In vitro degradation of a novel poly(lactide-co-glycolide) 75/25 foam

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Abstract

Macroporous poly(lactide-co-glycolide) PLGA 75/25 foams were prepared for application in bone tissue engineering. Their in vitro degradation behaviour was followed over a 30 week period at 37°C and at one of three pHs: (1) pH 5.0, which mimics the acidic environment produced by activated macrophages, (2) pH 7.4, which reproduces normal physiological conditions and (3) an intermediate pH 6.4. The degradation of the PLGA 75/25 foams was studied by measuring changes in mass, molecular weight and morphology. The degradation profile of foams maintained at pH 5.0, 6.4 and 7.4 was similar until week 16, after which foams maintained at pH 6.4 and 7.4 had comparable degradation patterns whereas foams maintained at pH 5.0 degraded faster. For example, mass loss was less than 3% for foams maintained at all three pHs until week 16; however, by week 30, foams maintained at pH 6.4 and 7.4 had lost 30% of their mass whereas foams maintained at pH 5.0 had lost 90% of their mass. Foams maintained at pH 6.4 and 7.4 showed a similar constant decrease in molecular weight over the entire degradation study. Foams maintained at pH 5.0 had a similar rate of molecular weight loss as those maintained at pH 6.4 and 7.4 until week 16, after which the rate of molecular weight loss of foams maintained at pH 5.0 was accelerated. The morphology of the foams maintained at pH 6.4 and 7.4 was unchanged for 25 weeks. Foams maintained at pH 5.0 collapsed after week 18. Thus the PLGA 75/25 foams, described herein, maintained their 3-D morphology at physiological pH for over 6 months, which is an important feature for tissue engineering applications. © 1999 Elsevier Science Ltd. All rights reserved

Keywords: Biodegradable polymers; Poly(lactide-co-glycolide); Foams

1. Introduction

Biodegradable aliphatic polyesters derived from lactide and glycolide are widely used in medical and pharmaceutical applications [1]. These biocompatible and non-cytotoxic polymers can be molded into virtually any shape and have found applications in vivo as suture materials, bone fracture fixation devices and drug delivery systems [2].

Biodegradable polymers have also been used as scaffolds for tissue engineering applications with several cell types including chondrocytes [3], hepatocytes [4] and most recently, bone marrow-derived cells [5, 6]. The concept of bone tissue engineering is to harvest osteogenic cells, seed them on a biodegradable 3-D foam and allow them to proliferate and differentiate to create a new tissue. Since the polymer scaffold degrades, no synthetic polymer remains in the final engineered tissue. The degradation rate of the scaffold should either be similar to or slower than the rate of tissue formation. Consequently, for tissue engineering applications, it is important to understand the degradation profile of a given polymer scaffold. The rate of hydrolytic degradation of PLGA is influenced by polymer molecular weight, polydispersity [7], crystallinity [8], shape and morphology [9]. The pH, ionic strength, temperature and buffering capacity of the medium in which the degradation occurs also influence degradation kinetics [10]. Consequently, previous studies underline the importance of investigating the degradation profile of new polymeric structures/morphologies that are intended for in vivo applications.

In vivo degradation is accelerated by enzymes [11], cellular activity [12] and cell-induced pH changes, such as those caused by activated macrophages [13]. While in vitro degradation models do not address these issues, they provide important insights into the hydrolytic degradation profile in vivo.

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We recently described the use of PLGA 75/25 foams for bone tissue engineering applications [14, 15]. While others have used PLGA foams in tissue engineering applications [16, 17], our foams had a completely different morphology, similar to that of trabecular bone. This unique geometry required that a thorough degradation study be conducted in order to better predict whether polymer degradation would be congruent with bone formation in vivo.

We assessed the degradation of these PLGA 75/25 foams at 37°C in sodium cacodylate buffers maintained at pH 5.0, 6.4 or 7.4. While not purporting to address all of the in vivo factors that influence degradation, the three pH values represent different in vivo milieus that affect degradation. The pH 5.0 solution mimics the pH in macrophage lysozomes [18]. The pH 7.4 solution simulates physiological conditions while the pH 6.4 solution reflects both the acidic extracellular environment around macrophages [19] and an intermediate pH between the very acidic lysozomal milieu and the ideal physiological pH. Cacodylate buffers were chosen as the degradation media because they are stable at all three pHs.

Degraded PLGA 75/25 foams were characterized by changes in mass, molecular weight and morphology. Molecular weight was followed by gel permeation chromatography (GPC) and morphology by light microscopy (LM) and scanning electron microscopy (SEM). LM data provided information on major morphological changes such as pore size and overall foam dimensions while SEM provided information on minor morphological changes such as cracks, surface roughness and bulk porosity.

2. Materials and methods

Deionized distilled water (ddH$_2$O) was obtained from Milli-RO 10 Plus and Milli-Q UF Plus apparatus (Bedford, MA) and used at 18 mΩ resistance. PLGA 75/25 (Birmingham Polymers, Inc. Birmingham, AL) had an intrinsic viscosity of 0.87 dL/g at 30°C in chloroform. The weight-average molecular weight (M_w) of the polymer was determined by gel permeation chromatography (GPC) to be 81 500 g/mol. The changes in polymer molecular weight were determined at each degradation time. Specifically, 50 μl of a PLGA 75/25 solution in tetrahydrofuran (THF) with 0.1% (w/w) tetrabutylammonium bromide was injected onto and eluted through a series configuration of columns (American Polymer Standards 10^5, 10^4, 500 Å, cross-linked polystyrene/divinylbenzene) at a flow rate of 0.8 ml/min. The GPC (Waters 2690, operating temp. 35°C) was equipped with a refractive index detector (Waters 410, operating temperature 32°C) and calibrated with polystyrene standards (Aldrich, Ont., Canada). Data were analyzed using Millennium version 2.15.01 software.

