

Less Harmful Acidic Degradation of Poly(lactic-co-glycolic acid) Bone Tissue Engineering Scaffolds Through Titania Nanoparticle Addition

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ABSTRACT

One main concern of the use of poly(lactic-co-glycolic acid) (PLGA) as scaffold materials in bone tissue engineering is that their degradation products reduce local pH, which in turn induces an inflammatory reaction and damages bone cell health at the implant site. Thus, the objective of the present *in vitro* study was to investigate the degradation behavior of PLGA when added with dispersed titania nanoparticles. The results of this study provided the first evidence that the increased dispersion of nanophase titania in PLGA decreased the harmful change in pH normal for PLGA degradation. Moreover, previous studies have demonstrated that the increased dispersion of titania nanoparticles into PLGA significantly improved osteoblast (bone-forming cell) functions (such as adhesion, alkaline phosphatase activity and calcium-containing minerals deposition). In this manner, nanophase titania/PLGA composites may be promising scaffold materials for more effective orthopedic tissue engineering applications.

Keywords: poly(lactic-co-glycolic acid), nanophase titania, degradation, orthopedic tissue engineering, nanocomposite, scaffold

1 INTRODUCTION

The use of biodegradable polyesters in orthopedic devices for proper fixation of long bone fractures was first clinically implemented in Finland in 1985 [1]. It was demonstrated that the exact union of the fracture fixed by poly(lactic-co-glycolic acid) (PLGA) was 5 days faster than the fracture fixed by conventional metallic implants [1]. Since the 1990s, the applications of poly(glycolic acid) (PGA), poly(lactic acid) (PLA), and their copolymers PLGA in bone tissue engineering have been investigated extensively [2]. Importantly, these polymers (PLA, PGA, and PLGAs) have been approved by the U.S. Food and Drug Administration (FDA) for certain human clinical applications because their degradation products are nontoxic, endogenous natural metabolites, and are eventually eliminated from the body in the form of carbon dioxide and water [3].

However, one main concern of the use of these polymers in bone tissue engineering is that their

intermediate degradation products (specifically, lactic acid and/or glycolic acid) by non-enzymatic hydrolysis of ester bonds in their backbone reduces the local pH, which in turn induces an inflammatory reaction and damages bone cell health at the implant site. Moreover, the rapid drop of pH *in vivo* may accelerate the polymer's degradation rate, thus, resulting in premature loss of mechanical properties before new bone formation occurs. Therefore, it is critical to decrease the adverse effects of PLGA degradation before this biomaterial can be used more effectively in orthopedic applications.

It has been reported that ceramic particles (such as Bioglass[®] and hydroxyapatite) used as additives to PLGA can provide a pH buffering effect at the polymer surface and, thus, avoid the formation of an unfavorable environment for new bone growth [4-5]. Specifically, the pH of the media containing PLGA/hydroxyapatite foams dropped 73% less than the pH of the media containing control PLGA foams after 50 days of incubation. The pH of the media containing PLGA/Bioglass[®] foams dropped 3% less than the pH of the media containing control PLGA foams after 30 days of incubation. However, the influence of titania nanoparticle addition and its dispersion status on the degradation kinetics of PLGA have not been completed so far. It is hypothesized that the addition of titania nanoparticles to PLGA may decrease the degradation rate of PLGA by interfering with water and acidic oligomer diffusion and, thus, decrease harmful pH variations during degradation.

Moreover, previous studies have demonstrated that the increased dispersion of titania nanoparticles into PLGA significantly enhanced osteoblast (bone-forming cell) functions (such as adhesion, collagen synthesis, alkaline phosphatase activity and calcium-containing minerals deposition) [6-7]. Mechanical advantages of combining nanophase titania into PLGA have also been reported [8]. Specifically, the bending modulus of composites of PLA with 50 wt. % nanophase (< 100 nm) titania was significantly greater than respective composite formulations with conventional coarser grained titania. That is, compared to a bending modulus of 60 ± 3 MPa for plain PLA and 870 ± 30 MPa for conventional titania/PLA composites, the bending modulus of nanophase titania/PLA composites was 1960 ± 250 MPa, which was in the same order of magnitude as trabecular bone [8]. In this manner, nanophase

titania/PLGA composites may be promising scaffold materials for more effective orthopedic tissue engineering applications.

