. Generally, the addition of ceramic or glass particles to polymer matrices should improve its mechanical properties, provided that there is good adhesion between the two phases. This has either been achieved through mechanical interlocking, which can be dependent on the size and surface roughness of the particulate filler or when there is a chemical bond as achieved through surface treatment. This study did not undertake surface treatment in order to improve adhesion between the PG filler and PLA matrix and the smooth surface finish of the glass particle led to low interfacial adhesion between the two phases. DMA on the bulk composites demonstrated that a significant increase in the storage modulus was only achieved by adding PG at 20 wt % (~12.5 vol %) compared to the other composite formulations and PLA matrix alone. Furthermore, since the foaming of PLA-20PG was difficult to achieve in

this study, as it compromised the size and homogeneity of pores, this composite was not considered for the foamed samples. In line with the DMA data, there were no significant differences in the quasistatic compressive modulus values of the composite foams with 5 wt % (~2.5 vol %) and 10 wt % PG (~5 vol %) and that of the PLA foams. Future work will investigate the effect of surface treatment of these glass particles, in order to improve the adhesion with the matrix and therefore the mechanical properties. The degradation of these glass particles was found to be much more rapid than that of the PLA matrix. However, since the degradation of these glasses can be altered through their chemistry,^{19,20} e.g., by adding other doping ion such as iron, future work will also assess the effect of varying the glass composition, along with inclusions of multiple glass compositions. Previous studies have shown that the properties of composites with different glass compositions in polycaprolactone can be controlled.^{37,38} The rate of change in these properties also

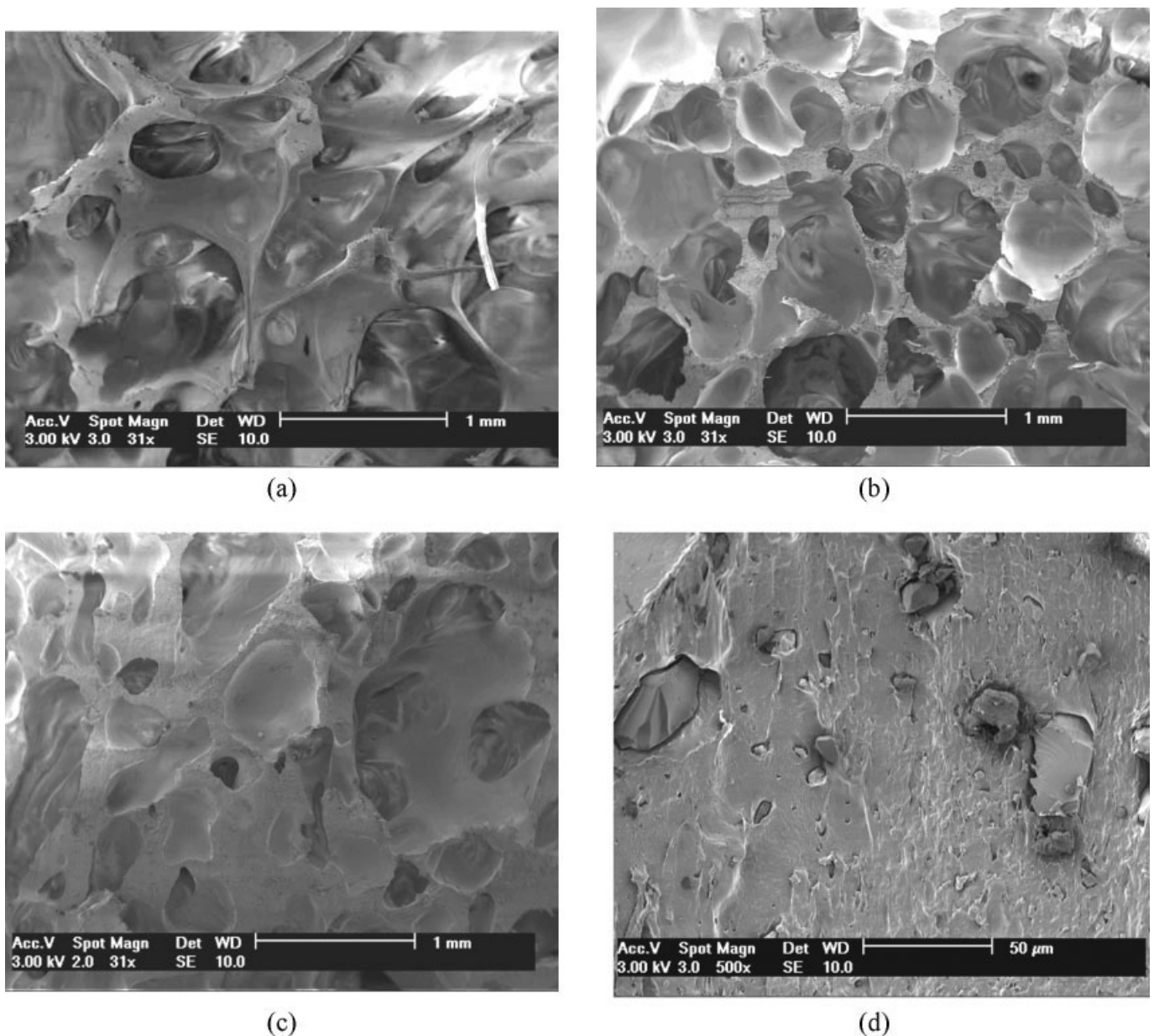


Figure 6. SEM image showing the effect of PG fillers on PLA foam morphology (a) Neat PLA foams (b) PLA-5PG foams (c) PLA-10PG foams (d) Close up of PG particles in a PLA foam wall.

showed a correlation with the amount of CaO incorporated in the glass composition, where the lower the CaO content in glass the greater the rate of change in properties.