Mass loss was measured using a Sartorius MC5 microbalance (Göttingen, Germany). At each time point, samples were weighed after drying and mass loss was calculated by comparing the initial mass (W_0) with that at a given time point (W_t), as shown in Eq. [1]. Measurements were made for samples maintained at all three pHs, the results of which are presented as the mean $\pm$ standard deviation ($n = 3$):

$$\text{Mass loss} = \frac{(W_0 - W_t)}{W_0} 100\%. \tag{1}$$

Light microscopy observations were made on a Leitz dissecting microscope (Heerbrugg, Switzerland). Foams were observed immediately after weighing under light microscopy using indirect light at magnifications of 16×, 25× and 40×. Scanning electron micrographs were taken on a Hitachi 2500 scanning electron microscope (SEM) at an acceleration voltage of 15 kV after sputter-coating the samples with gold under argon atmosphere (Polaron Instrument Inc., Doylestown, PA). Foams degraded for less than 22 weeks were cross-sectioned at a thickness of approximately 5 mm and observed by SEM. After 22 weeks of degradation, foams were too fragile to be sectioned thus entire foams were observed by SEM. Changes in foam dimensions were measured with a Max-Cal digital caliper (Labcor, Anjou, Québec).

For histomorphometry, foams were cryosectioned at −20°C using a Lab-tek cryostat (Elkhart, Indiana) and digitized images of the foam sections were analyzed using a NIH*image* analysis program.[1] The porosity of the polymer matrices was also estimated by mercury porosimetry (Quantachrome Autoscan 6). A solid penetrometer with 5 cm^3 cell stem volume was used for samples in the range of 0.015–0.020 g. The void volume was calculated from the mercury intrusion volume.

2.1. Preparation of polymer foams

PLGA 75/25 foams were prepared as previously described [14]. Briefly, glucose crystals were dispersed in a PLGA 75/25 solution in dimethylsulfoxide (DMSO, BDH, Toronto, ON). The polymer was precipitated and the glucose crystals were extracted from the precipitated polymer. Foams were dried to constant mass (0.01 mmHg, 72 h).

2.2. Preparation of buffers

Three different sodium cacodylate (Na cacodylate) [(CH$_3$)$_2$AsO$_2$Na·3H$_2$O] buffers were prepared. The pH

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[1] Developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/nih-image/.

5.0 Na cacodylate buffer was prepared by mixing 51.5 ml of 2.0 M Na cacodylate with 484.5 ml of 1.0 M HCl; the pH 6.4 buffer was prepared by mixing 73.1 ml of 2.0 M Na cacodylate with 269 ml of 1.0 M HCl; and the pH 7.4 buffer was prepared by mixing 94.6 ml of 2.0 M Na cacodylate with 53 ml of 1.0 M HCl. Ten ml of 10% sodium azide (NaN_3) was added to each solution to inhibit bacterial and fungal growth for the length of the degradation study, and the volume of all three solutions was brought up to 1 l.

2.3. Polymer degradation

Twenty-four cylindrical foams (1.5 cm × 3 cm) were prepared and cut into 8 sections with a razor blade. The size of each foam section was ∼1 cm in height and 0.4 cm^2 base. Each section was weighed, thereby providing the initial mass of each section (referred to as W_0). Sections were then placed in a perforated Eppendorf tube, and the mass of the tube containing the foam section was recorded so that even if the foams were not retrievable from the tube after degradation, a difference in total mass would indicate mass loss. Sections from each cylinder were either stored in an evacuated dessicator and used as controls or used as samples and stored at 37°C in one of the three buffer solutions: pH 7.4, 6.4 or 5.0. During the degradation study, comparisons were made between control and degraded samples derived from a same foam cylinder. Foam sections were immersed in large volumes of buffer, with a foam : buffer ratio of 1 : 1000 (w/v). Since the buffer solutions were changed every second week and the buffer pH was monitored weekly, the pH was maintained constant throughout the degradation study. All samples were maintained in the same incubator at 37°C. At each degradation time point, and prior to analysis, three samples per analytical technique were removed from the buffer, washed with ddH$_2$O in a sonicator (3 times, 20 min each) and then dried ($P = 0.01$ mmHg, 72 h, room temperature) using a lyophilizer (Labconco, Kansas City, MO). After week 22, samples were rinsed without sonication because they were fragile and then dried as described above.

3. Results

3.1. Initial polymer foams

Polymer foam samples were characterized prior to degradation in terms of mass, molecular weight and morphology. Foam sections had an initial mass ranging from 13 to 17 mg and an initial M_w of 81,500 g/mol and polydispersity index (PDI) of 1.7, as determined by GPC. The foam samples revealed a complex morphology, as seen by LM (Fig. 1a–c) and SEM (Fig. 1d and e). Light micrographs demonstrated a uniform distribution of
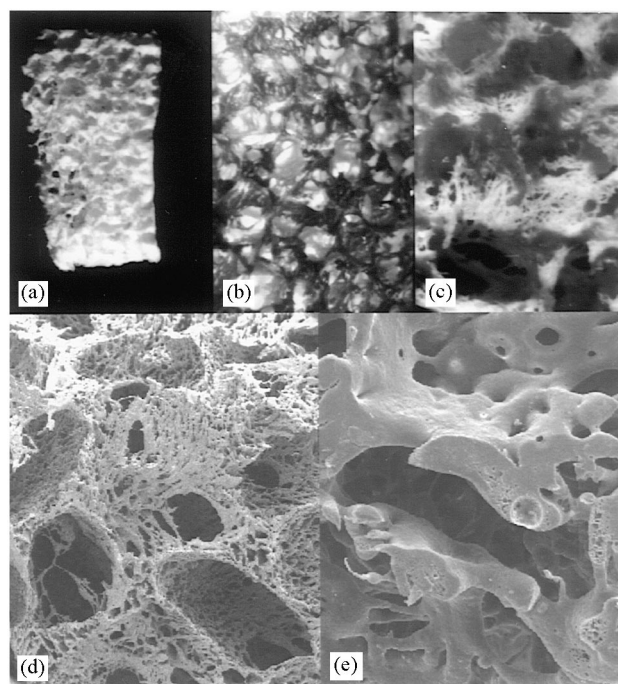

