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The influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterials

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The *in vitro* degradation of biodegradable polymer/ceramic composites was assessed in two different environments under both static and pseudodynamic conditions. The blends, consisting of polycaprolactone, poly(lactic-*co*-glycolic acid), and hydroxyapatite, have potential use in bone tissue engineering applications, thus it is essential to establish a standardized method of characterizing the degradation of new biomaterials. In this study, the variation in polymer blend ratio was examined to observe a change in degradation rate. The porous blends were degraded in water and serum-containing media. A previous study examined *in vitro* degradation in serum-free buffer. Molecular weight loss, gravimetric weight loss, pH changes and morphological changes were evaluated. The changes in porosity were observed with scanning electron microscopy and quantitatively assessed using image analysis. There was a significant difference in molecular weight loss and gravimetric weight loss between the blends after 10 weeks *in vitro*. Blends containing the greatest amount of poly(lactic-*co*-glycolic acid) degraded most rapidly.

1. Introduction

Biomaterials for tissue engineering applications include polymers, ceramics and polymer/ceramic composites [1]. A standardization of protocols for characterization of the degradation properties of these biomaterials is necessary. Agrawal has extensively characterized the *in vitro* degradation of biodegradable polymers such as poly (lactic-*co*-glycolic acid) (PLGA) [2, 3]. We have previously reported the fabrication of porous scaffolds consisting of a blend of two biodegradable polymers and hydroxyapatite [4]. We have examined this material both *in vitro* [5] and *in vivo* [6], and now we present our results of a systematic study of the factors that can affect degradation rate.

Our materials consist of two biodegradable polymers, polycaprolactone (PCL) and PLGA, and the bioceramic hydroxyapatite. The degradation rates of these composites can be controled by varying the material composition. An *in vitro* study aimed at determining the effects of altered polymer blend ratios on degradation rate was conducted. Both static and ''dynamic'' conditions were employed for the 10-week study. The degradation rate was determined by analysis of gravimetric weight loss, molecular weight loss, and morphological changes after incubating the polymer/ceramic materials at 37 °C in one of two solutions: water or serum-containing media. Additionally, the pH changes in the environment were determined. The effects of acidity of the degradation products on weight loss were assessed, as well as the effects of constant media changes. The changes in porosity were observed with scanning electron microscopy and quantitatively assessed using image analysis.

2. Materials and methods

2.1. Materials

Polycaprolactone [Aldrich (Mw 65 kDa)], poly(D,L-lactic acid-*co*-glycolic acid) [Mw 40-65 kDa, (65 : 35), Aldrich], hydroxyapatite [Aldrich], and tetrahydrofuran [Fisher] were all used as received. Phosphate-buffered saline (PBS) tablets were purchased from Sigma. NaCl [Aldrich] was sieved into particles of diameter 150–250 microns using ASTM-standard brass sieves (Fisher). Alpha minimum essential medium/Ham's F-12 nutrient mixture, supplemented calf serum, penicillin/streptomycin, and glutamine were purchased from Sigma.

2.2. Preparation of polymer scaffolds

Polymer scaffolds were prepared using a particulate-leaching technique as described by Mikos *et al*. [7]. The

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polymers were dissolved in tetrahydrofuran (THF) at room temperature (7–10% w/v). Sieved NaCl (150–250 μm particle size), and hydroxyapatite were suspended in the solution and sonicated for 60 s. The commercial, nonsintered hydroxyapatite [$Ca_{10}(PO_4)_6$-$(OH)_2$], was $\sim$ 10 microns in size (as analyzed using SEM) and the X-ray diffraction pattern demonstrated strong peaks, indicating a crystalline structure. The average specific surface area of HA was 67 m^2/g (BET analysis); based on the particle size, this indicates a porous structure. After evaporation of the solvent, the scaffolds were pressed at a pressure of 6000–10000 psi using a Carver hydraulic press, model 100. The applied pressure controlled the thickness of the scaffolds to 1 mm. The scaffolds were immersed in distilled water to dissolve the NaCl and dried. Blends of 10/90 and 40/60 were prepared (10% PCL and 90% PLGA; 40% PCL and 60% PLGA, w/w, respectively) with 10% HA (w/w). Porosity was 80% (as controlled by the amount of NaCl incorporated). The scaffolds were cut into 5 × 5 mm squares. The scaffolds were sterilized by washing with ethanol twice, sterile water four times, and sterile PBS buffer twice, all over a 3-h period. The scaffolds were dried under sterile conditions and the weights were recorded (20 $\pm$ 1 mg). SEM analysis revealed a homogeneous distribution of the hydroxyapatite throughout the composite.

