
Surface and bulk modifications to photocrosslinked polyanhydrides to control degradation behavior

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Abstract: A unique class of surface-eroding polyanhydrides was developed and explored for use in medical applications requiring high-strength biomaterials (e.g., orthopedics). In particular, dimethacrylated anhydride monomers were synthesized that photopolymerize quickly to render densely crosslinked polymer networks that degrade from the surface only by hydrolysis of labile anhydride linkages. Previous research on these materials has shown that the rate of hydrolysis of the degradable linkages is dependent on the hydrophobicity of the network composition. This article demonstrates the versatility in controlling the degradation process and resulting cellular response in these materials through the incorporation of new chemistries and the formation of polymer-polymer composite structures. Specifically, the rate of mass loss was controlled by the addition of hydrophobic linear polymers [e.g., poly(methyl methacry-

late)] or monovinyl monomers based on hydrophobic natural components (e.g., cholesterol, steric acid). In addition, a newly established photografting method was used to modify the network surface chemistry with cholesterol- and stearic acid-based polymer grafts to control the degradation front and cellular interactions at the polymer-tissue interface. Finally, a porogen leaching method was used to form porous polyanhydride constructs, which can be subsequently filled with osteoblasts photoencapsulated in a hydrogel, as potential synthetic allograft materials for tissue engineering bone. © 2000 John Wiley & Sons, Inc. *J Biomed Mater Res*, 51, 352–359, 2000.

Key words: polyanhydride; photopolymerization; surface erosion; photografting

INTRODUCTION

Synthetic polymers are used in numerous aspects of clinical medicine, ranging from nondegrading devices (e.g., stents, intravenous tubing) to degradable implants (e.g., drug delivery, fixation pins). The uniqueness of degradable polymer implants is their ability to function for a temporary period and subsequently degrade to allow full independence of the surrounding tissue. This biodegradability not only eliminates the need for a second surgery for removal of the implant, but allows improved healing, as viable tissue intimately interacts and grows into the degrading construct.

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Degradable materials have allowed for new approaches to treatments and therapies for diseases by improved drug delivery technology. In particular, direct implants allow local treatment and controlled release of many drug molecules^{1,2} and have enhanced the delivery of anticancer agents,^{3–5} antibiotics,^{6,7} growth factors,^{8,9} vaccines,^{10,11} steroids,¹² and hormones.¹³ Also, many researchers are studying high-strength and degradable polymers to fix and initially restore function to injured bones and subsequently degrade away to allow for bone in-growth and remodeling.^{14–17} More recently, advances in tissue engineering have led to increased efforts on bioresorbable meshes to facilitate cell growth and accelerated tissue healing.^{18,19} Clearly, the demands for biomaterials with controlled, predictable degradation kinetics are numerous and have led to research on a variety of synthetic and biopolymers engineered for use in a wide range of medical applications.

For the past 3 decades, the aliphatic polyesters have been one of the most widely used classes of biodegradable polymers in medical applications such as wound closure, tissue repair, and drug delivery.²⁰ The most common poly(α -hydroxy acids) [poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copoly-

mers (PLGA)] have contributed to numerous health care products that are already on the market (e.g., Dexon[®], Maxon[®], Biofix[®]) and have a bright future for continued application in clinical medicine. Despite this wide applicability, numerous researchers are exploring new directions in degradable polymers to address some of the current limitations with poly(α -hydroxy acids), especially related to their degradation mechanism. For example, polyanhydrides have found a niche in drug delivery applications where their surface erosion mechanism results in very predictable mass loss (and delivery) profiles.^{2,21,22} Poly(ortho esters), another class of surface-eroding polymers, have been examined by researchers as potential delivery vehicles and orthopedic materials.²³ In contrast to the carbon-carbon backbone polymers, the polyphosphazenes have an inorganic phosphorus-nitrogen backbone that can be synthetically modified to incorporate a wide range of organic substituents for applications ranging from matrices for tissue regeneration to controlled drug release.²⁴⁻²⁶ Inclusion of degradable pendant groups (e.g., imidazolyl or amino acid alkyl esters) results in a polymer which hydrolyzes into nontoxic phosphate and ammonia salts and side group compounds. In addition to the aforementioned hydrolytically degrading polymer systems, there are numerous polymers that are enzymatically degraded (e.g., modified polysaccharides and proteins, amino acid-derived polymers²⁷).