Fig. 1. Initial (non-degraded) control PLGA 75/25 foams as observed under (a, b and c) LM and (d, e) SEM. (a: field width = 13 mm, b: field width = 4.5 mm, c: field width = 3 mm, d: field width = 2.4 mm, e: field width = 0.44 mm).

interconnected macropores throughout the polymer matrix. The macropores varied in size from 0.8 to 1.5 mm and had irregular shapes. Macropores were connected by large interconnections (∼350 μm) and micropores (∼100 μm) within the pore walls. The thickness of these microporous walls was estimated at ∼300 μm (cf. Fig. 1c). The overall porosity was estimated from histomorphometry at 92% and confirmed by mercury porosimetry at ∼91%. Notwithstanding the corroboration between these data, mercury porosimetry was found to be unsuitable for our foam structures due to the inaccuracy of this technique for macroporous structures. Histomorphometry was unsuitable for our degraded foam samples due to their fragility at later time points when morphological changes were greatest.

When observed by SEM, the size of the macropores was confirmed to be approximately 1.5 mm (cf. Fig. 1d); the size of the micropores within the pore walls was confirmed to have an average size of 100 μm. Small concavities were scattered along the surface of the pore walls. The polymer surfaces were smooth and very few pores were seen within the bulk of the polymer walls (cf. Fig. 1e).

3.2. Change in mass

The mass loss data for foams degraded at pH 5.0, 6.4 and 7.4 are summarized in Fig. 2. During the first 16
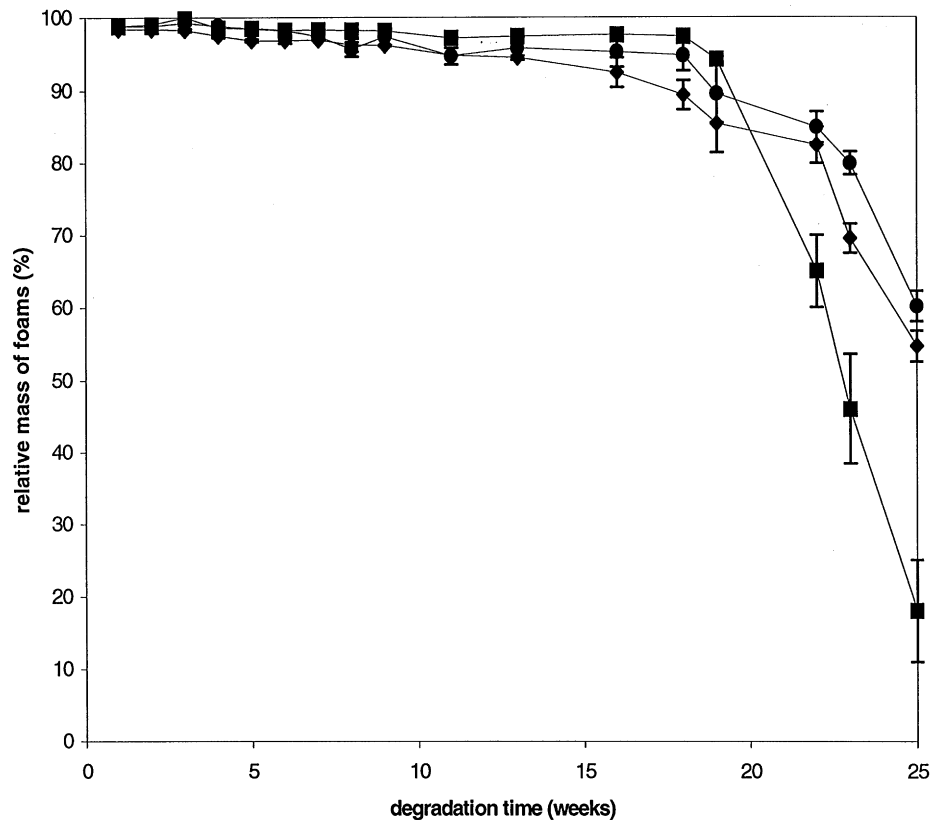
Fig. 2. The relative mass of PLGA 75/25 foams decreased over time due to degradation in the Na Cacodylate buffers maintained at: (■) pH 5.0, (●) pH 6.4 or (◆) pH 7.4.

weeks, a similar mass loss was observed for all foams maintained at pH 5.0, 6.4 and 7.4 and was lower than 3%. At week 16, samples maintained at pH 6.4 and 7.4 lost mass at a faster rate, losing a total of 45% of their original mass by week 25. From weeks 16 to 25, the degradation rate was estimated at 3.3% per week. Samples maintained at pH 5.0 showed an accelerated mass loss rate after 18 weeks of degradation, losing a total of 82% by week 25. From week 18 to week 25, the degradation rate was approximated at 9.6% per week. The mass loss rate of foams maintained at pH 5.0 was ∼3 times higher than that of foams maintained at pH 6.4 and 7.4.

3.3. Change in molecular weight

As shown in Fig. 3, the molecular weight (M_w) of the foams decreased with time, as determined by GPC. The molecular weight of all foams decreased at a constant rate of ∼3500 g/mol/week until week 16. After week 16, the molecular weight of foams maintained at pH 6.4 and 7.4 showed a reduced rate of molecular weight loss of 1300 g/mol/week, whereas foams maintained at pH 5.0 kept the same rate of molecular weight loss of 3500 g/mol/week.

3.4. Change in foam dimensions

Changes in dimension were monitored in terms of the overall height of the foam over 25 weeks for samples maintained at all three pHs (cf. Fig. 4). Until week 8, all foams lost less than 5% of their overall height and no noticeable differences were seen between foams maintained at any of the three pHs. At week 18, foams maintained at pH 5.0 had collapsed, losing 32% of their original height. Foams maintained at pH 6.4 and 7.4 also shrank between weeks 18 and 19, but lost only between 15 and 20% of their original height. By 25 weeks, foams maintained at pH 5.0 decreased further by 40% of their original height whereas those maintained at pH 6.4 and 7.4 decreased by 20%.

