Technique to Control pH in Vicinity of Biodegrading PLA-PGA Implants

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Abstract: This *in vitro* study was performed to examine if the pH decrease in the vicinity of degrading polylactic acid (PLA) and polyglycolic acid (PGA) polymers can be offset by incorporation of basic salts within PLA-PGA implants. It has been suggested that such pH lowering results in adverse effects, which may be responsible for biocompatibility concerns raised recently about PLA and PGA polymers. The results indicated that all three salts investigated in this study were successful in controlling the decrease in pH due to the acidic degradation products of the copolymer. The pH of the test media for the control group fell to a value of 3.0 at 9 weeks. Implants containing calcium carbonate maintained the pH value between 7.4 and 6.3 throughout the degradation process. Implants with calcium hydroxyapatite and sodium bicarbonate controlled the pH values between 6.9 and 4.3 and 8.2 and 4.5, respectively. At 3 weeks, marked swelling of implants containing calcium carbonate or sodium bicarbonate was observed relative to the control implants. The molecular weight and mass changes in the implants did not show any significant differences at 9 weeks. Thus, results from this *in vitro* model show that a significant decrease in pH in the vicinity of PLA-PGA implants can be avoided by incorporating basic salts. © 1997 John Wiley & Sons, Inc. J Biomed Mater Res (Appl Biomater) 38: 105–114, 1997

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INTRODUCTION

As the fields of biotechnology and tissue engineering are gaining more prominence, the use of polylactic acid (PLA) and polyglycolic acid (PGA) materials to assist in tissue healing is also growing. Although they are considered to be generally biocompatible, several recent studies raised some questions.[1–6] For example, Böstman et al.[2] used PGA rods to repair malleolar fractures in 67 patients and reported the formation of an aseptic sinus in 25% of these cases. This occurred approximately 2 to 4 months after the operation and was accompanied by painful swelling at the ankle. However, no long-term effects were detected. In other studies, Böstman et al.[3,4] reported that on the average the incidence of such aseptic sinus formation occurred in approximately 7.9% of the patient population treated with biodegradable fixation devices. Bergsma et al.[5] treated displaced zygomatic fractures using PLA screws and plates and reported complaints of intermittent swelling at the site of implantation 3 years postoperatively. Osteolytic changes in the bone in the proximity of degrading PGA implants were reported in both humans and rabbits.[2,6] Other instances of the adverse effects of PLA-PGA materials were reviewed by Agrawal et al.[7] and Athanasiou et al.[8] Occurrences of a sterile sinus discharge or inflammatory discharge related to PLA-PGA devices were also reported in the field of podiatry.[9,10] Most complications outlined above occurred weeks or months postoperatively. Because infection was ruled out as a cause in all these cases, it is likely that the adverse response is related to the biodegradation of the PLA-PGA implants.

PLA, PGA, and their copolymers are polyesters belonging to the poly(α-hydroxy acids) group.[7] Upon degradation by hydrolysis, PLA and PGA produce lactic acid and glycolic acid, respectively, which are incorporated in the tricarboxylic acid cycle and are excreted as carbon dioxide and water. Because PLA-PGA materials undergo bulk degradation, the molecular weight of the polymer commences to decrease immediately upon contact with water, but its mass does not change significantly until the molecular chains are reduced enough in size to freely diffuse out of the polymer matrix.[11,12] This phenomenon often results in an accelerated decrease in implant mass weeks or months after the surgery, at which time the implants are reduced to particulate form. Accompanying this disintegration is a

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simultaneous increase in the release of acidic by-products of degradation. The delayed adverse biological response so often reported in humans is perhaps related to this voluminous release of particles or acidic by-products.

Suganuma and Alexander[13] studied the biological response of intramedullary bone to poly-L-lactic acid in a canine model and showed that while the poly-L-lactic acid initially exhibited excellent bone biocompatibility, its ''biocompatibility decreased dramatically'' upon implant degradation. The authors reported bone resorption in and around poly-L-lactic acid at long time periods and concluded that these deleterious effects were caused by degradation by-products. They also showed that this adverse response was not due to 40-μm polymer particles and speculated that a local decrease in pH around the implants was the cause. Daniels et al.[14,15] used a bioluminescent bacteria acute toxicity assay to evaluate the accumulated degradation products of several biodegradable polymers. They determined that both PLA and PGA produced toxic solutions, probably due to the acidic nature of their degradation by-products. These tests were performed *in vitro* and as a result ignored the buffering effects provided by body fluids. Nevertheless, it is possible that when PLA-PGA materials are implanted in large sizes or in anatomical regions without access to sufficient quantities of body fluids, the resulting release of large amounts of acidic degradation by-products might overwhelm the body's capacity to provide adequate local buffering. Such a scenario would lead to a lowering of the local pH and could be detrimental to the surrounding cells and tissue.

Therefore, the goal of the present study was to examine techniques to maintain the pH at nontoxic levels in the vicinity of degrading PLA-PGA implants. Methods to incorporate various buffering agents in PLA-PGA implants were developed and their efficacy in controlling the local pH was examined *in vitro.* Additionally, the degradation characteristics of these buffered implants were also examined.