Although the above list is not all-inclusive, it demonstrates the flexibility and diversity in the polymer chemistry that has been investigated to produce biomaterials with controlled structures, physical and mechanical properties, and degradation behavior. For example, the degradation rate of linear poly(α -hydroxy acids) is controlled by many chemical and structural factors, including polymer composition and morphology, molecular weight, size and shape, and crystallinity.²⁸ In particular, PLA degrades considerably slower than PGA or polydioxanone (PDS), and researchers have shown that polyesters preferentially degrade in amorphous regions (thus, crystallinity reduces the overall degradation rate).²⁹ In addition, degradation is autocatalyzed by the carboxyl end groups formed during the hydrolysis reaction of the degradable ester linkages, and the limited diffusion of these end groups within the polymer core results in heterogeneous degradation or faster internal degradation.³⁰ Furthermore, these complexities in the degradation behavior can result in dramatic morphologic changes which may compromise the macroscopic properties of the final polyester implant.

In a similar manner, other investigators have aimed to control the degradation rate of newly developed polymers by variations in the polymer chemistry, but not without affecting the final mechanical or morphological properties. For example, the rate of hydrolysis

of the degradable linkages in surface-eroding polyanhydrides or poly(ortho ester) is controlled by the polymer backbone chemistry.^{23,31} In addition, polyphosphazenes have versatile degradation rates that can be controlled by the nature of the side chain (e.g., imidazole) and its percent substitution. However, altering the polymer backbone or side chain chemistry inherently leads to changes (some positive and others detrimental) in the molecular weight distribution, glass transition temperature, and crystallinity, all of which ultimately influence the final mechanical and physical properties.

To address issues related to the independent control of degradation and other material properties, this work presents a unique class of hydrolytically degradable polymers synthesized from photopolymerizable multimethacrylate monomers that react to produce densely crosslinked polyanhydride networks. The resulting polymers are high strength and degrade from the surface by hydrolysis of the anhydride linkages.³² One advantage of this synthetic design is the ability to alter the degradation time scale dramatically by simple modifications to the monomer backbone chemistry, while independently controlling the final mechanical properties with changes in the network structure (i.e., crosslinking density). Whereas we have illustrated that the mass loss of these materials can be altered from days to a year depending on the hydrophobicity of the network composition,³² this work demonstrates novel ways to control the degradation (e.g., time scale and direction of front) and cell interactions through bulk and surface chemical modifications to these networks. Specifically, we can engineer crosslinked polyanhydride constructs for a diverse number of biomedical applications by (a) modifying the network composition through the addition of linear polymers and monovinyl monomers (to control the rate of mass loss), (b) photografting on the network surface (to change the direction of the degradation front and control the acute cytocompatibility), and (c) inducing pore formation in the polyanhydride networks to produce complex, composite structures for bone tissue engineering.

EXPERIMENTAL

Methacrylated sebacic acid (MSA), methacrylated *bis*(*p*-carboxyphenoxy) propane (MCP), and methacrylated *bis*(*p*-carboxyphenoxy) hexane (MCPH) monomers were synthesized from methacrylic anhydride and characterized as described elsewhere.³² Saturated fatty acids (e.g., stearic acid) were methacrylated similarly with methacrylic anhydride at 80°C. Methacrylated cholesterol was synthesized via acyl attack at the 3-hydroxyl group with 1.1M equivalents of methacryloyl chloride and 1.1M triethylamine. Reactants were added dropwise to a solution of cholesterol in chloro-

form at 0°C. After warming to room temperature and stirring overnight, the chloroform solution was filtered for salts and immiscibles and washed with cold 0.5M sodium bicarbonate solution and cold water, and dried with magnesium sulfate. The purified chloroform solution was evaporated under vacuum to a solid following vacuum filtration of the magnesium salts. Methacrylated monomers were characterized with ¹H-nuclear magnetic resonance imaging (NMR) to examine the extent of methacrylation.³³ All methacrylated monomers (Fig. 1) were stored under argon and at 4°C to minimize hydrolysis.

Linear polyanhydrides [poly(CPH) and poly(CPP:CPH)] were synthesized by melt condensation of acetylated monomers, as described elsewhere.^{31,34} The acetylated diacids were characterized by Fourier transform infrared spectroscopy (FTIR) for the disappearance of the carboxylic acid peak. The acetylated diacids were heated to 180°C and polymerized by melt condensation. Poly(CPP:CPH) was synthesized with equal molar amounts of the acetylated CPP and CPH diacids. Poly(methyl methacrylate) and poly(lactic acid) were used as received.