2.3. *In vitro* degradation studies

Weight loss during storage at 37 °C in sterile water, PBS (pH 7.4) [4] or serum-containing media was determined for the scaffolds. Serum-containing media consisted of alpha minimum essential medium/Ham's F-12 nutrient mixture (alpha/F12, 1/1) containing 10% supplemented calf serum, penicillin/streptomycin (50 U/mL/50 μg/mL), and 2 mM glutamine. For static studies, the water remained unchanged for 1, 2, 4, 8, and 10 weeks, and the media remained unchanged for 1, 2 and 4 weeks. The pH of the water and the media was recorded at the end of the time points with an Accumet® pH/conductivity meter, model 20. For the dynamic studies, the water/media was changed every 2–3 days as adapted from studies previously described [2–4]. At the end of the study, the scaffold was removed, rinsed with distilled water, and dried for measurement of weight loss at 1, 2, 4, 8, and 10 weeks. The results are reported as the mean $\pm$ standard error of the mean of six measurements. The specimens were then examined for molecular weight loss and morphological changes.

2.3.1. Molecular weight determination

Gel permeation chromatography (GPC) was carried out using a Styrogel column equipped with a Waters 510 programmable pump and a Waters 410 differential refractometer. Molecular weights are relative to monodisperse polystyrene standards (Waters). The solvent used was THF and the hydroxyapatite was removed with a 0.45-micron syringe filter prior to injection. The results are reported as the mean $\pm$ standard error of the mean of four measurements.

2.3.2. Electron microscopy analysis and preparation

Specimens for scanning electron microscopy (SEM) were mounted on aluminum specimen stubs and coated with gold using a Pelco SC-2 sputter coater. The samples were viewed using a Hitachi H-2460N SEM at 5 keV. Images were digitally recorded (TIFF image format) using a PC-based Quartz PCI image management system (Quartz Imaging Corporation, Vancouver, Canada).

2.3.3. Image analysis

TIFF images of scaffold cross-sections obtained with SEM were transferred into NIH image 1.61. The porosity of the polymer was determined by dividing the number of pixels on the surface by the total number of pixels in the image. The results are reported as the mean $\pm$ standard error of the mean of the analysis of four cross-sections per sample.

2.4. Statistical analysis

Comparisons of means representing multiple measurements over time, were analyzed using repeated-measures analysis of variance (ANOVA) followed by protected *t*-test. Comparisons of means within a single time period were analyzed by one-way ANOVA. Mean differences were determined via Tukey's multiple comparison procedure. The threshold for statistical significance was set at $p \leq 0.05$.

3. Results

By simply altering the polymer blend ratio, we hoped to obtain a significant difference in molecular weight loss, gravimetric weight loss, and porosity, as well as link those changes to the surrounding pH of the environment.

3.1. Molecular weight loss

Fig. 1 indicates the molecular weight loss of the composites. Gel permeation chromatographs displayed a single modal spectrum until 8 weeks. The molecular weight of PCL and PLGA are similar thus the GPC resulted in a single peak with polydispersity indices of 1.66 for 10/90 and 1.65 for 40/60. After 8 weeks degradation, however, a bimodal peak appeared for 40/60, indicating the slower degradation of the more abundant PCL.

There was a dramatic decrease in the molecular weight after 8 weeks in water under static conditions. After 10 weeks in water, there was not a significant difference in weight loss between static vs. dynamic conditions for 10/90 ($p = 0.17$), but there was a significant difference for 40/60 ($p \leq 0.05$). Furthermore, there was a significant difference between the two composites under both conditions ($p < 0.001$, static and dynamic).