MATERIALS AND METHODS

A 50:50 PLA-PGA copolymer (Birmingham Polymers Inc., Birmingham, AL) with an initial molecular weight of 53 kDa was used for this study. Three different basic compounds, calcium carbonate (CC), sodium bicarbonate (SBC), and calcium hydroxyapatite (HA) (Sigma Chemical Co., St. Louis, MO) were used in conjunction with this polymer to fabricate specimens. The polymer was first dissolved in acetone and precipitated in ethanol. Details of implant production were previously reported elsewhere.[16,17] A measured quantity (30% by volume of the dry polymer) of the appropriate buffering compound was then added to the gummy precipitate and mixed thoroughly to ensure a uniform distribution. These corresponded to the following inorganic weight fractions (basic compound mass/implant mass): 0.42 for CC, 0.35 for SBC, and 0.44 for HA. The composite was then packed into cylindrical cavities in a Teflon® mold and subjected to a regime of temperature and pressure to produce specimens with dimensions of 5 $\times$ 5 mm (height $\times$ diameter).

A total of 96 specimens were fabricated in this fashion. These specimens were divided into four groups of 24 specimens each: a control group and three test groups corresponding to the three basic compounds. Each of these groups was further subdivided into four sets corresponding to test periods of 0, 3, 6, and 9 weeks. Each specimen from the 3, 6, and 9 week sets was weighed and then immersed in 10 mL of distilled water and maintained at 37 °C. The average mass of the control specimens was measured to be 25.10 $\pm$ 3.03 mg. The pH of the medium was monitored every 2 days. At the end of each test period, the corresponding set was removed and the specimens dried in a vacuum for 72 h before analysis for mass, molecular weight, mechanical properties, and surface morphology.

The mass of the specimens was determined using an electronic balance (Mettler) with an accuracy of 1 μg. Molecular weight was estimated using gel permeation chromatography with chloroform at 34°C as the mobile phase and polystyrene standards. The mechanical properties of the specimens were measured under creep indentation conditions, using an automated indentation apparatus.[18] This system allowed the measurement of specimen indentation at 1 h under 0.083 N applied by a 1-mm diameter porous indenter tip. Deformation was then normalized with respect to specimen height to yield specimen surface axial strain, which is indicative of the specimen's compressive stiffness.[17]

RESULTS

Figure 1 shows the temporal variations of media pH for the four specimens groups. The control specimens maintained relatively neutral pH values up to approximately 24 days followed by a rapid decrease at a rate of 0.23 per day for 16 days. After approximately 40 days the media pH of the control specimens stabilized at an approximate value of 3. From all groups, the CC specimens exhibited the least variation in their media pH. At 0 days the CC pH was 7.4; following an approximately linear but small total decrease, the pH was 6.3 at the end of 9 weeks. The HA specimens exhibited an almost linear decrease in their media pH from 7.0 to 4.3 at a rate of 0.042 per day. The SBC specimens started out at a media pH of about 8.0 that changed to 7.0 by 38 days. However, between 5.5 and 7 weeks there was a precipitous decrease to 4.5 in SBC media pH, followed by a relatively constant value thereafter.

Figure 2 shows differences in mass loss as a function of time among the four groups. Mass changes were normalized with respect to each specimen's mass at 0 days. The control specimens experienced a 5% mass loss at 3 weeks, followed by a 55 and 99.5% mass loss at 6 and 9 weeks, respectively. The CC specimens experienced 2, 35, and