Samples were polymerized with either longwave ultraviolet light (Model B100AP; 115 V, 2.5A Black Ray) or a high-intensity mercury arc lamp (EFOS; Ultracure 100SS) equipped with a bandpass filter (EFOS; UC100SS Plus, 365 nm) that transmits 325- to 400-nm light. Photopolymerizations were initiated with 0.1–1.0 wt % 2,2-dimethoxy-2-phenylacetophenone (DMPA), a common initiator used in ultraviolet photopolymerization applications.³⁵

The surfaces of the resulting crosslinked networks were modified via a multistep photografting method. First, the bulk monomer was melted and polymerized with *N,N,N',N'*-tetraethylthiuram disulfide (TED) and DMPA. Specifically, TED dissociates upon irradiation and generates dithiocarbamyl radicals, which may then terminate propagating carbon radicals initiated from the DMPA. The termination results in a carbon-dithiocarbamyl linkage that can be reinitiated upon further exposure to ultraviolet light.³⁶ Hence, after the bulk monomer was completely polymerized in this manner, a drop of the desired monomer to be grafted was applied to the polymer surface (without additional initiator)

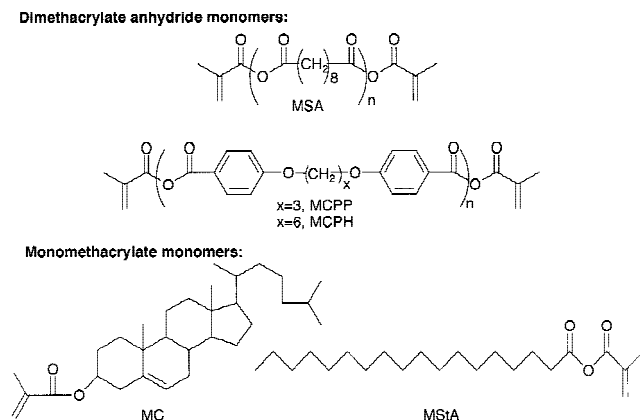


Figure 1. Photoreactive monomer structures: methacrylated sebacic acid (MSA), methacrylated *bis*(*p*-carboxyphenoxy) propane (MCPH), methacrylated *bis*(*p*-carboxyphenoxy) hexane (MCPH), methacrylated cholesterol (MC), and methacrylated stearic acid (MStA).

and exposed to light to initiate chain growth from the dithiocarbamyl radicals at the surface. The photografted samples were polymerized with 1.0 wt % DMPA and 0.5 wt % TED.

To evaluate the degradation mechanism and kinetics, disks (*d* = 12 mm, *t* = 1.4 mm) were prepared in Teflon molds. Mass loss was measured as a function of time in phosphate-buffered saline under simulated *in vivo* conditions (i.e., pH 7.4 and 37°C) with continuous orbital shaking at 80 rpm. Each sample was degraded in 250 mL of buffered saline, and the saline was replaced when the pH dropped below 7.4, maintaining sink conditions. Disk dimensions were also monitored to determine the mode and direction of the degradation front.

Porous samples were constructed according to the salt leaching method described by Mikos et al.^{37–39} Methacrylated anhydride monomers were combined with 70 wt % milled and sieved sodium chloride, placed in Teflon molds, and photopolymerized. The constructs were subsequently placed in phosphate-buffered saline to leach the salt (~48 h) and degraded. Scanning electron microscopy (SEM) was used to monitor the distribution of pore sizes.

Polyanhydride samples were implanted subcutaneously into the backs of adult male Sprague–Dawley rats weighing in the range of 375–399 g. Two incisions were made dorsally and two pockets formed laterally from each incision by blunt dissection techniques. One polymer sample was placed in each pocket, and the incisions were subsequently closed with surgical staples. National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Pub. No. 85-23 Rev. 1985) were observed. The rats were sacrificed after 7 days and the implants and surrounding tissue harvested for histological analysis. Samples were fixed in 10% buffered formalin, dehydrated to wax, sectioned, and stained with hematoxylin and eosin.

Osteoblasts were obtained through collagenase digestion of neonatal (<1-day-old) rat calvaria, as described elsewhere.⁴⁰ After culturing (four passages) in supplemented Dulbecco's modified Eagle's medium, cell suspensions were prepared at 50×10^6 cells/mL of 10 wt % poly(ethylene oxide) dimethacrylate (PEODM) in phosphate-buffered saline. This suspension was mixed with 0.05 wt % 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone (Ciba-Geigy; Irgacure 2959), a water-soluble ultraviolet initiator at a cytocompatible concentration, and subsequently photopolymerized for 6 min with 10 mW/cm² ultraviolet light (Model B100AP; 115 V, 2.5A Black Ray). Assays and stains were used to measure cell viability and activity immediately after cell encapsulation and after 7 days in culture. Cell viability was characterized with a Live/Dead® Viability/Cytotoxicity Assay Kit, whereby live cells fluoresce green and dead cells fluoresce red. In addition, cell constructs were dehydrated through ethanol, embedded in wax, sectioned to 10 μm, and stained with a modified von Kossa's silver nitrate stain⁴¹ for calcium deposition.