3.5. Change in morphology

For the first 8 weeks, no significant morphological differences were observed by LM between control and degraded foams maintained at any of the three pHs. Signs of degradation were only visible by SEM. As shown in Fig. 5a and b, cracks in the polymer structure were observed on all foams. An increase in bulk porosity was
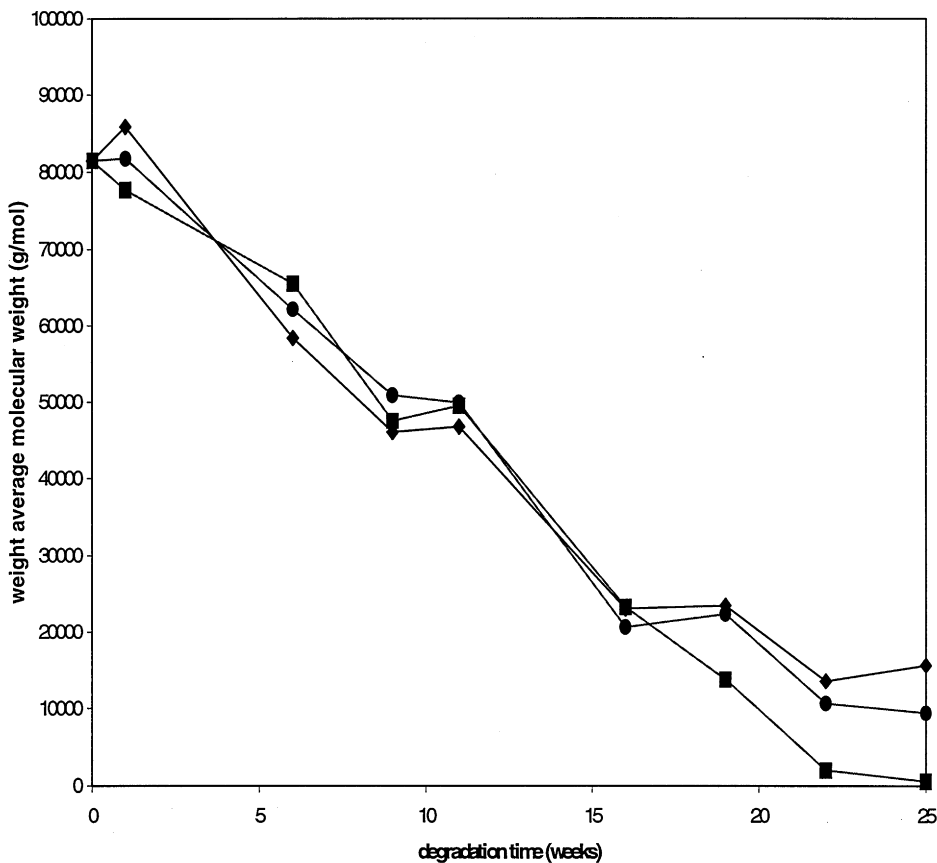
Fig. 3. The weight average molecular weight of PLGA 75/25 foams decreased over time due to degradation in the Na Cacodylate buffers maintained at: (■) pH 5.0, (●) pH 6.4 or (◆) pH 7.4.

also observed between control and degraded foams, as shown in Fig. 5c and d (arrows). Foams maintained at pH 5.0, 6.4 or 7.4 showed a similar extent of degradation and had a similar polymer skin morphology.

Morphological changes due to degradation were observed by LM only after week 18. As shown in Fig. 6, the overall morphology of the foams maintained at pH 6.4 and 7.4 was similar to that of control foams; however, the pore size decreased by $\sim 10\%$ and the pore wall appeared more compact, *as if* the foam had been compressed under a load (cf. Fig. 6a and b). Morphological changes observed on foams maintained at pH 5.0 were more pronounced than those observed on the foams maintained at pH 6.4 and 7.4. No resemblance between control and pH 5-degraded foams was observed. The original open cell morphology was now a closed-cell morphology, with a melted and shiny appearance. Small pores of ~ 0.1 mm were scattered throughout the polymer block (cf. Fig. 6c).

SEM observations revealed other signs of degradation at all three pHs. Within the bulk of the pore wall, the porosity of foams maintained at pH 6.4 and 7.4 increased over time with a noticeable difference observed between 8 and 18 weeks. Bulk porosity (i.e., within the pore wall)

was estimated by analyzing scanning electron micrographs with the NIH*image* analysis software and the areas occupied by pores vs. bulk polymer were measured. The amount of bulk porosity was thereby estimated to be $\sim 15\%$ after 8 weeks, and $\sim 35\%$ after 18 weeks (cf. Fig. 6d and e). The difference in bulk porosity between pH 6.4 foams and pH 7.4 foams was insignificant. However, a difference was observed in the smoothness of the polymer skin on the foams maintained at pH 6.4 and 7.4. While the polymer skin on pH 7.4 foams remained smooth, that on pH 6.4 foams was rough and had a wavy appearance; this was the first difference noticed between foams maintained at pH 6.4 and 7.4. At that same degradation time (18 weeks) pH 5.0 foams showed major morphological differences with respect to control foams or pH 6.4 and 7.4 foams. The foams maintained at pH 5.0 had a melted appearance with reduced macropores, ranging in size from 100 to 250 μm, that were bound by thick, smooth pore walls. Pores within the bulk of the polymer walls were observed to be perfectly round and may have resulted from trapped air/water bubbles rather than from degradation (cf. Fig. 6f).