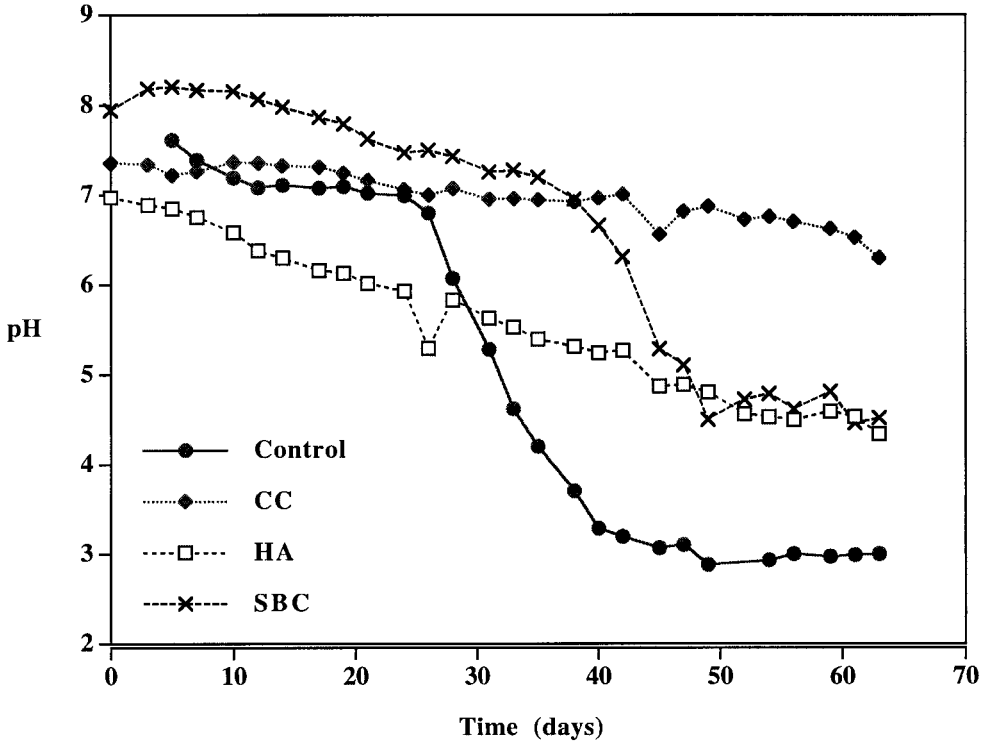


Figure 1. pH of the media in the vicinity of degrading control and buffered PLA-PGA specimens shown as a function of time.

89% mass loss at 3, 6, and 9 weeks, respectively. Similarly, the HA specimens experienced 8, 39, and 82% mass loss at 3, 6, and 9 weeks, respectively. The SBC mass loss was 28, 72, and 97% at 3, 6, and 9 weeks, respectively. Of all four groups, the control specimens followed the most linear decrease in their mass.

The temporal variations in the weight average molecular weight (MW) of controls and experimental groups are shown in Figure 3. After specimen fabrication, the initial MW of all groups was approximately 53 kDa, which did not differ significantly from the virgin polymer. The control specimens exhibited an approximate 61% reduction in MW

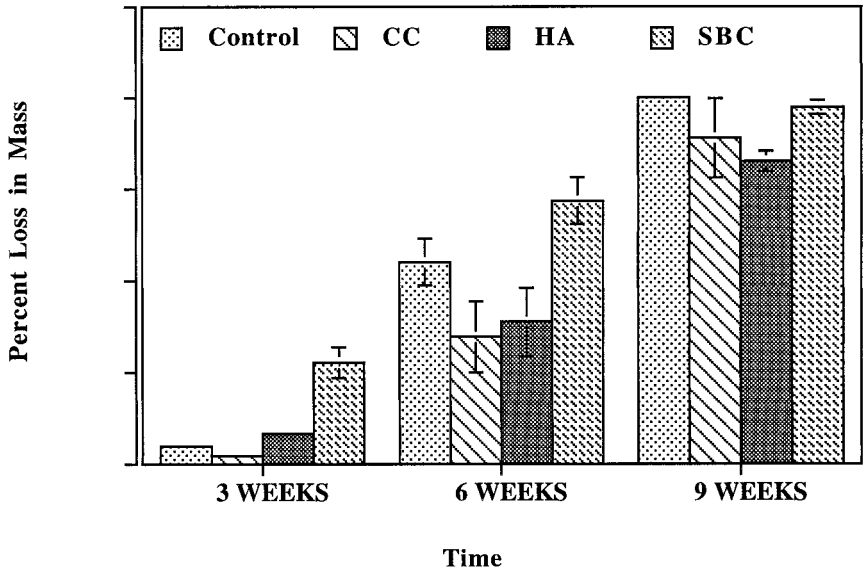


Figure 2. Percent mass loss as a function of time for degrading PLA-PGA specimens with different basic compounds.

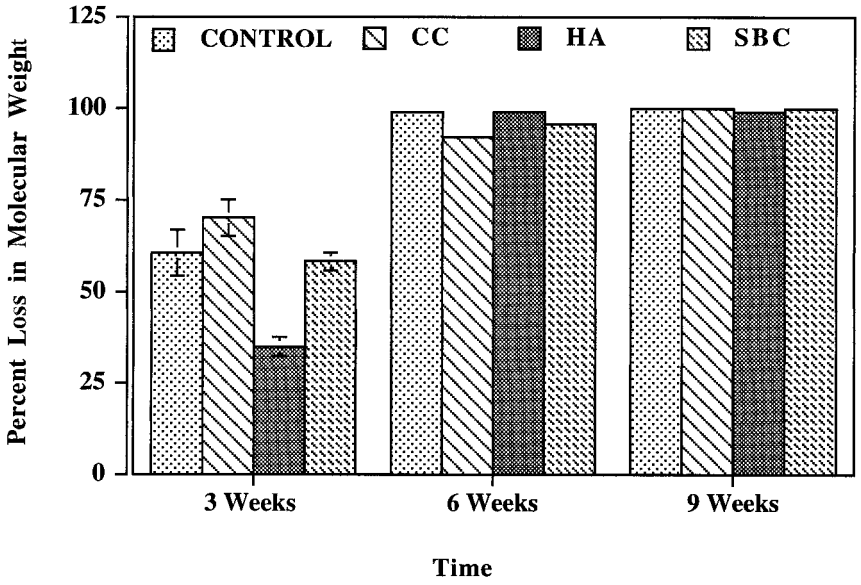


Figure 3. Temporal variations in percent loss in molecular weight of control and buffered PLA-PGA specimens.

