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A review of photocrosslinked polyanhydrides: in situ forming degradable networks

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Abstract

Many orthopaedic injuries could benefit from a high-strength and degradable material with good tissue compatibility. In addition, there is a great clinical need for materials which are easily contoured or placed into complex-shaped defects by a surgeon. We have rationally designed a new class of photocrosslinkable polyanhydride monomers which in situ form high-strength and surface eroding networks of complex geometries. This paper highlights the advantages of these materials for orthopaedic applications and the technique of photopolymerization for reacting these monomers under physiological conditions. The rationale for the material design, photopolymerization kinetics, degradation behavior, and histology in subcutaneous tissue and a model bone defect are presented.

Keywords: Photopolymerization; Polyanhydride; In situ forming; Surface eroding

1. Introduction

Numerous medical applications benefit from the ability to form polymers in situ. For example, dentists photopolymerize dimethacrylate monomers in the presence of silica particle fillers in tooth caries to render tooth-colored composite restorations as alternatives to mercury amalgam fillings [1,2]. Orthopedic surgeons internally fix implants with bone cements that react in situ. The reaction is initiated via the mixing of a powder containing poly(methyl methacrylate), barium sulfate, and benzoyl peroxide with liquid methyl methacrylate and *N,N* dimethyl *p*-toluidine [3,4]. In addition to these long-standing applications, recent advances in polymer chemistry and processing have led to many newfound methods and applications for forming biomaterials in situ.

Atrix Laboratories markets an in situ forming, biodegradable polymer for guided tissue regeneration [5]. In their technology, solutions of poly(DL-lactide) in a biocompatible solvent (*N*-methyl-2-pyrrolidone) are precipitated by contact with aqueous fluids in the body to form drug delivering barriers at periodontal sites. Focal uses photoinitiated polymerizations to in situ seal air leaks associated with lung surgery and fluid leaks from the dura after neurosurgery with degradable hydrogels [6]. The uniqueness to their approach relates to a two part process that first stains the tissue with photoinitiator molecules and subsequently introduces a reactive macromer that is polymerized from the tissue interface in a controlled and adherent fashion. In the pharmaceutical industry, injectable gels are synthesized from triblock copolymers of poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide), Pluronics. This polymer is readily soluble in water, but the solution gels at higher temperatures (i.e., 37°C) via hydrophobic associations to form a physically crosslinked network [7,8]. Hence, drug-loaded solutions can be injected subcutaneously and upon gelation allow for the sustained delivery of active agents. Fibrin glue has also received widespread attention, especially in Europe, as an in situ forming tissue adhesive [9]. Fibrin glue is formed from blood-derived products, specifically, thrombin, in the presence of calcium chloride and Factor XIIIa, and react with fibrinogen to produce a hydrogel.

While the above list is not exhaustive, one can appreciate the breadth of chemistries and processing tools used to synthesize polymers in situ. Despite this versatility, the challenges of in situ polymer formation are many, and when designing new materials and methods to form

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polymers in vivo, several issues must be considered. In particular, the reaction conditions for in vivo applications are quite stringent, including a narrow range of physiologically acceptable temperatures, requirement for nontoxic monomers and/or solvents, moist and oxygen-rich environments, the need for rapid processing, and clinically suitable rates of polymerization. In this work, we are particularly interested in the development of in situ forming orthopaedic biomaterials, especially a high-strength degradable material, through photoinitiated polymerizations.

Development of a photopolymerizable, degradable orthopaedic biomaterial would provide many advantages. First, photoinitiated reactions overcome many of the limitations for in vivo polymerizations and provide rapid polymerization rates at physiological temperatures, while allowing spatial and temporal control of the process. Second, because the initial materials are liquid solutions or moldable putties, the systems are easily placed in complex shapes and subsequently reacted to form a polymer of exactly the required dimensions. Little, if any, additional shaping or modification of the implant is required. Third, the adhesion of the polymer to surrounding tissue is generally significantly improved because of intimate contact of the polymer with the tissue during formation and the mechanical interlocking that can result from surface roughness. Finally, the invasiveness of surgical techniques can be minimized as the macromer is easily introduced at the defect and can be photocured with fiber optic cables, or even through tissues, as will be demonstrated in this work.