No further changes were observed for pH 6.4 and 7.4 foams after 22 weeks. Foams maintained at pH 5.0,
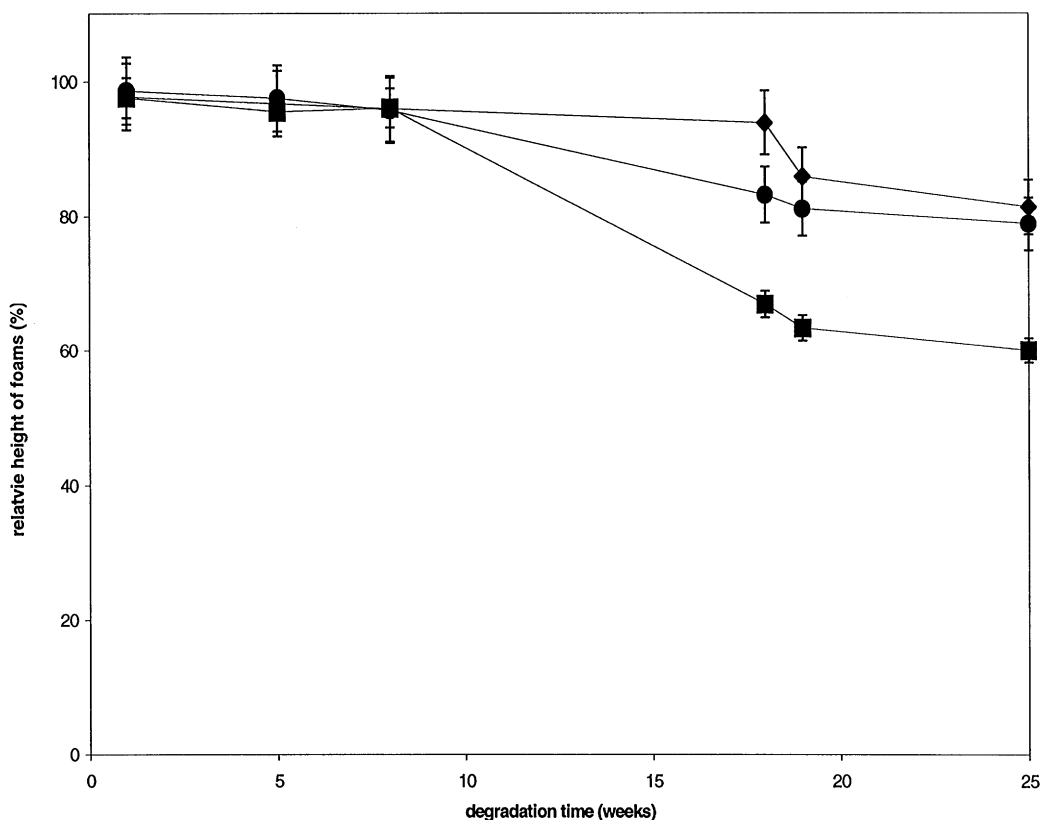
Fig. 4. The relative height of PLGA foams decreased over time due to degradation in Na Cacodylate buffers maintained at: (■) pH 5.0, (●) pH 6.4 or (◆) pH 7.4.

however, revealed a glassy outer appearance and an irregular shape; the superficial pores observed after 18 weeks had disappeared (cf. Fig. 7a and b). When observed by SEM, no differences in structure were found between the pH 5.0 foams that had degraded for 22 weeks and those that had degraded for 18 weeks.

After 25 weeks, pH 6.4 and 7.4 foams maintained their morphology yet were fragile and difficult to handle, making sectioning of the samples impossible; pH 5.0 foams had degraded to a granular structure. When observed by SEM, the polymer skin of the pH 7.4 foams was not smooth and resembled that of pH 6.4 foams after 18 weeks, which confirmed a slower but similar degradation profile for foams maintained at pH 7.4 and 6.4 (cf. Fig. 8). At week 25, the polymer skin of pH 6.4 foams was as porous as the bulk of the polymer, showing more degradation than the pH 7.4 foams. After 25 weeks, pH 5.0 foams collapsed into small particles; SEM observations revealed very thin film-like structures with rough surfaces.

4. Discussion

The degradation profile of PLGA 75/25 foams was followed in vitro at three constant pHs: pH 5.0, 6.4 and 7.4. As explained above, these pHs mimic three in vivo environments. Therefore, after different implantation periods in vivo, the PLGA 75/25 foams may experience either one or a combination of all three pHs. The in vitro mass, molecular weight and morphology changes were followed at each pH and over time to predict the in vivo degradation profiles of our foams.

As has been shown previously using other polyester devices [9], the molecular weight of our polymer foams decreased prior to their mass. Until week 16, the rate of mass loss of the polymer was insignificant whereas the rate of molecular weight loss was constant at 3500 g/mol/week; the mass of the polymer did not decrease significantly because degraded polymer chains may not have been able to leach out of the polymer foams. Mass loss for all foams became significant as M_w approached $= 20\,000$ g/mol, ca. week 16. From week 16 onwards, the mass of the foams diminished accordingly.

Morphological changes were followed by LM and SEM. Other techniques, such as mercury porosimetry and histomorphometry were unsuitable due to the macroporous (> 1 mm) nature of our foam samples and their increased fragility after 18 weeks, which precluded extensive handling. By LM and SEM observations, some new pores were observed in the bulk of the polymer structures of all foams after 8 weeks of degradation. The surface of
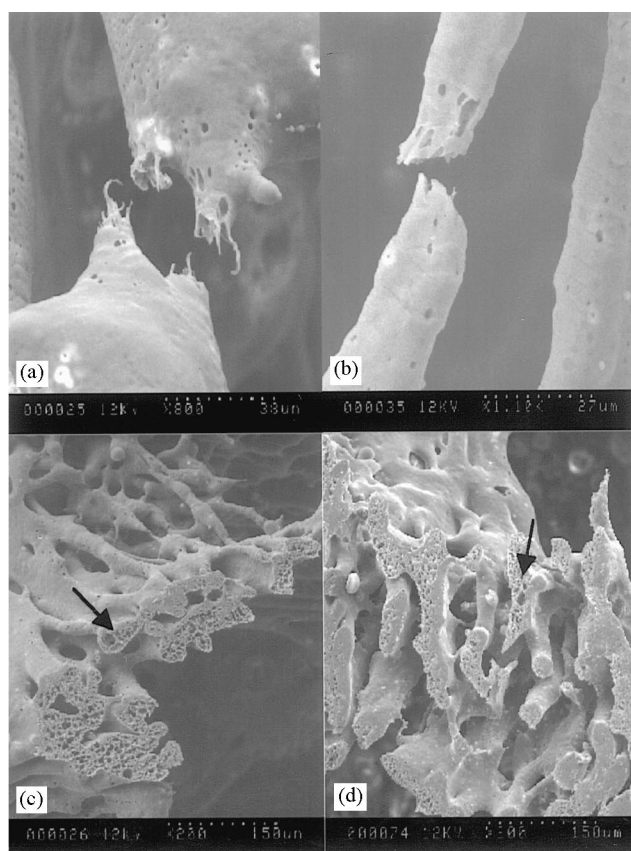
Fig. 5. Foams degraded for 8 weeks in all three pH buffers had cracks and micropores (arrows) in the bulk of the pore walls: (a) pH 5.0, (b) pH 6.4, (c) pH 5.0 and (d) pH 7.4. No differences were observed between the foams degraded at the different pHs.