RESULTS AND DISCUSSION

Multifunctional methacrylated monomers that polymerize to form densely crosslinked and surface eroding networks were synthesized for orthopedic

medical applications. In particular, the monomer chemistry was designed from linear polyanhydrides, a class of surface-eroding polymers recently approved by the Federal Food and Drug Administration for local delivery of anticancer agents to patients with brain tumors.³ Whereas linear polyanhydrides are synthesized from acetylated monomers reacted at high temperatures and low pressures, the aforementioned multimethacrylate anhydride monomers can be reacted under physiological conditions by photopolymerization. Photopolymerizations are advantageous for medical applications, where rapid photoinitiation rates overcome oxygen inhibition and solvent effects (e.g., water). In addition, the temporal and spatial control of the photoinitiation process bring in the powerful capacity to polymerize complex shapes *in vivo*, which may offer less invasive surgical procedures.

We functionalized these anhydride-based monomers with methacrylate groups to form photoreactive biomaterials. Methacrylate groups polymerized by a radical chain mechanism are used in many medical applications (e.g., bone cements, dental restorations).⁴² When combined with a suitable photoinitiator and exposed to visible or ultraviolet light, liquid methacrylated monomers can be reacted into rigid solids in seconds to minutes under ambient conditions.⁴² In this study, the anhydride monomers were equipped with two methacrylate end groups per molecule to facilitate crosslinking during the photopolymerization, and the monomer molecular weight was used to control the crosslinking density. Crosslinking increases the final network mechanical properties and controls transport of water into the polymer matrix. These materials photopolymerize to form highly crosslinked, rigid polymer networks with hydrolytically labile anhydride linkages. A detailed description of the photopolymerization behavior of these monomers was published elsewhere.³³

Dimethacrylated monomers were synthesized from diacids of varying hydrophobicity (Fig. 1). In general, the hydrophobicity of the monomer backbone is sufficient to prevent penetration of water into the core of the final polymer network. Therefore, the rate of hydrolysis of anhydride linkages is much greater at the polymer surface than in the bulk, resulting in a surface degradation mechanism. Similar to linear polyanhydrides and poly(ortho ester)s, these crosslinked polyanhydrides have a degradation rate that is controlled by the hydrophobicity of the backbone chemistry. Specifically, we demonstrated that the rate of mass loss in these networks can be controlled from several days to approximately 1 year by compositional changes.³² A disk ($d = 16$, $t = 1.7$) synthesized from a hydrophobic monomer, MCPH, degrades ~ 150 times slower than a disk polymerized of MSA, a more hydrophilic, aliphatic monomer. Simply by copolymerizing varying ratios of MSA and MCPH, polyanhydride networks

can be fabricated which span degradation times between 2 days and 1 year.

While precise control of the degradation rate is important for numerous applications, this work explores new, alternative mechanisms to modify the surface and bulk chemistries of polymer networks to impart desirable degradation characteristics, structural properties, and cellular responses. For example, semi-interpenetrating networks (semi-IPNs) were synthesized by incorporating linear polymers within the crosslinked network to control the overall material hydrophobicity. The degradation behavior as a function of time for three different semi-IPN chemistries compared to the degradation of a crosslinked network of poly(MSA) is shown in Figure 2. Whereas the poly(MSA) disk was completely degraded in approximately 50 h, a disk of similar dimensions but loaded with 10 wt % poly(methyl methacrylate) (PMMA), was only 23% degraded at that time. Semi-IPNs were fabricated with PMMA because it is the major component in thermally polymerizing bone cements⁴³ and has been shown to have good cytocompatibility.⁴⁴ However, its nondegrading nature compromises healing in orthopedic injuries (e.g., limits vascularization and bone remodeling) and complicates the degradation profile in these semi-IPNs. Initially, the rate of mass loss is linear at approximately 6%/h. However, during the late stages of degradation, the rate significantly slows as the sample composition approaches purely hydrophobic, nondegrading PMMA chains.