Fig. 2 displays the molecular weight loss in media. This study was conducted for 4 weeks and as with water, there was not a significant difference in weight loss between static vs. dynamic conditions for 10/90 ($p = 0.08$), but there was a significant difference for 40/60 ($p < 0.05$) in media at 4 weeks. There was a

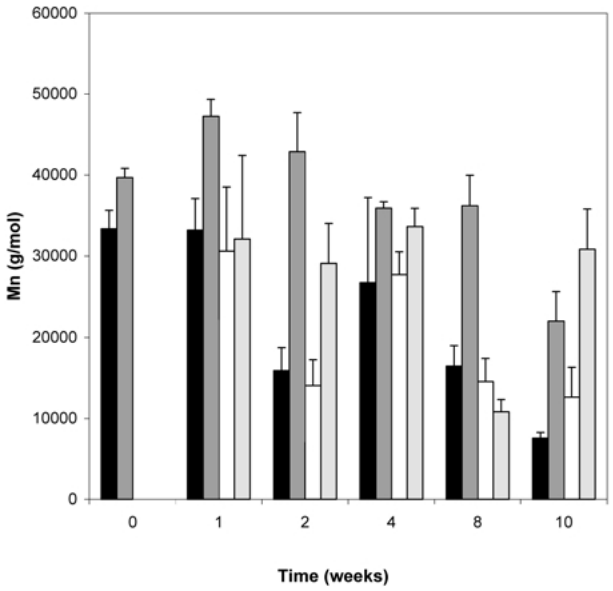

Figure 1 Molecular weight loss in water. The number average molecular weight of the polymer/ceramic composite treatment groups: ■ = 10/90 dynamic conditions, ▩ = 40/60 dynamic conditions, □ = 10/90 static conditions, ▨ = 40/60 static conditions, were determined by GPC. Bars represent the standard error of the mean of four measurements.

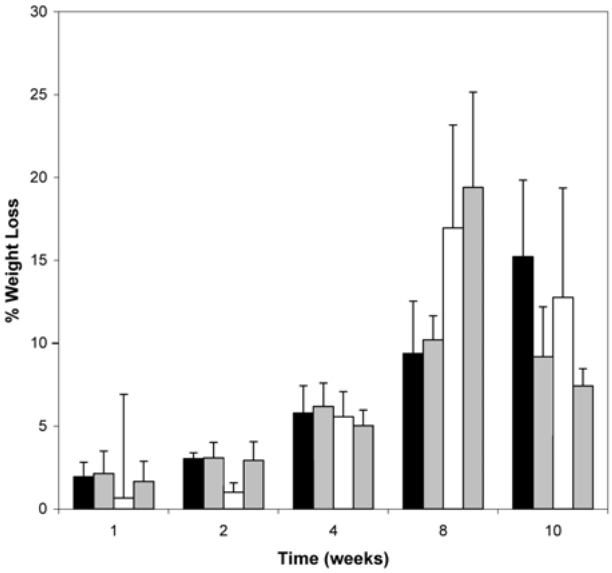

Figure 3 Gravimetric Weight Loss in Water. The mass loss of the polymer/ceramic composites was measured for treatment groups: ■ = 10/90 Dynamic conditions, ▩ = 40/60 Dynamic conditions, □ = 10/90 Static conditions, ▨ = 40/60 Static Conditions. Bars represent the standard error of the mean of six measurements.

significant difference between the two composites under both conditions ($p < 0.001$, static and dynamic).

3.2. Gravimetric weight loss

Fig. 3 displays the gravimetric weight loss of the composites in water. There is not a significant difference between composites until week 10 ($p = 0.002$ static, $p = 0.0001$ dynamic). Additionally, there is a significant difference between static vs. dynamic conditions in water during week 8 ($p < 0.001$).

3.3. Morphological changes

Fig. 4 displays the changes in porosity at 4 and 10 weeks of the composites (initially 80% porous), in water. At 4 weeks, porosity has slightly increased. At 10 weeks, there is no significant difference in static vs. dynamic conditions in water ($p = 0.49$ and 0.33, respectively), but the 10/90 composite is significantly more porous than 40/60 under dynamic conditions ($p = 0.047$).