the polymer (i.e., the polymer skin) started to show signs of degradation only after 18 weeks for foams maintained at pH 6.4 and 25 weeks for foam samples maintained at pH 7.4. Despite their unique three-dimensional structure and morphology, our foams demonstrated a bulk degradation profile similar to that observed for other polyesters. While PLGA is known to swell in aqueous media and, indeed, our foams seemed swollen while immersed in the buffers, no swelling experiments were conducted due to the complexity of the foams. (In order to perform a swelling experiment, excess water not absorbed by the polymer is removed by blotting; however, because our foams had a complex morphology, excess water would have likely remained trapped in the foam structure thereby producing misleading results.)

Given that: (1) molecular weight loss was observed prior to mass loss, (2) PLGA 75/25 does swell in aqueous conditions and (3) a bulk degradation profile was observed, it is likely that our foams degraded by an autocatalytic mechanism.

Previous studies have shown that the degradation of polyesters is dependent upon the morphology of the polymer device [9]. The morphology of foams main-

tained at pH 6.4 and 7.4 remained similar until the end of the degradation study, whereas that of foams maintained at pH 5.0 changed significantly after week 18. The pH 6.4 and 7.4 foams maintained a fiber-like pore wall structure for 25 weeks whereas the pH 5.0 foams adopted a 'melted' morphology after week 18. No large pores were visible in pH 5.0 foams beyond week 18 as the foams had collapsed. These samples were compact with very few small pores. The degradation profile of these compact samples was accelerated compared to that of other foam samples; this acceleration may result, in part, from an increase of bulk polymer volume in the samples. Such an increase was reflected by an increase in the pore wall thickness of foams which resulted in an autocatalyzed degradation profile.

The large macropores of foams maintained at pH 6.4 and 7.4 shrank after 18 weeks of degradation. Since the bulk porosity in the pore walls was very high ($\sim 35\%$) after 18 weeks, the pore walls may have been weakened by degradation and thereby affected by the drying procedure. Since the high vacuum used during the drying process may have changed the shape and overall size of the foam, the shrinkage observed may be an artifact.

The accelerated mass loss of PLGA 75/25 foams maintained at pH 5.0 after 18 weeks of degradation may have important implications in vivo. While it is unlikely that the in vivo pH would remain as low as pH 5.0 for extended periods of time, a sudden rise in lactic acid concentration would result in a sudden drop in the local pH. Degradation of PLLA/PLGA implants has been associated with delayed inflammatory responses at implantation sites, and it has been hypothesized that slow degrading polymers would not induce as intense an inflammatory response as fast degrading polymers [20]. Ricci [21] observed, using an in vivo canine chamber model, that newly formed bone tissue showed lower mineralization levels when grown between two surfaces of PLLA than between two surfaces of hydroxyapatite, commercially pure titanium or cobalt–chromium–molybdenum alloy. Surfaces of dimethyl-trimethyl carbonate or poly(desaminotyrosyl–tyrosine ethyl ester carbonate)—the degradation products of which are not acidic—also showed more bone ingrowth over a 6-week period. Ricci attributed the lack of mineralization observed between PLLA surfaces to by-products due to the degradation of the polymer. This acidity has major drawbacks for tissue engineering applications yet can be overcome by using a blend of PLGA 75/25 with varying molecular weights, as was demonstrated by Von Recum et al. [7] or using buffering salts within the polymer constructs [22].