Traditional bone cements based on methyl methacrylate (MMA) could be reacted in situ with photoinitiated chemistries by incorporation of a photoinitiator molecule instead of the thermal initiator, benzoyl peroxide. Photoinitiated bone cements might provide several processing advantages, especially with respect to temporal control of the reaction; however, the resulting polymers are non-degrading which can cause further complications with healing (e.g., limit blood flow). In addition, the high concentration of reactive groups in methyl methacrylate leads to high temperatures during polymerization and subsequent tissue necrosis [10]. Thus, we are particularly interested in developing a *degradable* orthopaedic biomaterial that could be formed in situ. Since bone has the capacity to heal itself, a degradable polymer that restores function temporarily and then degrades with a rate tuned to the healing process would provide many benefits for the treatment of skeletal defects.

Despite the benefits, relatively few polymers exist that can be reacted in situ and possess suitable properties for orthopaedic applications (e.g., high strength and controlled degradation rate). Mikos et al. [11,12] have developed one system based on poly(propylene fumarate) chemistry that can be reacted in bone defects by radically crosslinking this unsaturated linear polyester with a vinyl monomer such as *N*-vinyl pyrrolidinone. In addition, we have recently developed multifunctional monomers based on anhydride chemistry that react to form highly crosslinked networks that surface degrade [13,14]. This chemistry provides specific advantages, particularly for several orthopaedic applications, which will be illustrated in this contribution. Specifically, we will demonstrate the ability to: control the time scale of the polymerization, which is critical for clinical placement and to minimize the exothermic temperature rise; polymerize through tissues to minimize the invasiveness of certain surgical procedures; control the degradation time scale from days to months through changes in the network chemistry; and fabricate thick, three-dimensional and complex-shaped polymers in situ in a bone defect.

2. Experimental

2.1. Materials

Dimethacrylated anhydride monomers were synthesized from diacid precursors by reaction with methacrylic anhydride (Aldrich) at elevated temperatures and purified as described elsewhere [13,15]. For example, sebacic acid (Aldrich) was reacted with 2.5 M equivalents of methacrylic anhydride for 1.5 h and the resulting monomer, methacrylated sebacic acid (MSA), was subsequently purified by precipitation in anhydrous petroleum ether. Methacrylated monomers were characterized using ^{1}H-NMR and Fourier transform infrared spectroscopy (FTIR) for the presence of methacrylate groups. Methacrylate $=CH_2$ protons were confirmed with ^{1}H-NMR at approximately $\delta = 6.0$ and 6.5 parts per million and at 1637 cm^{-1} with FTIR. Monovinyl monomers of methacrylated stearic acid (MStA) and methacrylated cholesterol (MC) were synthesized by reaction of stearic acid (Aldrich) and cholesterol (Aldrich) with methacryloyl chloride (Aldrich) and triethylamine (Aldrich). A linear copolymer, poly(CPP : CPH), was synthesized as described elsewhere from acetylated diacids of *bis*(*p*-carboxyphenoxy)propane (CPP) and *bis*(*p*-carboxyphenoxy)hexane (CPH) [16]. A list of abbreviations is shown in Table 1.

Methacrylated monomers were combined with a photoinitiator, and the mixture subsequently transferred into a mold. Photopolymerizations were initiated with ultraviolet (365 nm) or visible light (400–500 nm) from a high-intensity light source (EFOS, Ultracure 100SS), with the light intensity controlled by neutral density filters (Melles-Griot) and wavelength controlled by monochromatic or broad band filters (EFOS). The visible light initiating system chosen for this work consisted of camphorquinone (CQ, Aldrich) and triethanolamine (TEA, Aldrich). This system is typical for many