by 3 weeks. This rapid decrease in MW of the control specimens was followed by another rapid decline in MW, such that by 6 weeks the MW decreased to less than 1 kDa. The CC specimen group experienced similar declines in MW from 70% at 3 weeks, to 92% at 6 weeks, to virtually 100% at 9 weeks. In contrast, HA specimens showed the smallest decline, 35%, in MW at 3 weeks. This was followed by a 99% decrease in MW by 6 weeks. The SBC specimens experienced 58, 96, and 99% decreases in their MWs at 3, 6, and 9 weeks, respectively. At 3 weeks the HA group showed the smallest decline in MW and the CC group exhibited the largest. However, by 6 weeks all specimen groups showed a virtually complete reduction in MW.

Figure 4 shows variations of specimen surface axial strain for all groups between 0 and 3 weeks. During this time period, significant stiffening was observed in the control and HA specimens, while both CC and SBC specimens became more compliant. By 6 weeks deformation data could not be obtained because all specimens had already undergone drastic losses in structural integrity. At 0 weeks CC specimens were the stiffest (1.4%) and control specimens were the most compliant (4.4%). In contrast, at 3 weeks control specimens were the stiffest (0.2%) while

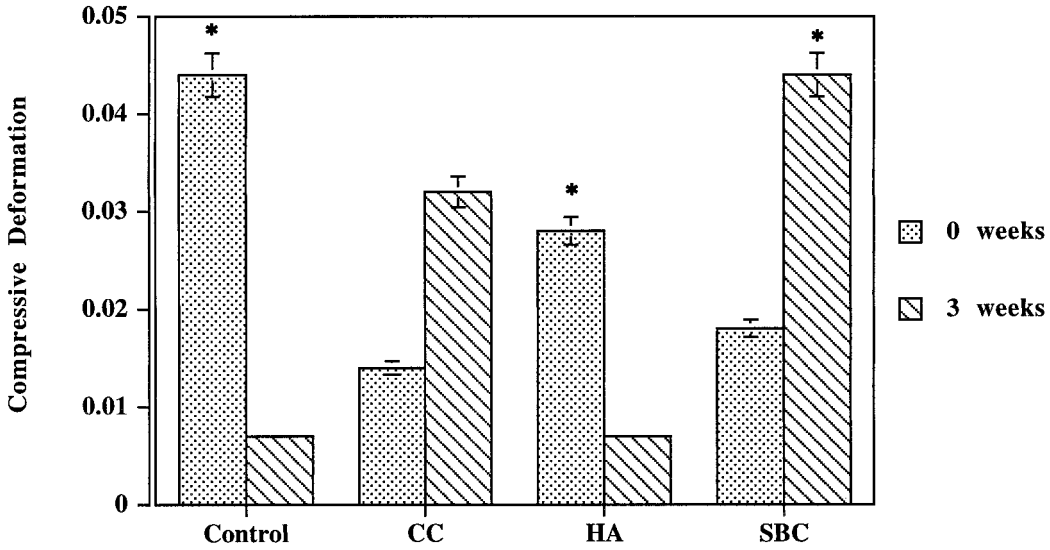


Figure 4. Changes in the surface axial strain as a function of time for PLA-PGA specimens containing different basic compounds.