However, to provide a complete story of the usefulness of nanophase titania/PLGA composites to improve orthopedic implant effectiveness, the objective of the present study was to investigate the degradation behavior of PLGA as influenced by the dispersion of titania nanoparticles.

2 MATERIALS AND METHODS

2.1 Materials Preparation

PLGA pellets (50/50 wt. % poly(dl-lactic/glycolic acid); Polysciences, Warrington, PA, U.S.A.) were dissolved in chloroform while nanophase titania powder (Nanophase Technologies, Romeoville, IL, U.S.A.) was then added to the PLGA solution to give a 30/70 ceramic/polymer weight ratio. The composite mixture was then sonicated using a W-380 sonicator (Heat Systems - Ultrasonics, Farmingdale, NY, U.S.A.) with output power settings at 118.75 W, 166.25 W, 213.75 W, and 332.5 W (termed PTC25, PTC35, PTC45, and PTC70 respectively). After sonication, the suspension was cast into a Teflon petri dish, evaporated in air at room temperature for 24 hours and dried in an air vacuum chamber at room temperature for 48 hours.

Pure PLGA and nanophase titania compacts were used as controls. Nanophase titania compacts were prepared by dry pressing titania powders in a tool-steel die via a uniaxial pressing cycle from 0.6 to 3 GPa over a 10 minute period. The green (unsintered) titania compacts were then heated in air at a rate of 10 °C/minute from room temperature to a final temperature of 600 °C, sintered at 600 °C for 2 hours and were cooled down at the same rate as the heating rate. These compacts were termed sintered titania compacts (TCS).

2.2 Material Characterization

Surface topographies of the nanophase titania/PLGA composites were characterized according to standard scanning electron microscopy techniques using a JEOL JSM-840 Scanning Electron Microscope (SEM) at a 5 kV accelerating voltage. Substrates were sputter-coated with a thin layer of gold-palladium using a Hummer I Sputter Coater (Technics, Alexandria, VA, U.S.A.) in a 100 millitorr vacuum argon environment for 3 minutes with 0.01 Amp of current. SEM images taken at 15 kX magnifications were used to determine differences in topography and titania coverage of the nanophase titania/PLGA composites.

2.3 Degradation Experiments

For degradation experiments, 12 specimens with dimensions of 10 mm × 10 mm × 0.3 mm were used for each composite and control sample. These specimens were divided into 3 sets according to incubation times. Initial dry substrates of interest were weighed (W_0) and sterilized. Then, all the substrates were immersed into phosphate buffered saline (PBS; a solution containing 8 g NaCl, 0.2 g KCl, 1.5 g Na_2HPO_4 , and 0.2 g KH_2PO_4 in 1000 ml deionized water adjusted to a pH of 7.4; all chemicals from Sigma) (along with blank PBS as a reference) and were incubated under standard cell culture conditions, that is 37°C, 5% CO_2 /95% humidified air environment. After 21, 28, and 35 days, specimens were removed from PBS, abundantly rinsed with deionized water to remove the soluble inorganic salt, and dried in an air vacuum chamber at room temperature for 48 hours to reach constant mass. At each time point, samples were weighed (W_t) and the percentage of weight loss (%WL) with respect to incubation time was calculated according to the following equation.

$$\%WL = \frac{(W_0 - W_t)}{W_0} \times 100\% \quad (1)$$

The pH of the supernatant buffer was monitored three times a week during the experiments using a Corning 430 pH meter (Corning Incorporated, Acton, MA, U.S.A.).

2.4 Statistical Analysis

Numerical data were analyzed using standard analysis of variance (ANOVA) techniques; statistical significance was considered at $p < 0.05$.