TABLE III. Porosity and Compressive Modulus of Foamed PLA-PG Composites, Measured Parallel or Perpendicularly to the Foaming Direction

Material	Porosity (%)	Modulus (MPa)	
		Parallel	Perpendicular
PLA	88.3 ± 1.2	115.1 ± 30.9	109.5 ± 2.8
PLA-5G	81.2 ± 1.5	80.0 ± 19.7	100.0 ± 28.1
PLA-10G	81.7 ± 1.2	87.0 ± 12.6	116.5 ± 12.5

CONCLUSIONS

Bulk and foamed composites of PLA-PG were produced for bone tissue engineering. In the bulk, the addition of 20 wt %PG gave higher mean storage modulus values compared to the other compositions. The effect of thermal processing tended to increase the PLA- T_g in the composites. Elemental analysis showed that the increase of calcium and phosphorous on the surface of the PLA-PG composites were less than what was added to the bulk.

Aging in deionized water showed initial water absorption for all materials. Thereafter, all materials showed a decreased in weight, which was dependent on the amount of PG in the composites. This was attributed to a controlled ion-diffusion

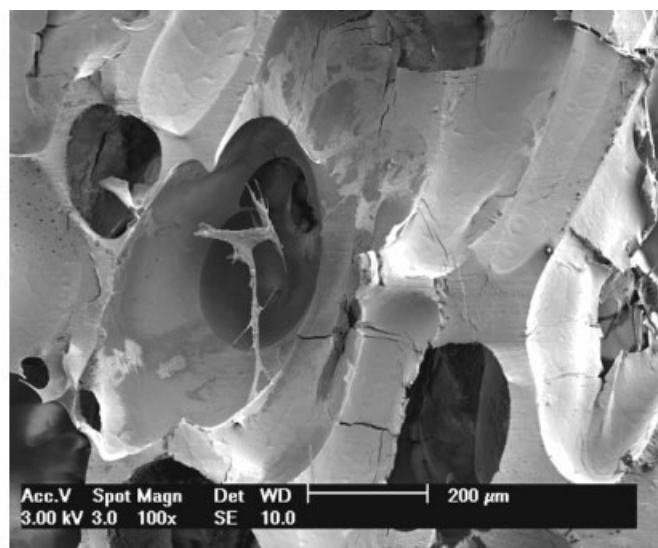
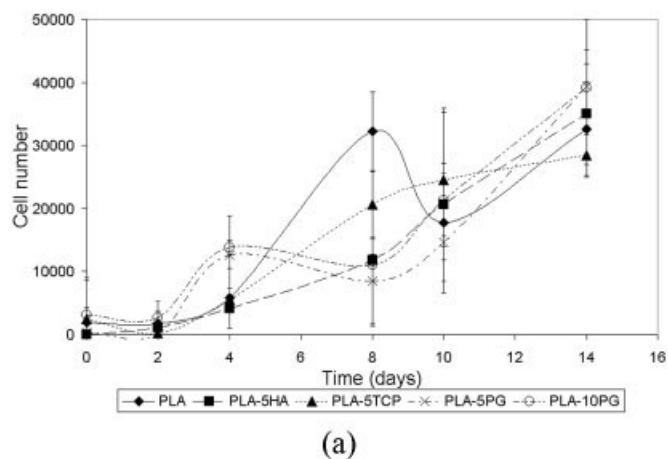


Figure 7. Biocompatibility assessment using a proliferation study on composite foams (a) Cell proliferation as measured by CellTiter, compared with other composites of PLA-5 wt % HA or with PLA-5 wt % β -TCP (b) SEM image showing a cross-section of a PLA-5PG after 2 weeks of cell culture.

process and subsequent breakdown of the glass. The large drop in pH for PLA-20PG was attributed to the overriding effect of PO_4^{3-} release over Na^+ and Ca^{2+} . The rate of ion release increased relative to glass addition, which correlated with the changes in weight loss and pH relative to each composite.

The foam morphology was shown to vary with glass addition. The results showed that smaller pores were created as a function of glass addition and the foaming of PLA-20PG composites was not efficient. Foam densities increased with the addition of fillers; however, the required level of porosity for bone tissue engineering applications was still maintained above 75%. Thermal analysis showed that the PLA- T_g in the composite foams increased compared to those obtained for the bulk because of the foam-processing conditions. The compressive modulus values of the PLA-PG composite foams showed no significant reinforcing effect at the filler levels of 5 and 10 wt % PG when tested in either expansion direction for the porous foams. Biocompatibility data showed that proliferation of human fetal bone cells was more rapid for PLA foams compared to the composite foams of PLA-PG. Changes in surface topography as a result of rapid degradation of PG filler restrict cell attachment, thus, reducing the proliferation rate of cells on PLA-PG composites. However, the proliferation rate of these PLA-PG composite foams were similar to those obtained for foams of PLA with either HA or β -TCP particles.

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