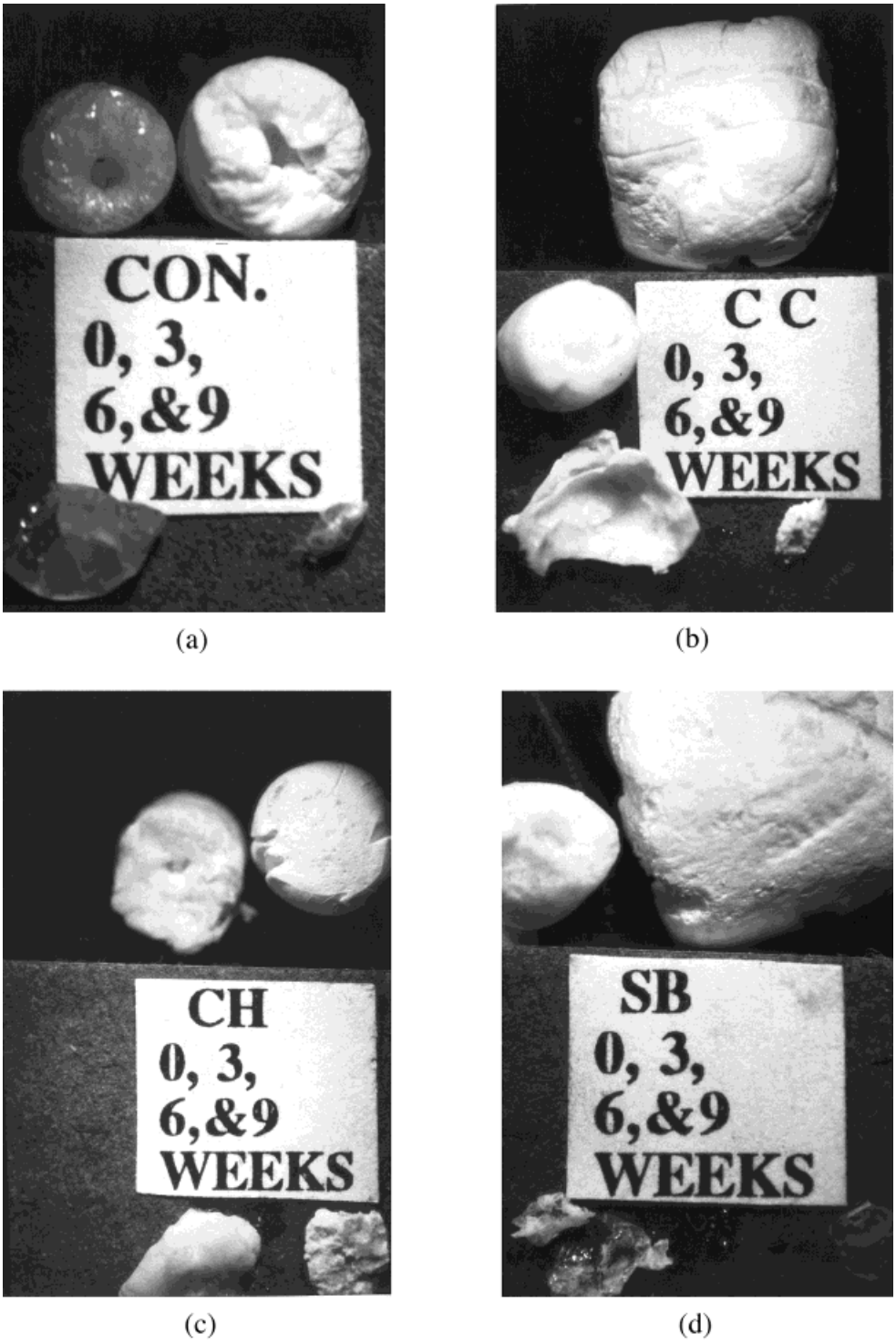
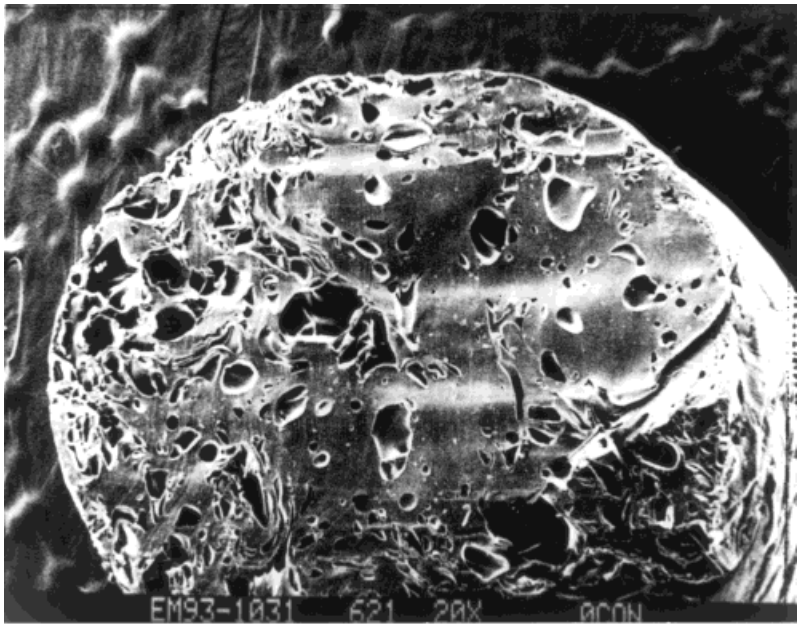


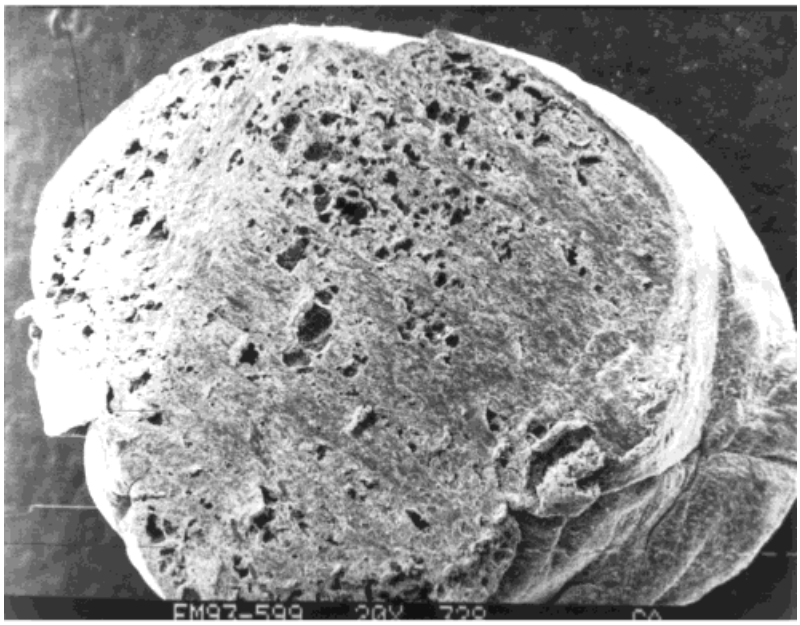
Figure 5. Time related changes in the appearance of specimens: (a) control, (b) CC, (c) HA, and (d) SBC. Both CC and SBC specimens exhibited dramatic swelling at 3 weeks. Photographs taken originally at 1 × 1 magnification.