Completely degradable semi-IPN samples were also fabricated by reacting the multimethacrylated anhydride monomers in the presence of linear polyanhydride chains. Figure 2 also illustrates the degradation profiles for disks of poly(MSA) with 25 wt % poly(CPH) and 50 wt % poly(CPP:CPH). The former semi-

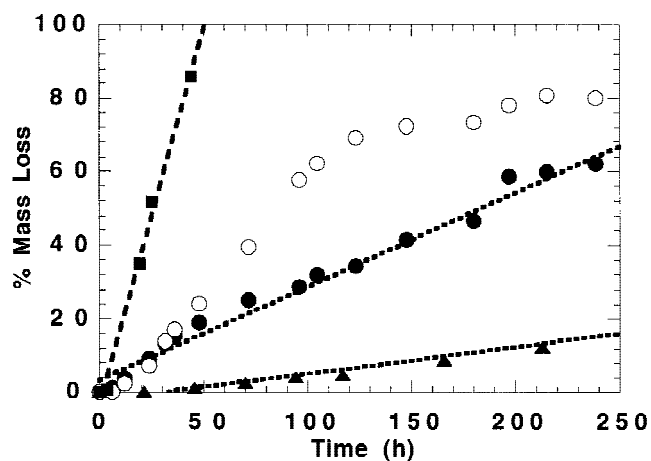


Figure 2. Cumulative percent mass loss as a function of degradation time for a homopolymer of poly(MSA) (■) compared to three semi-IPN compositions: poly(MSA) with 10 wt % PMMA (○); 25 wt % poly(CPH) (●), and 50 wt % poly(CPP:CPH) (▲).

IPN was not quite 20% degraded after 50 h and would be completely eroded in ~ 17 days assuming surface erosion were maintained. For the latter composition, the degradation time was even further reduced and would take nearly 60 days to erode completely. In synthesizing semi-IPNs, these linear polymer chains act to increase the degradation time dramatically by imparting hydrophobicity to the network, but also provide other significant advantages. For example, the addition of linear polymer chains to the bulk monomer composition serves to decrease the concentration of reactive functionalities, which reduces the polymerization exotherm and volume shrinkage; allows control of the solution viscosity and overall handling properties; and reduces the concentration of the hydrophilic poly(methacrylic acid) (PMAA) degradation product.

To expand on the methacrylate monomer chemistry and provide another mechanism to control the polymer degradation behavior, monovinyl monomers were synthesized from naturally occurring hydrophobic compounds. In particular, we synthesized photo-reactive monomers from cholesterol and stearic acid (Fig. 1), which are minimally soluble in water and found naturally in cell and lipid membranes. Figure 3 compares the mass loss profiles of a homopolymer network of poly(MSA) to copolymer networks polymerized from 75 wt % MSA and 25 wt % methacrylated steric acid (MStA) or 25 wt % methacrylated cholesterol (MC). The hydrophobicity of the aforementioned monomethacrylate monomers was sufficient to prevent diffusion of water into the polyanhydride network; thus, the surface erosion mechanism was maintained. For example, the degradation profile for a disk of 25/75 (w/w) MStA/MSA was linear, and complete degradation of the sample would take ~ 15 days. The 25/75 MC/MSA network degraded even more slowly,

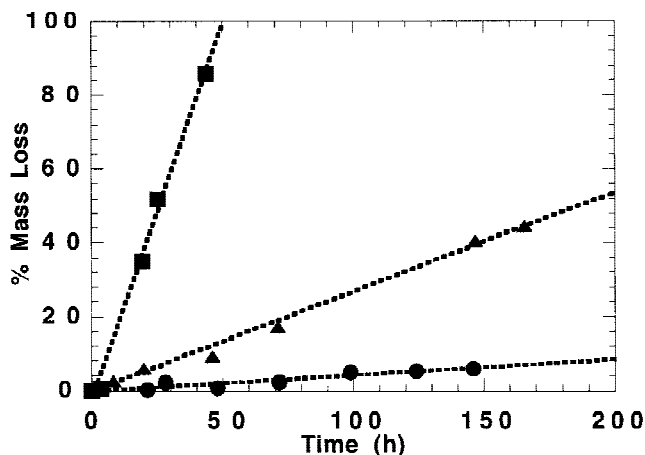


Figure 3. Cumulative percent mass loss as a function of degradation time for a homopolymer of poly(MSA) (■) compared to copolymers polymerized from MSA and 25 wt % MC (●) and 25 wt % MStA (▲).

with only 7% mass loss in 1 week. Thus, by copolymerizing these hydrophobic monomers with dimethacrylated anhydride monomers, we can effectively slow the erosion rate of the final polymer, provide a mechanism to control the crosslinking density and structural properties, and potentially control cellular responses at the polymer–tissue interface.