Table 1
List of abbreviated materials

List of abbreviations used	
CPP	1,3-*bis*(*p*-Carboxyphenoxy) propane
CPH	1,6-*bis*(*p*-Carboxyphenoxy) hexane
CQ	Camphorquinone
DMPA	2,2-Dimethoxy-2-phenylacetophenone
MC	Methacrylated cholesterol
MCPH	Methacrylated 1,6-*bis*(*p*-carboxyphenoxy) hexane
MCPP	Methacrylated 1,3-*bis*(*p*-carboxyphenoxy) propane
MMA	Methyl methacrylate
MSA	Methacrylated sebacic acid
MStA	Methacrylated stearic acid
PMAA	Poly(methacrylic acid)
TEA	Triethanolamine

current dental applications and provides an alternative to using ultraviolet radiation to initiate polymerization. Samples were polymerized with 0.25–0.5 wt% CQ and 0.25–0.5 wt% TEA. All initiators were used as received.

2.2. Polymerization

Polymerization rate profiles were monitored as a function of irradiation time using a differential scanning calorimeter equipped with a photocalorimetric accessory (DPC, Perkin-Elmer DSC7). Samples of monomer and initiator weighing 5–10 mg were placed in aluminum pans, and the polymerization rate was monitored isothermally at 37°C in the presence of oxygen to simulate physiological conditions. The heat flux, measured by the DSC, was converted to a rate of polymerization using the characteristic heat of reaction per methacrylate double bond reacted (13.1 kcal/mol) [17].

UV–Vis spectrophotometry (Model 8452, Hewlett Packard) was used to measure the absorbance spectra of initiator solutions as a function of wavelength and exposure time. A dilute solution of CQ was prepared in 1-hexanol (Aldrich), placed in a disposable cuvette, and irradiated using a blue light curing unit (DenMat, Marathon Two, Model 3940) at approximately 150 mW/cm^2. The solution was placed in the spectrophotometer to obtain the absorbance spectra as a function of time. In addition, the molar absorptivity ε, as a function of wavelength and irradiation time, was calculated according to the relationship

$$A = \varepsilon[\mathrm{In}]b' \tag{1}$$

where A is the absorbance at a specific wavelength, [In] is the initiator concentration, and b' is the path length of the cuvette (1 cm). The molar absorptivity at 470 nm was used to calculate light attenuation in the crosslinked networks.

2.3. Degradation

Uniform polymer disks ($d = 12.0$ mm, $t = 1.4$ mm) were used to simulate one-dimensional degradation and follow the degradation kinetics and mechanism. Samples were placed in a phosphate-buffered solution at simulated physiological conditions (e.g., pH 7.4 and 37°C with constant orbital shaking (80 rpm)) and monitored for mass loss with time. In addition, the disk dimensions were monitored with time to further confirm the surface erosion mechanism; Disk diameters did not change significantly with degradation.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (PerSeptive Biosystems Voyager-DE STR) was used to measure the molecular weight distribution (MWD) of poly(methacrylic acid) (PMAA) degradation products. PMAA samples were separated from other degradation products as described elsewhere [15], dissolved in methanol or water ($\sim$10 mg/ml), and combined with the matrix material in a 1 : 3 ratio (sample : matrix) by volume. Generally, 1 μl of this solution was spotted per well on the sample plate, and the contents of the well were ionized with a nitrogen laser emitting at 337 nm (3 ns pulse width). Irradiance intensity and accelerating voltage (10–20 kV) were optimized for each sample run, and negative ions were detected using sinapinic acid (Hewlett-Packard) as the matrix molecule. The number fraction of n-mers was calculated by peak heights of the MALDI-TOF distribution.

2.4. Histology

Photopolymerized disks ($d = 12.0$ mm, $t = 1.4$ mm) were placed in the subcutaneous tissue of male Sprague–Dawley rats and examined histologically for an inflammatory response. After 28 days, the samples and surrounding tissue were harvested and fixed in 10% formalin solution for at least 24 h. Samples were embedded in paraffin, and 5–10 μm sections were cut by rotary microtomy (Leica) and subsequently stained with hematoxylin (Electron Microscopy Sciences) and eosin (Polysciences).