SBC and CC specimens were the softest: 4.4 and 3.2%, respectively. Thus, control specimens showed a significant reduction in surface axial strain from 4.4 to 0.2%. In contrast, CC specimens became compliant (1.4% at 0 weeks to 3.2% at 3 weeks). HA specimens became stiffer with time (2.9% at 0 weeks to 0.7% at 3 weeks) and SBC specimens became significantly compliant (1.8% at 0 weeks to 4.4% at 3 weeks).

Gross morphologically, the four groups differed significantly from each other at each time point. Similarly, the



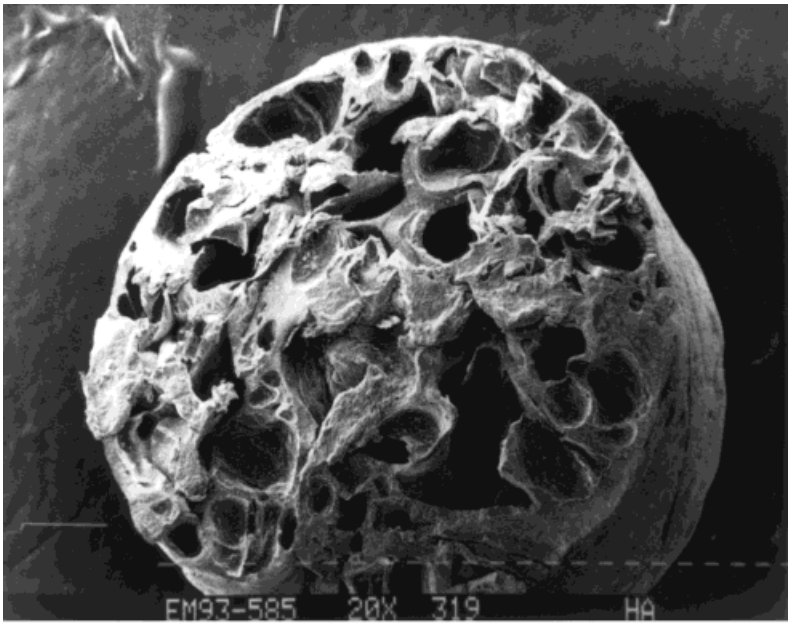
(a)



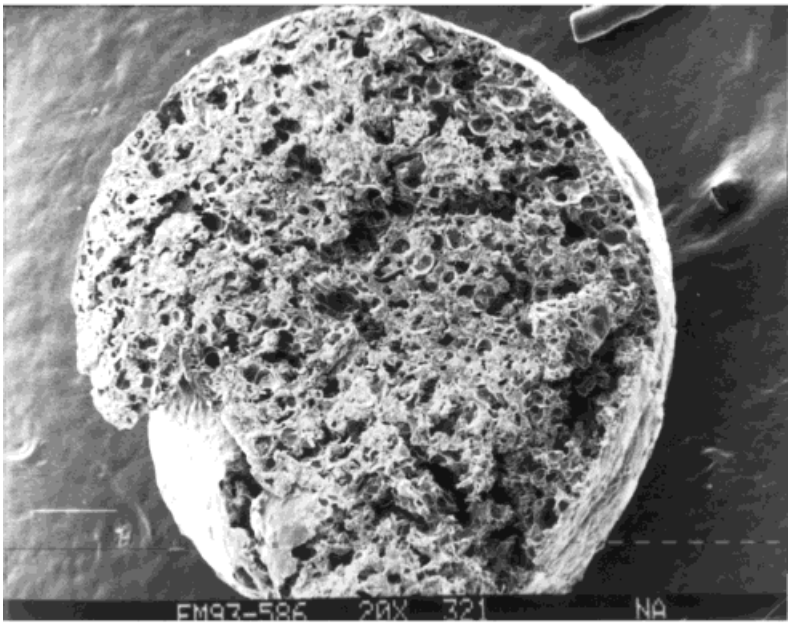
(b)

Figure 6. SEM micrographs of the cross-section of specimens as fabricated (0 weeks): (a) control, (b) CC, (c) HA, and (d) SBC. Original magnification ×20.

temporal variations in their appearance also followed a distinct path for each group. At 0 weeks all specimens were approximately cylindrical with dimensions of 5 × 5 mm. The three buffered groups were chalky white, while control specimens were relatively translucent. At 3 weeks specimens from all groups maintained their overall cylindrical shape and appeared chalky white, but different degrees of swelling were noted among the groups (Fig. 5). The least swelling at 3 weeks was exhibited by control and HA specimens. The highest degree of swelling was experienced by SBC specimens. Specifically, at 3 weeks SBC specimens experienced a three- to fourfold increase in their volume, CC specimens doubled their volume, the control specimens swelled marginally, and HA specimens exhibited no significant swelling. At 6 weeks not only did the swelling disappear but specimens were also approximately half the size of their original dimensions. They also appeared compliant, sticky, and easily malleable as a result of significant



(c)



(d)

Figure 6. (continued)

loss of original MW and mechanical stiffness. At 9 weeks all specimens exhibited virtually complete degradation.