3 RESULTS

3.1 Material Characterization

Titania particles of different agglomeration sizes were visible on the surface of the composites, as shown in Figure 1. Scanning electron micrographs suggested that the distribution of ceramic particles was different on the surface of the composites depending on the sonication power utilized; specifically, there were more titania particles on the surface of each composite after sonication with higher power. Finer titania particles were also observed on the surface with increasing sonication powers. That is, larger ceramic agglomerations tended to break into smaller particles in the polymer solution after higher powers of sonication. Because of this, the amount of surface area occupied by titania increased on the surface of the composites with higher sonication powers. Specifically, 10.6%, 10.2%, and 10.1% compared to 5.7% of the surface area occupied was titania on the PTC70, PTC45, PTC35

and PTC25 composites, respectively. At higher sonication powers, titania particles became smaller and were more evenly dispersed in the PLGA matrix. However, there were no significant differences in terms of titania surface coverage for PTC45 and PTC70 compared to PTC35.

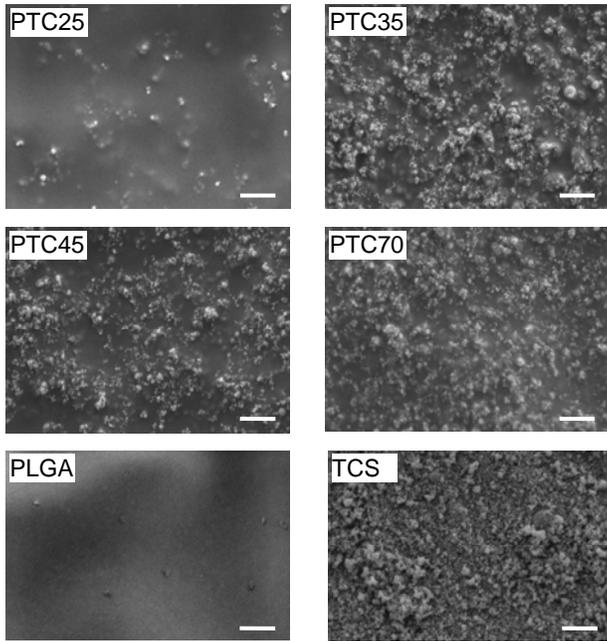


Figure 1. SEM micrographs of materials of interest: PTC25, PTC35, PTC45, PTC70, PLGA, and TCS. Original magnification: 15 kX; magnification bars: 1 μ m.

3.2 Decreased Degradation for Nanophase Titania/PLGA Composites

Results of the degradation study showed that the weight loss percentage of pure PLGA was the greatest among all the substrates tested after respective days (Figure 2). As expected, no weight loss was observed on titania compacts. Specifically, the weight loss of PTC35, PTC45 and PTC70 was approximately 20% less than pure PLGA at 21 days of incubation; 30% less than pure PLGA at 28 days of incubation; and 50% less than pure PLGA after 35 days of incubation. Among all the composites, the weight loss of PTC25 was greater than the others at 35 days of incubation. This indicated that the dispersion status of nanophase titania in PLGA played an important role in decreasing the degradation behavior of these nano-composite.

Moreover, the buffering effect of titania particles towards PLGA weight loss was more significant after longer time periods of incubation; this correlated to the pH buffering effect of titania particles. Specifically, the pH drop was less than 16% for all the composites during the first 21 days of incubation while it was 19% for pure PLGA (Figure 3). During 22 to 35 days of incubation, the pH drop for all the composites and PLGA was faster than the first 3

weeks of incubation. Specifically, the pH drop was 70% for pure PLGA while it was 68% for PTC25, 56% for PTC35, and only 43% for PTC70 after 35 days of incubation.