5. Conclusion

The PLGA 75/25 macroporous foams maintained their morphology for more than 6 months at 37°C in Na
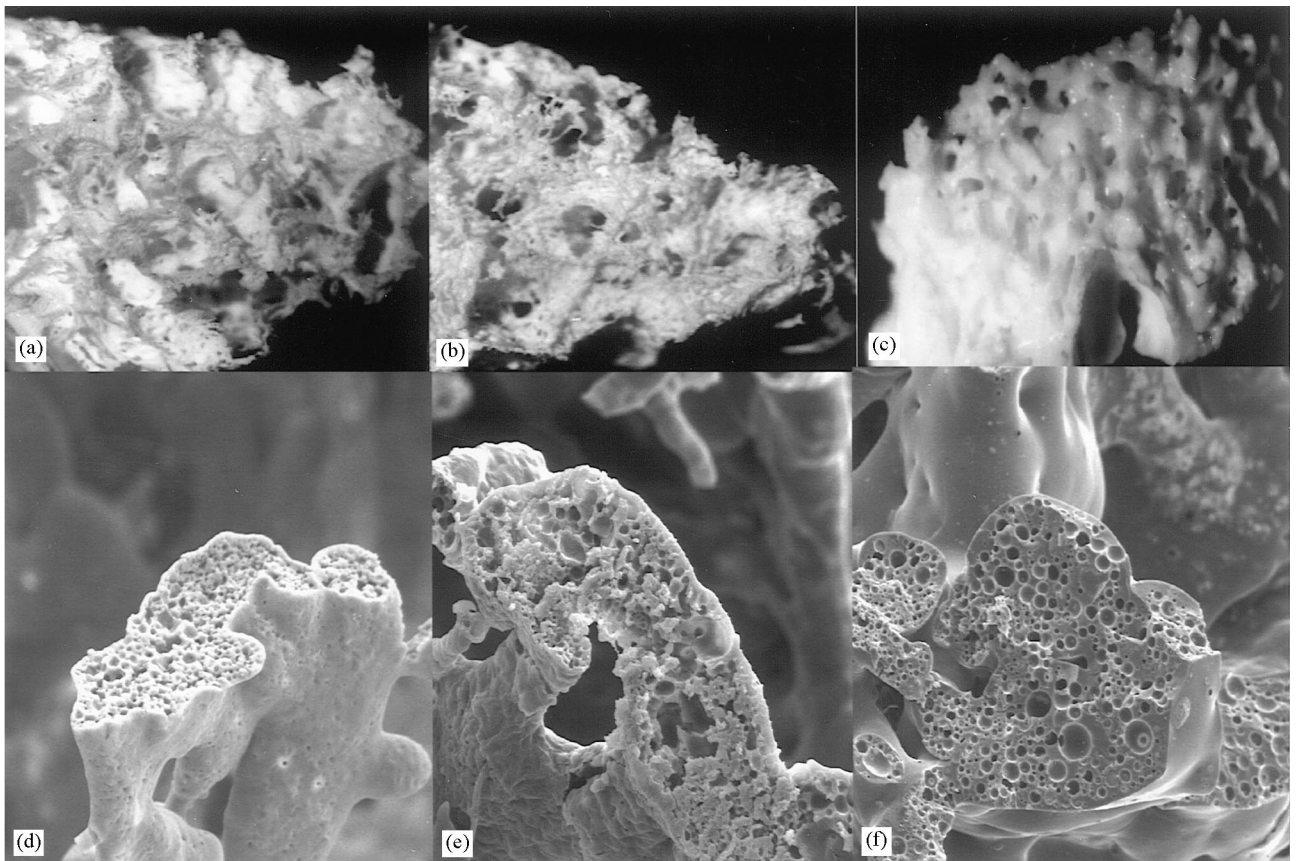
Fig. 6. The morphology of the foams degraded for 18 weeks in the different pH buffers was observed by LM (field width = 5 mm) and SEM: (a) pH 7.4 (LM); (b) pH 6.4 (LM); (c) pH 5.0 (LM); (d) pH 7.4 (SEM); (e) pH 6.4 (SEM); (f) pH 5.0 (SEM). The light micrographs indicate changes in the surface morphology of the foams. Those in pH 7.4 remain smooth, those in pH 6.4 are rougher and those in pH 5.0 are unrecognizable, having a 'melted' appearance (d: field width = 184 μm, e: field width = 273 μm, f: field width = 389 μm).
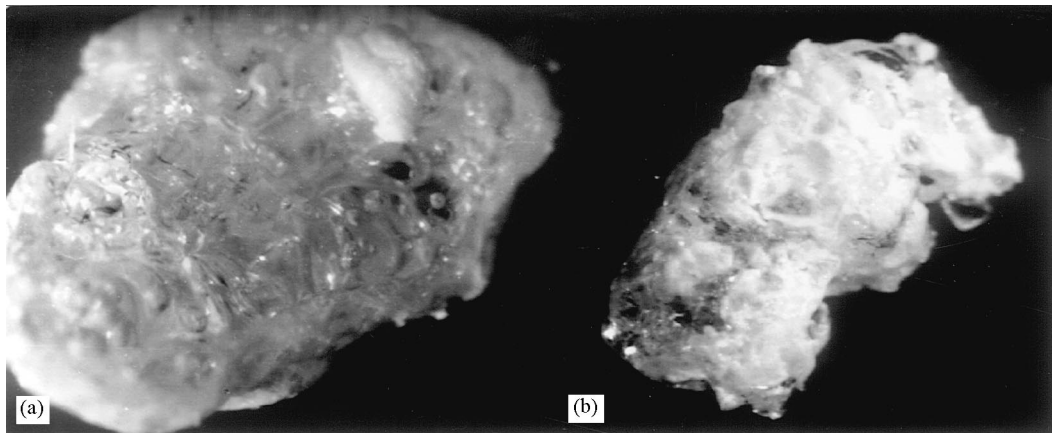

Fig. 7. Foams degraded for 22 weeks in the pH 5.0 buffer had a new, glass-like appearance as observed by LM (a: field width = 4.3 mm, b: field width = 3.3 mm). The foams shrank by approximately 40%.

cacodylate buffer solutions at pH 6.4 and 7.4. This indicates that these foams can be used in tissue engineering applications for tissues that regenerate within a similar timeframe. However, if an acidic environment persists at the implant site, as modeled in our in vitro study by the

pH 5.0 degradation profile, the timeframe for tissue engineering applications would be reduced to $\sim 4\frac{1}{2}$ months.