SEM analysis indicated that incorporation of the three basic compounds had a significant effect on the microscopic appearance of the specimens (Fig. 6). It also verified that substantially different degradation pathways were followed by the four groups. Control specimens appeared porous at 0 and 3 weeks, turning into amorphous masses with few pores by 6 weeks, and essentially disappearing by 9 weeks. SBC specimens were also porous at 0 weeks and became significantly more porous at 3 weeks as a result of the significant amount of swelling they experienced. By 6 weeks, however, SBC specimens had few pores and lost their shape due to significant degradation. All SBC specimens were almost fully degraded by 9 weeks. Between 0 and 3 weeks CC specimens exhibited SEM characteristics similar to those of the SBC specimens. However, by 6 weeks CC specimens exhibited the formation of needle

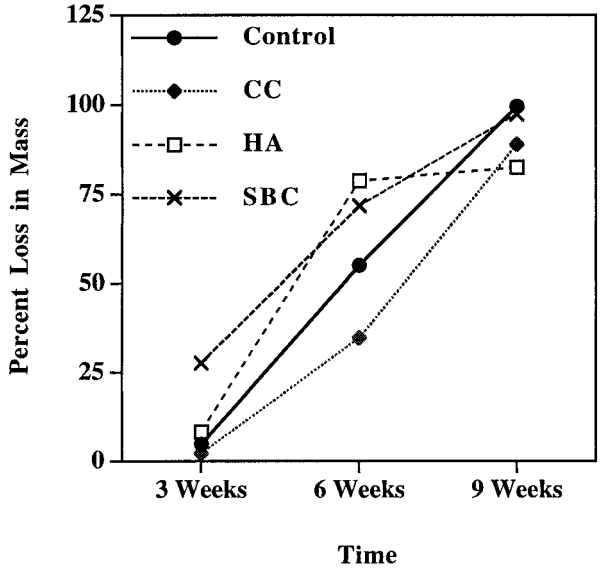


Figure 7. Relative rates of percent mass loss exhibited by specimens containing different basic compounds.

shaped crystals, which were not evident at 0 or 3 weeks. By 9 weeks all CC specimens were significantly degraded. At 0 weeks HA specimens were equally porous but the pore distribution was less homogeneous than in the other groups. For example, areas of nonporous material were surrounded by large pores (on the order of 0.5–1.0 mm). By 3 weeks HA specimens were more homogeneously porous. As in the other groups, by 6 weeks HA specimens lost their shape and were significantly disintegrated by 9 weeks. Based on gross morphological and SEM observations, it was also interesting to note the presence of a nonporous ''skin'' layer on the outer layers of specimens in all groups. This skin became slightly more porous with time, but it continued to be significantly less porous than the interior surfaces of all specimens.

DISCUSSION

MW

The four test groups exhibited different degrees of loss in MW at 3 weeks. For the control, CC, and SBC groups, this loss was in the range of 58 to 70%. In sharp contrast, the HA group underwent an average loss of only 35%. This occurred despite the fact that the HA specimens initially appeared to be more porous compared to the control and CC specimens. It is possible that the swelling of the control and CC specimens and the concomitant increase in volume at 3 weeks influenced their degradation kinetics. The HA specimens exhibited the least degree of swelling and also the least decrease in MW.

By 6 weeks all the groups tested exhibited greater than 90% loss in their MW and there were no significant differences between the groups. Earlier studies[17,19] on specimens similar to the controls in this study also demonstrated the rapid degradation in MW between 4 and 6 weeks. It is noteworthy that in the present study the pH of the media in the vicinity of the control specimens also decreased precipitously, starting at approximately the 4-week time point. This decrease in pH could result from the rapid breakdown of the polymer and the resulting elution of acidic degradation products during the same time period.

Mass

The mass loss exhibited by the control specimens was approximately linear with time (Fig. 7). The SBC group demonstrated significantly higher mass loss at both 3 and 6 weeks compared to the other groups. This decrease in mass might be related to the excellent solubility of the SBC in water: upon immersion of the specimen the salt accessible to the media immediately goes into solution, thereby reducing the weight of the specimen. This phenomenon is also reflected in the slightly elevated pH of the media during the first 12 days. At all time points the CC and HA specimens exhibited slightly lower mass loss compared to the other groups. At 9 weeks, although there was an approximate complete degradation of MW, the specimens from these two groups retained some mass, probably due to undissolved compounds.