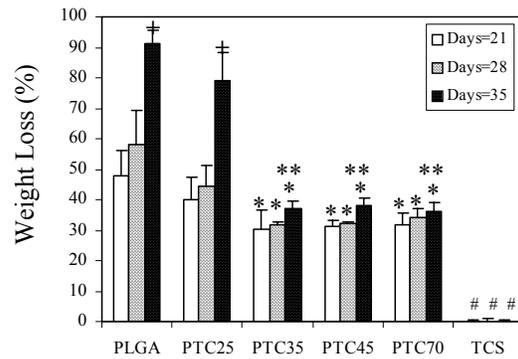


Figure 2: Percent weight loss for PTC25, PTC35, PTC45, PTC70, PLGA, and TCS incubated in PBS under standard cell culture conditions. Values are mean \pm SEM; $n = 3$; * $p < 0.05$ compared to PLGA at respective days; # $p < 0.05$ compared to all the composites at respective days; ** $p < 0.05$ compared to PTC25 at respective days; † $p < 0.05$ compared to respective materials at earlier days.

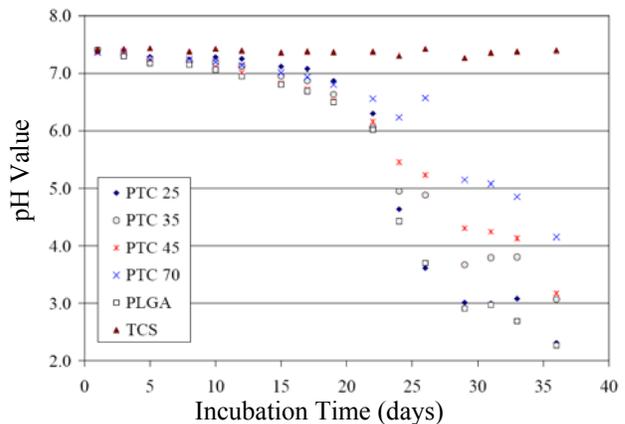


Figure 3: pH variation with incubation time for PTC25, PTC35, PTC45, PTC70, PLGA, and TCS incubated in PBS under standard cell culture conditions.

4 DISCUSSION AND CONCLUSIONS

The PLGA degradation process involves three steps that begin at the outer perimeter of the scaffolds and moves gradually into the interior, followed by catastrophic disintegration [9]. In step 1, water diffuses into the polymer chain and hydrolytic random chain scission of ester bonds begins. Since it undergoes hydrolytic cleavage, PLGA has more predictable degradation rates *in vivo* than polymers whose degradation is mediated predominantly by enzymes; this is because the levels of enzymatic activity may vary widely not only among different patients but also among different implantation sites. But the availability of water is virtually constant in all soft/hard tissues and varies little

from patient to patient. In step 2, the molecular weight decreases and low-molecular-weight oligomers in the inner part of the matrix begin to diffuse out of the thinning outer layer. At this stage, an acidic environment is formed. When the molecular weight of these oligomers is low enough to allow solubilization in the medium, weight loss begins. In the final step 3, a polymer shell remains after the oligomers solubilize and slow degradation of the shell takes place. Degradation of PLGA demonstrates random scission mode under normal conditions (i.e., in water or phosphate buffer medium of pH 7.4 at 37 °C), while unzipping mode (chain-end scission) under harsh conditions (such as high acidity, high temperature, or high energy radiation) [10].

This study provided the first evidence that nanophase titania dispersed in PLGA can improve the structural stability, buffer the harmful pH variations, and decrease the weight loss of the scaffolds. The possible reasons are that titania nanoparticles dispersed in PLGA may hinder water diffusion into the polymer chain and, thus, slow down hydrolytic cleavage; moreover, titania nanoparticles may interfere with the diffusion of intermediate degradation products (oligomers) out of the polymer and, thus, slow down the pH drop. The buffering effect of titania particles on pH was more significant at the later stage of the degradation, that is, after 21 days of incubation. At the early stage of degradation, the pH reduction might be attributed to the residual monomers and oligomers in the original PLGA. The significant pH drop started when the molecular chain was small enough to be able to diffuse out of the scaffold into the PBS solution. Data demonstrated that the dispersion of nanophase titania in PLGA decreased the degradation rate of PLGA which may be favorable to new bone growth.

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