It is important to note that this in vitro study did not account for degradation associated with cell activity such
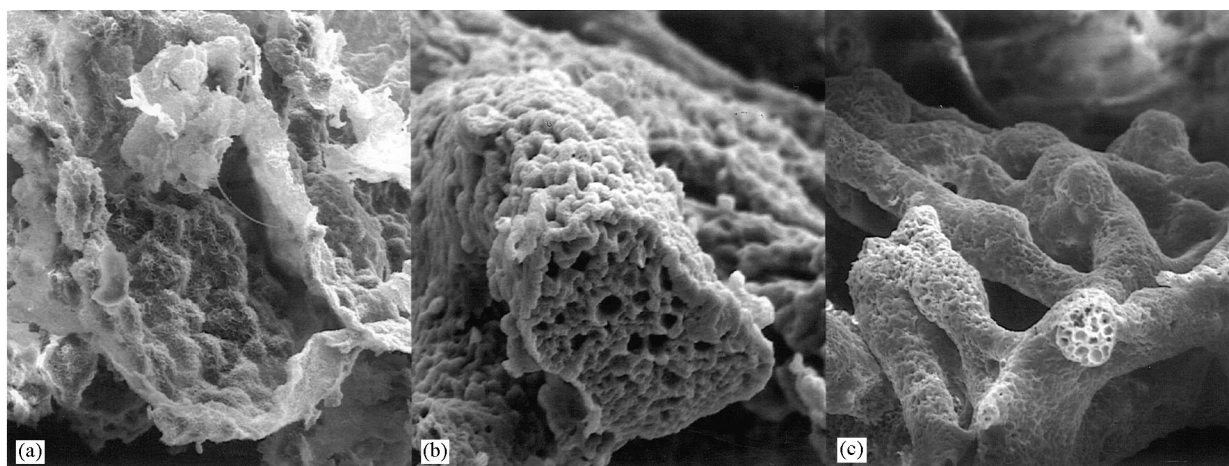
Fig. 8. The morphology of foams degraded for 25 weeks was observed by SEM: (a) foams degraded at pH 5.0 seemed to consist of small polymer fragments; (b) foams degraded at pH 6.4 had a completely porous surface; (c) foams degraded at pH 7.4 had a rougher surface morphology relative to that observed for pH 7.4 foams at 18 weeks (a: field width = 120 μm, b: field width = 97 μm, c: field width = 297 μm).

as contraction of the scaffold when colonized with fibroblast-like cells or enzymatic degradation. These biological phenomena may considerably modify the degradation profile reported herein.

References

[1] Engleberg I, Kohn J. Physico–chemical properties of degradable polymers used in medical applications: a comparative study. Biomaterials 1991;12:292–304.

[2] Vert M. Bioresorbable polymers for temporary therapeutic applications. Angew Makromol Chem 1989;166:155–8.

[3] Freed LE, Marquis JC, Nohria A, Emmanual J, Mikos AG, Langer R. Neocartilage formation in vitro and in vivo using cells cultured on synthetic biodegradable polymers. J Biomed Mater Res 1993;27:11–3.

[4] Mooney DJ, Park S, Kaufmann PM, Sano K, McNamara K, Vacanti PJ, Langer R. Biodegradable sponges for hepatocyte transplantation. J Biomed Mater Res 1995;29:959–65.

[5] Ishaug SL, Crane GM, Miller MJ, Yasko AW, Yaszemski MJ, Mikos AG. Bone formation by three-dimensional stromal osteoblasts culture in biodegradable polymer scaffolds. J Biomed Mater Res 1997;36:17–28.

[6] Holy CE, Shoichet MS, Davies JE. Bone marrow cell colonization of, and extracellular matrix expression on, biodegradable polymers. Cells Mater 1997;7:223–34.

[7] Von Recum HA, Cleek RL, Eskin SG, Mikos AG. Degradation of polydispersed poly(L-lactic acid) to modulate lactic acid release. Biomaterials 1995;16:441–7.

[8] Pistner H, Bendix DR, Muehling J. Reuther JF. Poly(L-lactide): a long-term degradation study in vivo. Biomaterials 1993;14: 291–8.

[9] Grizzi I, Garreau H, Li S, Vert M. Hydrolytic degradation of devices based on poly(DL-lactic acid) size-dependence. Biomaterials 1995;16:305–11.

[10] Vert M. Degradation of polymeric biomaterials with respect to temporary therapeutic applications: tricks and treats. In: Barenberg SA, Brash JL, Narayan R, Redpath AE, editors. Biodegradable materials. Boca Raton: CRC Press, 1990:11–37.

[11] Smith R, Oliver C, Williams DF. The enzyme degradation of polymers in vitro. J Biomed Mater Res 1987;21:1149–66.

[12] Williams DF. Mechanisms of biodegradation of implantable polymers. Clin Mater 1992;10:9–12.

[13] Black CM, Paliescheskey M, Beaman BL, Donovan RM, Goldstein E. Acidification of phagosomes in murine macrophages: blockage by Nocardia asteroids. J Infect Dis 1986;154(6):952–8.

[14] Holy CE, Davies JE, Shoichet MS. Bone tissue engineering on biodegradable polymers: preparation of a novel poly(lactide-co-glycolide) foam. In: Peppas NA, Mooney DJ, Mikos AG, Brannon-Peppas L. editors. Biomaterials, carriers for drug delivery, and scaffolds for tissue engineering. New York: AIChE 1997:272–4.

[15] Holy CE, Shoichet MS, Davies JE. Bone ingrowth on a novel PLGA 75/25 foam. Trans Soc Biomater 1998;21:145.

[16] Mikos AG, Sarakinos G, Leite SM, Vacanti JP, Langer R. Laminated three-dimensional biodegradable foams for use in tissue engineering. Biomaterials 1993;14:323–30.

[17] Thomson RC, Yaszemski MJ, Powers JM, Mikos AG. Fabrication of biodegradable polymer scaffolds to engineer trabecular bone. J Biomater Sci Polym Ed 1995;7:23–38.

[18] Geisow MJ. Fluorescein conjugates as indicators of subcellular pH. A critical evaluation. Exp Cell Res 1984;150:29–35.

[19] Geisow MJ. pH in the endosome: measurements during pinocytosis and receptor-mediated endocytosis. Exp Cell Res 1984;150:36–46.

[20] Boestman OM. Current concepts review—Absorbable implants for the fixation of fractures. J Bone Joint Surg 1991;73A:148–53.

[21] Ricci JL. Evaluation of degradation and biocompatibility of orthopaedic implant materials in the canine chamber model. Trans Soc Biomater 1997;20:116.

[22] Agrawal CM, Athanasiou KA. Technique to control pH in vicinity of biodegrading PLA–PGA implants. J Biomed Mater Res (Appl Biomater) 1997;38:105–14.