Recognizing the potential benefits, with respect to cytocompatibility, of bulk-modified networks containing cholesterol and stearic acid, we also pursued a novel photografting technique to modify our polyanhydride surfaces with these natural based monomers. The technique uses living radical polymerizations to modify the surface chemistry of the polymer networks. The benefits of this surface modification are twofold. First, photografting hydrophobic monomers such as MC and MStA onto the circular surfaces of poly(MSA) disks changes the direction of the degradation front. Specifically, the anhydride linkages protected by the hydrophobic grafts do not readily hydrolyze, whereas the anhydride linkages exposed on the disk's circumference degrade according to the kinetics for poly(MSA). Second, photografting can be used to change the polymer's surface chemistry to enhance the immediate cell response at the tissue–polymer interface.

Figure 4 compares the mass loss as a function of time for disks of poly(MSA) to disks of poly(MSA) with grafted surfaces of poly(MC) and poly(MStA). For both surface-modified samples, the rate of erosion was reduced [compared to the unmodified poly(MSA) sample] owing to the decrease in surface area available for anhydride hydrolysis. Also, although bulk-modified networks indicated that MC is significantly more hydrophobic than MStA when copolymerized

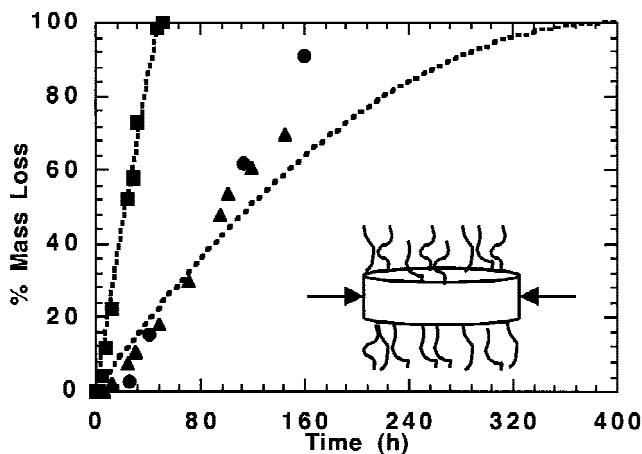


Figure 4. Cumulative percent mass loss as a function of degradation time for a sample of poly(MSA) (■) compared to samples of poly(MSA) with surface grafts of poly(MC) (●) and poly(MStA) (▲). The dashed line indicates the predicted mass loss of a sample of poly(MSA), where hydrolysis of anhydride linkages occurs only on the circumferential area (i.e., radial degradation).

with MSA, both surface-modified poly(MSA) disks degraded at nearly the same rate. Hence, photografting serves mainly to control the surface area available for degradation, which is nearly identical in both samples. The dashed line in Figure 4 represents the theoretical curve for mass loss of a sample of poly(MSA) where degradation of anhydride linkages occurs only at the circumferential surface (i.e., radial degradation front). The kinetics of the degradation of poly(MSA) was previously characterized and is $\sim 1.3 \times 10^{-2}$ mm/h.³² The prediction fits the data well, deviating slightly at higher mass losses, but confirms the general radial degradation mechanism. The ability to control the degradation direction may be beneficial for orthopedic applications such as implanting in a bone defect where the surface adjacent to the muscle could be modified to prevent degradation from that surface.

To assess the cellular response to these surface-modified polyanhydride networks, disks fabricated from a semi-IPN of 50/50 (w/w) poly(MSA)/poly(CPP:CPH) with poly(MStA) grafts on the circular surfaces were implanted subcutaneously in rats. Figure 5 shows the histological results from 7-day implants evaluated for an acute inflammatory reaction by hematoxylin and eosin staining and light microscopy. Figure 5 clearly shows the grafted layer (unstained and indicated by arrows) and the bulk polymer (portions darkly stained). The micrograph illustrates the graft-tissue interface, indicating healthy fibroblastic cells at the modified polymer surface. The presence of few macrophages and many vascular structures confirm a normal response to a surgical procedure and degrading polymer implant. Further studies are under way to examine the ability of varied graft chemistries [e.g., poly(acrylic acid) and poly(ethylene glycol), as well as MC and MStA] to modulate acute cell responses at modified polymer surfaces.