Axial Strain

The surface axial strain for the control and HA groups decreased significantly between 0 and 3 weeks, indicating a stiffening of the specimens. Because 50:50 PLA-PGA is not a substantially crystalline material, this increase could not result from changes in crystallinity. It is possible, however, that there was retained solvent within the specimens that rendered them compliant. Upon immersion of the specimens in the test media, this solvent was likely to be diluted or washed out, leading to a stiffening of the specimen structures. In contrast to the control and HA specimens, at 3 weeks the surface axial strain for the CC and SBC specimens increased compared to the values at 0 weeks. As mentioned earlier, these specimens also underwent significant swelling. Any increase in stiffness due to the elimination of trapped solvent was perhaps offset by the increase in volume, salt dissolution, and the ensuing increase in compliance.

pH

The pH of the media for the control specimens showed a slight decrease for the first 12 days and then remained steady until 24 days. The initial decrease was perhaps due to the release of oligomers and other low MW fractions from the specimens upon immersion. The pH then remained constant until degradation reduced the MW of the polymer to an extent where a significant number of the

molecular chains were small enough to freely diffuse out of the specimen into the surrounding media. At this point the pH fell precipitously. Gibbons[20] suggested that the chains have significant solubility only when their MW has decreased to very low values (about 5 kDa). The inclusion of HA in the specimens resulted in a more linear decrease in pH and eliminated the sharp decrease as exhibited by the control group. Additionally, the HA maintained the pH at a level significantly higher than for the control group.

As mentioned earlier, the pH of the media for the SBC group first exhibited a slight increase for up to approximately 12 days, followed by a relatively slow rate of decrease up to 5.5 weeks. The pH then fell sharply to a value of approximately 4.5 and then remained steady. Thus, it is obvious that the SBC eluting from the specimens could control the pH only until 5.5 weeks, after which the amount of acidic degradation products exceeded the buffering capability of this compound. It is possible that the reservoir of SBC incorporated in the specimens was exhausted by 5.5 weeks because the SBC specimens showed a high degree of mass loss at both 3 and 6 weeks. On the other hand, the CC group maintained the pH of the media in the range of 7.4 to 6.3 over the test period of 9 weeks and was the most successful in controlling the pH.

Morphology

The implant production technique employed in this study resulted in implants exhibiting a significant, nonporous, or slightly porous skin on their surface. In other words, in all groups it was observed that internal porosity was substantially higher than surface porosity. As a result, it is expected that the skin will provide an impermeable barrier between the implant's bulk and surrounding media or tissues, which can significantly hinder evacuation of degradation by-products from the implant's interior. Accumulation of acidic by-products in the implant's interior may accelerate local autocatalysis. It may also result in rapid lowering of the pH in the implant's vicinity, once the skin is significantly permeabilized. On the other hand, a skinless implant may allow for continuous evacuation of acidic by-products from the implant's interior, reduce local autocatalysis, provide faster and easier pathways for cell infiltration and tissue in growth, and behave overall as a more suitable scaffold for tissue engineering. The buffered implants of this study also exhibited varying degrees of swelling, which may not be desirable in applications where exact geometrical dimensions are critical. On the other hand, swelling may be beneficial in obtaining better tissue–implant juxtaposition in press-fit situations.

Study Limitations and Future Work

A limitation of this study is the use of unbuffered, distilled water as the medium of degradation. This lack of buffering is nonphysiological because natural buffering agents are present *in situ,* which may ameliorate the sudden decrease in pH in the vicinity of the biodegrading implant. Furthermore, the degradation medium (10 mL distilled water plus degradation by-products) of each implant was not changed in this study; instead it was monitored unperturbed for the duration of the study. Again, such a scheme does not allow for fluid flow and by-products evacuation, which may be intrinsic at the *in vivo* site of implantation. However, the degree of by-product evacuation will depend on the anatomical site of implantation and may not be significant in highly avascular tissues such as articular cartilage. The objective of this study was to compare the three groups of implants with pH-controlling agents to controls under standardized conditions. Simulating *in vivo* buffering and fluid flow in this *in vitro* study would be a much better design, but unfortunately critical parameters defining the *in vivo* and *in situ* situation are lacking. To alleviate such shortcomings, it is imperative that studies be performed to define *in vivo* and *in situ* buffering and fluid flow parameters, which need to be incorporated in *in vitro* models of degradation.

In the present study the amount of basic compounds used per specimen was 30% by volume. This amount was chosen to ensure interconnectivity between the particles of the compound to obtain good diffusion characteristics. However, lesser quantities may be sufficient *in vivo,* where the body itself will provide assistance in buffering the acidic by-products. Thus, further *in vivo* studies are required to investigate the efficacy of buffering PLA-PGA materials and the effects of the buffering agents on the surrounding tissue. If in future studies it is ascertained that the drop in local pH caused by PLA-PGA specimens *in vivo* indeed is deleterious to the surrounding tissue, then the techniques described in this study could be further developed and fine-tuned to successfully address that problem.

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