3.4. pH changes

Fig. 5 indicates the pH changes of water after 10 weeks. After 1 week, there was a significant decrease in the pH of both composites ($p < 0.001$), and at 8 weeks, the pH was lowest at 4.57 and 4.84 for the 10/90 and 40/60 composites, respectively. There was a significant difference between static vs. dynamic conditions ($p < 0.001$), as well between the composites, at 8 weeks ($p < 0.001$). However, there was not a significant difference between the composites at 10 weeks, under both conditions ($p = 0.78$ and 0.20, 10/90 and 40/60,

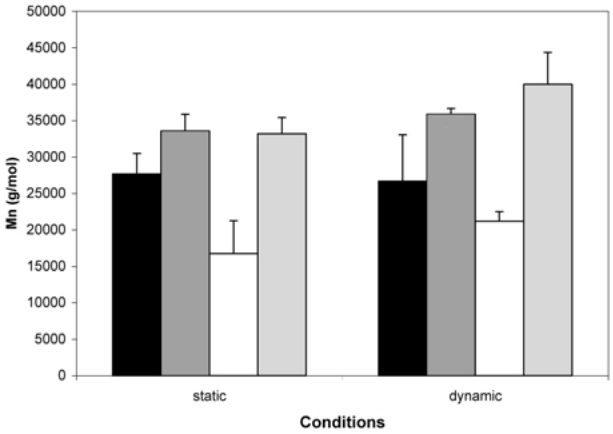

Figure 2 Molecular weight loss in water vs. media at 4 weeks. The number average molecular weight of the polymer/ceramic composite treatment groups: ■ = 10/90 water, ▩ = 40/60 water, □ = 10/90 media, ▨ = 40/60 media, were determined by GPC. Bars represent the standard error of the mean of four measurements.

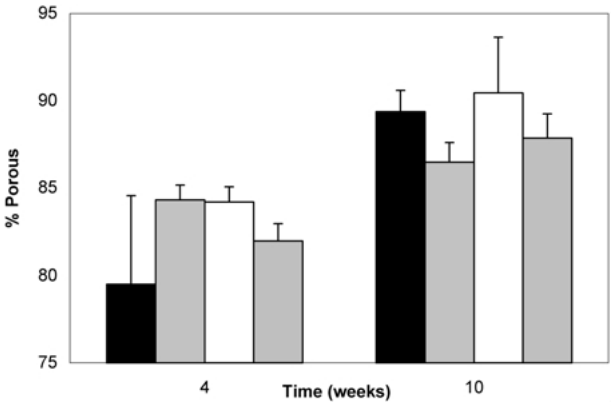

Figure 4 Porosity changes water. The porosity of the polymer/ceramic treatment groups: ■ = 10/90 dynamic conditions, ▩ = 40/60 dynamic conditions, □ = 10/90 static conditions, ▨ = 40/60 Static conditions, was determined using image analysis of SEM images. Bars represent the standard error of the mean of four measurements.

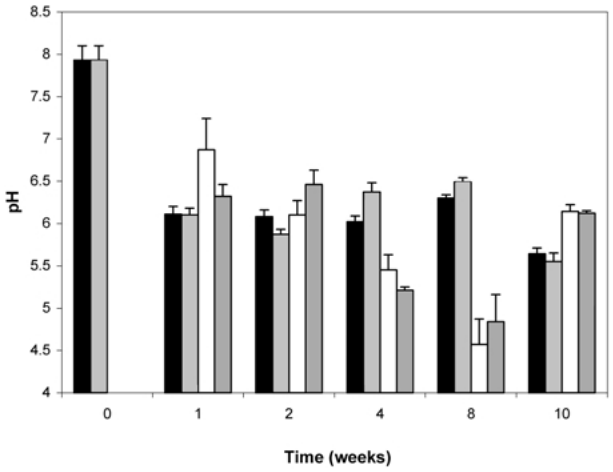

Figure 5 pH changes in water. pH was measured at various time points for polymer/ceramic treatment groups: ■ = 10/90 dynamic conditions, ▩ = 40/60 dynamic conditions, □ = 10/90 static conditions, ▨ = 40/60 static conditions, in both static and dynamic water conditions. Bars represent the standard error of the mean of six measurements.

respectively). However, there was a significant difference *between the conditions*, with dynamic conditions creating a significantly lower pH for both composites at 10 weeks ($p < 0.001$).