Monomer was photopolymerized in a model bone defect created in a rat tibia to illustrate the feasibility of in situ forming these polymers and to examine the influence of the polymerization reaction on local tissue. Briefly, the anteromedial tibial metaphasis was exposed, and a 2.3 mm diameter hole was drilled proximally near the cortex using a hand-press drill. A solution of 25 wt% MC and 75 wt% MSA was packed into the defect and photopolymerized in situ using a blue light source (DenMat, Marathon Two, Model 3940) at $\sim$50 mW/cm^2. An initiator concentration of 0.25 wt% CQ and 0.25 wt% TEA was used. After 5 days, the tibias were retrieved and fixed in 10% buffered formalin for approximately 20 h. Following decalcification with 10% formic acid at 37°C, the

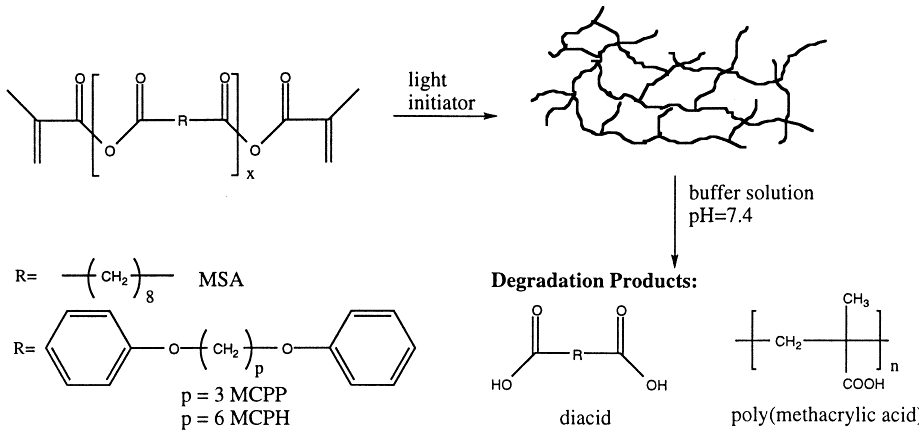

Fig. 1. Dimethacrylated anhydride monomers, methacrylated sebacic acid (MSA), methacrylated 1,3-*bis*(*p*-carboxyphenoxy) propane (MCPP) and methacrylated 1,6-*bis*(*p*-carboxyphenoxy) hexane (MCPH)), as well as a general polymerization and degradation scheme.

excess muscle was removed, and the samples were dehydrated to paraffin wax. Sections were cut to 5–10 μm and stained with hematoxylin and eosin.

3. Results and discussion

3.1. Material development

Fig. 1 shows the monomer structures, polymer network formation, and final degradation products. Our material design was based upon a class of FDA approved linear polyanhydrides used for drug delivery [18–20]; however, we rationally modified this chemistry to synthesize multifunctional monomers that react to form degradable networks with properties that address several key issues for orthopaedic biomaterials. Specifically, these monomers are photocurable, which allows for easy processing and in situ polymerization; form highly crosslinked networks upon polymerization, which improves mechanical properties; and result in polymer networks that degrade controllably and from the surface only, which allows maintenance of structural integrity with degradation.

The core of the macromer is based upon linear polyanhydride chemistry [18] wherein hydrophobic diacid molecules (e.g., sebacic acid and *bis*(*p*-carboxyphenoxy) propane) are linked via hydrolytically unstable anhydride bonds. The hydrophobicity of the backbone limits the transport of water in the polymer, and hence, only the anhydride linkages at the surface of the material are subject to hydrolytic cleavage and degradation. Furthermore, the degradation rate is readily modified from days to months by copolymerizing acids of varying hydrophobicity (i.e., R-groups, Fig. 1). While this controlled degradation mechanism has obvious advantages as a drug delivery vehicle, we hypothesize that the surface erosion mechanism may be ideal for orthopaedic applications. In particular, a surface degradable device could initially restore mechanical integrity to an injured bone and subsequently erode in a fashion, which maintains the strength of the device with mass loss.