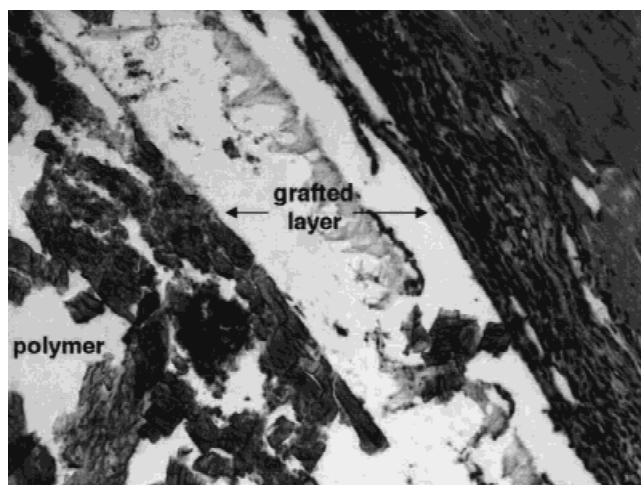


Figure 5. Hematoxylin and eosin stained micrograph of the cell-polymer interface for a sample of poly(MSA) surface-modified with poly(MStA) grafts after 7 days in rat subcutaneous tissue.

Finally, porous polyanhydride constructs were fabricated using a porogen leaching method.³⁹ Specifically, sodium chloride or gelatin was added to the multimethacrylate monomers and the resulting mixture photopolymerized. Depending on the porogen loading and network degradation rate, the controlled formation of pores for guided tissue growth or increased cell migration into the polyanhydride construct could be achieved. Figure 6 is a scanning electron micrograph demonstrating the distribution of pore sizes for a composite material of 70 wt % sodium chloride (milled and sieved) and 30 wt % 50/50 poly(MSA)/poly(CPP:CPH) which had degraded for ~ 48 h in buffered saline. The micrograph shows a cross section of the disk as the porogen leached from the construct and the solvent front moved through the sample; After 48 h of the degradation ($\sim 75\%$ mass loss), the aqueous front had dissolved the sodium chloride to a depth of ~ 800 μm in the network. Researchers have demonstrated that pore sizes of several hundred microns are required for uniform bone growth into polymer-coated metallic implants.⁴⁵ In these porogen-polymer composites, the pore size is a function of the initial size of the salt crystals and the extent of network degradation. After 48 h, pore sizes in the salt-leached polyanhydride constructs ranged from tens to several hundreds of microns.

Whereas high porosities tend to compromise final network mechanical properties, these *in situ* forming porous composites may be promising materials for non-load-bearing orthopedic applications (e.g., maxillofacial, cranial) or, alternatively, as scaffolds for bone tissue engineering. For example, we envision that the pores of a polyanhydride implant could be filled with osteoblasts, encapsulated in hydrogels, that

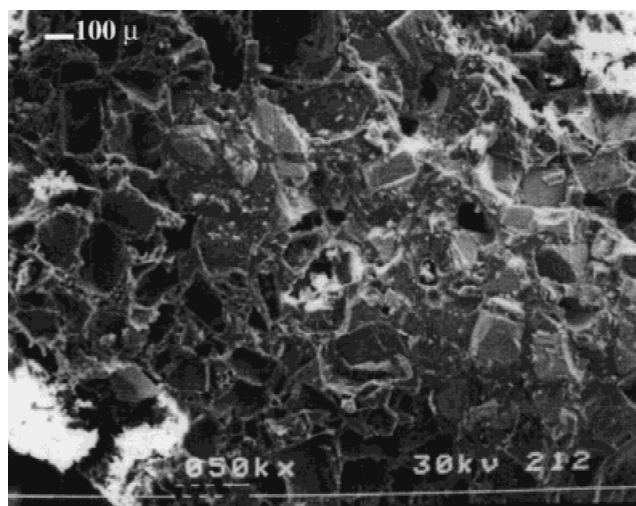
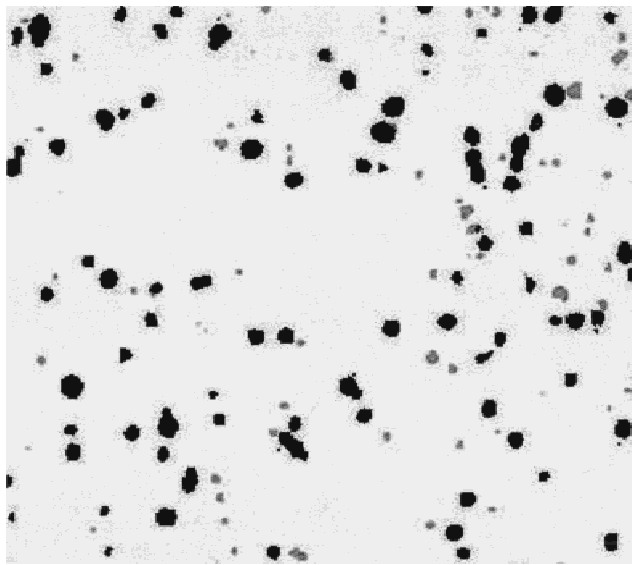