4. Discussion

The area of bone tissue engineering is a rapidly growing field. As such, the evaluation of biodegradable scaffolds both *in vitro* and *in vivo* is being examined. Numerous groups have examined the *in vitro* degradation of polymeric scaffolds in water or buffer solutions [2–4, 8–18]. Polymer blend degradation as well as polymer/ceramic composite degradation has also been examined. We have examined PCL, PLGA and HA composites and reported gravimetric weight loss in buffer over 8 weeks [4]. In an attempt to further characterize the degradation of these polymer/ceramic composites, a more comprehensive study was conducted. Porous polymer/ceramic scaffolds were degraded in serum-containing media as well as water. Each scaffold contains 10% HA by weight. The HA is evenly distributed throughout the scaffolds. The 10/90 scaffold consists of 10% PCL and 90% PLGA whereas the 40/60 scaffold consists of 40% PCL and 60% PLGA. We hypothesize that by simply altering the polymer blend ratio, we can obtain a significant difference in gravimetric and molecular weight loss, and porosity since the polymers have different degradation rates (PCL degrades more slowly than PLGA). We also expected to observe a correlation between the pH of the surrounding environment and those changes. Recently, Agrawal *et al*. has blended PLGA with varying molecular weights in an attempt to control the degradation rate [3]. The addition of basic calcium-containing salts to PLGA was also examined by Agrawal *et al*. in a successful attempt to increase the pH of the surrounding medium [2]. In our study, the pH of the surrounding medium was measured at the end of each time period. Molecular weight loss was determined by GPC as shown in Fig. 1. In water, 10/90 demonstrated a greater weight loss than 40/60 after 10 weeks under static conditions. This was expected due to the greater content of PLGA in 10/90. Under ''dynamic'' conditions, (the water was changed every 2–3 days), 10/90 demonstrated the highest molecular weight loss. This also corresponds to a significant increase in porosity for 10/90 at 10 weeks, regardless of conditions. The composites were initially 80% porous, and after 10 weeks in water, 10/90 was 89–90% porous while 40/60 became 86–87% porous.

Fig. 2 displays the results of the molecular weight loss in media vs. water. After four weeks, 10/90 degraded more rapidly than 40/60, regardless of medium or conditions. Media was used as surrounding medium in an effort to more closely mimic physiological conditions. Water, although not physiologically significant, did enable us to obtain useful data. The media study was concluded at 4 weeks due to mold growth in the samples. There have been very few reports of extended *in vitro* degradation studies of polymeric scaffolds in serum-containing media. Renier and Kohn examined polymer degradation in PBS vs. fetal bovine serum up to 72 h [19].

At 8 weeks, 10/90 and 40/60 had undergone 16.95% (± 6.2) and 19.4% (± 5.7) mass loss, respectively, under static conditions in water (Fig. 3). This does not represent a significant difference, but there is a significant difference between static vs. dynamic conditions for both composites. The mass loss was greater under static conditions. This corresponds to the low pH of the static water at 8 weeks ($4.57[10/90]$ and $4.84[40/60]$) (Fig. 5). This also corresponds to the increased molecular weight loss observed at eight weeks (Fig. 1). The 10/90 composite consists of a greater amount of PLGA than 40/60; therefore, we expected the 10/90 composite to demonstrate a lower pH (due to the acidic nature of the PLGA degradation products: lactic acid and glycolic acid). We are currently examining the effect of pH on the mechanical properties of the degrading composites. Mainil-Varlet *et al*. examined the degradation of low molecular weight poly(lactic acid) in PBS and found that the pH drop of the surrounding environment did not affect mechanical properties [20]. We are also examining *in vivo* degradation of the composites, and the effect of hydroxyapatite on the surrounding pH. There are reports of the comparison of *in vivo* vs. *in vitro* degradation rates. Tracy *et al*. has examined the degradation of PLGA microspheres and determined that the spheres degraded more quickly *in vivo* [21].

5. Conclusions

In the rapidly growing field of tissue engineering, novel biomaterials are intensely being examined. It is essential to begin to establish a standardized method of characterizing the degradation properties of these new biomaterials. We have been developing polymer/ceramic composites that have potential use as bone substitutes. We have characterized the degradation of these materials *in vitro* by analysis of molecular weight loss, gravimetric weight loss, and morphological changes as well as correlate these changes with the pH of the surrounding environment.

Future work includes the mechanical testing of these

materials during degradation as well as *in vivo* degradation and toxicity studies.

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