Attached to the core of the macromer via anhydride linkages are methacrylate end groups, which are widely used in acrylic bone cements [4] and dental restorative materials [21]. Since each molecule contains two methacrylate groups, the monomer can be reacted through a photoinitiated radical chain crosslinking polymerization to form a densely crosslinked network. Crosslinking not only aids to increase the final network properties, but further supports the surface erosion mechanism as the highly crosslinked network resists swelling. The photopolymerization reaction allows in situ formation of the polymer under physiological conditions with spatial and temporal control. In addition, the initial concentration of methacrylate double bonds in the system, which is controlled through changes to the macromer molecular weight, dictates the final network's crosslinking density and mechanical properties. Thus, the degradation rate and mechanical properties of the polymer are readily tunable and decoupled.

Upon hydrolytic degradation, small molecular weight diacid molecules (from the macromer core) and linear chains of poly(methacrylic acid) (formed during the radical polymerization of the methacrylate groups) are released. The toxicology of the diacid molecules in Fig. 1 has been extensively studied, as these molecules are used to synthesize linear polyanhydrides for the delivery of chemotherapy agents to brain tumors [20,22,23]. While poly(methacrylic acid) is a water-soluble polymer used in hydrogels for drug delivery applications, its biocompatibility and molecular weight distribution in the crosslinked polyanhydride materials are unknown and examined in this work.

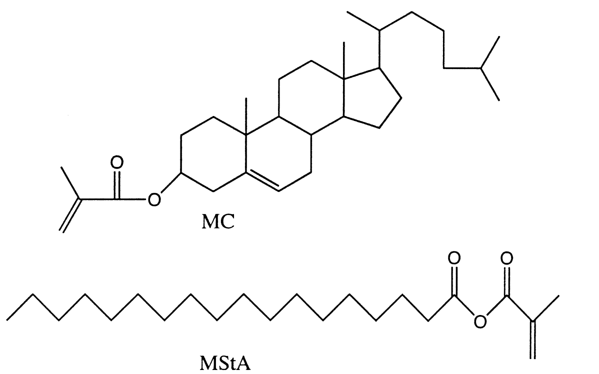

Fig. 2. Chemical structures of monovinyl monomers, methacrylated cholesterol (MC) and methacrylated stearic acid (MStA).

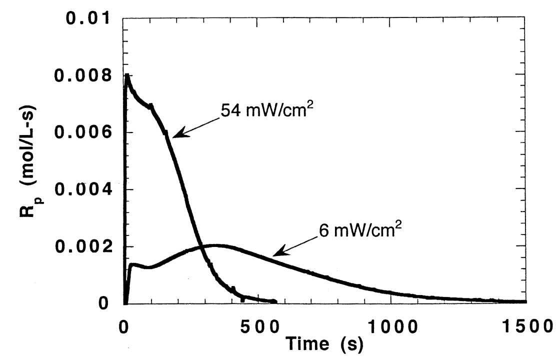

Fig. 3. Effect of light intensity on the rate of photopolymerization as a function of time for MSA polymerized with 0.5 wt% CQ and 0.5 wt% TEA.

In addition to these multifunctional anhydride monomers, we are also investigating monomethacrylated monomers based upon natural products (e.g., cholesterol and stearic acid) (Fig. 2). Cholesterol is a vital constituent of cell membranes and a precursor molecule to steroid hormones and bile salts. In addition, cholesterol has a hydroxyl group (i.e., at C-3) available for the attachment of a photoreactive methacrylate group. Similarly, stearic acid, a common fatty acid, has a carboxylic acid group available for synthetic modification and is a major component of various hydrophobic lipids. Reaction of these hydrophobic, monofunctional monomers into the polyanhydride networks can be used to control the overall network hydrophobicity, reduce the concentration of reactive groups, or alter the cytocompatibility of these materials.