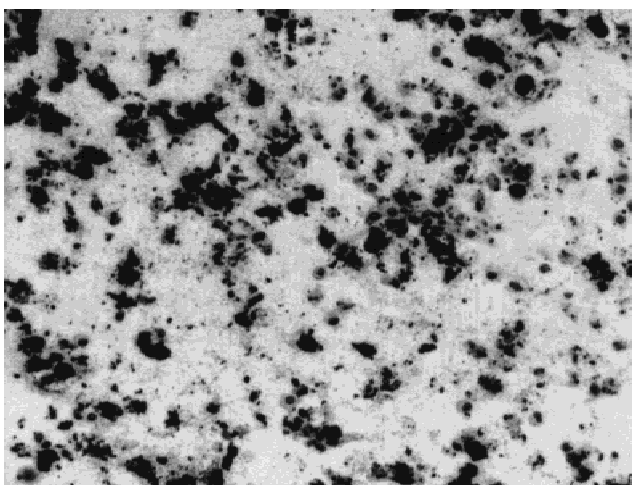
Figure 6. Scanning electron micrograph demonstrating the salt-leached pores in a cross section of a disk that initially consisted of 70 wt % sodium chloride in a semi-IPN of 50/50 poly(MSA)/poly(CPP:CPH) after ~ 48 h of degradation.

would subsequently proliferate and mineralize in the pore structures, while the polyanhydride "sponge" would degrade away. In particular, our group has recently confirmed that osteoblasts can be suspended in poly(ethylene oxide) dimethacrylate (PEODM) monomer solutions, and the mixture photopolymerized to render a photo-encapsulated cell-hydrogel construct.

Figure 7(A,B) illustrates the viability of osteoblasts after 7 days of incubation in a crosslinked PEODM hydrogel. A Live/Dead[®] cell assay [Fig. 7(A)] was used to confirm osteoblast viability in the construct; Numerous live cells were distinguished by their intense green fluorescence [represented in black in Fig. 7(A)] some dead cells produced a bright red fluorescence (represented in gray). In general, the majority of osteoblasts within the hydrogel were viable, indicat-



(A)



(B)

Figure 7. Micrographs illustrating the viability and activity of osteoblasts encapsulated in a hydrogel of crosslinked PEODM after 7 days of incubation: (A) Live/Dead[®] cell assay, and (B) von Kossa's silver nitrate stain.

ing that photoencapsulation may be a nontoxic technique for producing cell-polymer composite materials. In addition, a silver staining method (i.e., modified von Kossa's⁴¹) was used to demonstrate the deposition of calcium and other salts by the osteoblasts in the PEODM construct. Figure 7(B) demonstrates the presence of healthy osteoblast nuclei and cytoplasm, and surrounding bone mineral, blackened by the silver nitrate, in the PEODM hydrogel at 7 days. The results in these figures indicate that osteoblasts encapsulated in a hydrogel network survive photo-encapsulation and quickly begin to form minerals. Thus, polymer-polymer composites of osteoblast-hydrogel microparticles encapsulated in pores of a photocrosslinkable polyanhydride network may provide numerous benefits as a bone allograft material with respect to mechanics, degradation mechanism, processing, and osteoinduction and accelerated bone healing.

CONCLUSIONS

Polyanhydride networks were designed from photocrosslinkable multimethacrylate monomers for high-strength medical applications. The degradation process can be dramatically affected by changes in the chemical composition, whereas the final mechanical properties are independently controlled with changes to the network structure. In this work, we illustrated a diverse range of tools available to control the degradation process in these high-strength surface-eroding biomaterials. In particular, we made changes to the bulk polymer (by the addition of linear polymers, hydrophobic monovinyl monomers, and porogens) and surface chemistry (by photografting natural based materials) to control varied aspects of the degradation behavior. As a result, we can control the rate of mass loss, direction of degradation, cell-surface interactions, and porosities in these polyanhydride systems. Finally, we fabricated photoencapsulated osteoblast hydrogels which could be introduced into the pores of a photocrosslinked implant to accelerate and guide tissue growth and minimize the healing time of certain orthopedic injuries.

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