3.2. Polymerization behavior

Differential scanning photocalorimetry was used to follow the rate of polymerization of the aforementioned methacrylate monomers under simulated in vivo conditions (i.e., in air and at 37°C). From a clinical perspective, determining how fast these materials react, controlling the reaction rate, and quantifying the heat released and exothermic temperature rise during polymerization are important. Similarly, monitoring the double bond conversion, especially for these in situ forming highly crosslinked networks, is of further interest as unreacted monomer may leach out of the polymer and harm nearby cellular material or adversely affect mechanical properties by plasticizing the network.

Fig. 3 shows the typical reaction behavior of MSA when polymerized with a visible light photoinitiating system, CQ and TEA, with 400–500 nm light at two incident light intensities (6 and 54 mW/cm^2). In general, these degradable, dimethacrylate anhydride monomers exhibit behavior typical of multifunctional monomer polymerizations [24,25]. In particular, the low gel point conversion, combined with the restrictions on mobility as the densely crosslinked network evolves, leads to autoacceleration and autodeceleration, radical trapping, unequal reactivity of functional groups, and limiting double bond conversions. While the kinetics of these reactions are not studied in detail here [14], several important features are readily obtainable from these data.

Fig. 3 demonstrates the feasibility of reacting these materials on a clinically acceptable time scale, and this time scale is readily controlled through changes in the initiation rate (e.g., light intensity and initiator concentration). For example, the reaction of MSA initiated with 0.5 wt% CQ and 0.5 wt% TEA with blue light at 54 mW/cm^2 was polymerized in $\sim$8 min whereas the system exposed to 6 mW/cm^2 was complete in $\sim$20 min. These polymerization times are comparable to currently used bone cements, and thus, provide a clinically acceptable time frame. Furthermore, in typical dental applications, several hundred mW/cm^2 of 470–490 nm light are used to polymerize dimethacrylates in composite materials to render tooth colored restorations [26]. Thus, the reaction time for these polymers can be further reduced by utilizing higher light intensities.

From an application standpoint, controlling the rate of polymerization (and, therefore, the rate of heat released) is a significant advantage of photopolymerization over thermally polymerizing bone cements. Common bone cements, which thermally initiate the polymerization of methyl methacrylate with benzoyl peroxide in the presence of an accelerator, can lead to temperature rises of 40–50°C at the cement-bone interface [27]. As a result, cell necrosis often occurs. While the maximum exothermic temperature rise is affected by the reaction mass and concentration of initiator and monomer, the temperature dependence of the kinetics, especially the activation energy for initiation, inherently leads to temperature excursions. For example, as the exothermic polymerization begins, the temperature of the reacting solution increases. As the temperature increases, the rate of initiation is increased, which increases the rate of polymerization and the rate of heat generation. Unless a large heat sink is available or an external temperature controller, the process is inherently non-isothermal.

In contrast, photoinitiated polymerizations are much more easily controlled. By shuttering the light source, initiation is stopped, and the polymerization drops to diminishingly small rates. Hence, temperature excursions can be controlled and the polymerization rate matched to the heat transfer rate. In addition, the activation energy for photoinitiators is much lower than that of thermal initiators, hence, significantly reducing the temperature dependence of the initiation kinetics. For example, our studies have shown that with slow rates of photoinitiation, the temperature rise at the material surface can be controlled to less than 5°C. In addition to providing advantages with respect to minimizing exothermic temperature rises, shuttering the light source also allows processing advantages such as the production of contourable fracture fixation plates [28].

While polymerization of these methacrylated monomers can be achieved with fairly low incident light intensities for open surgical applications, certain applications might benefit from the ability to polymerize endoscopically or through tissues. For example, an endoscopic polymerization may be ideal for maxillofacial or cranial applications, where a viscous monomer could be introduced into a fracture by a syringe and polymerized in vivo by exposing the exterior surface of the skin to the initiating light (i.e., without any incision). To examine the feasibility of such a minimally invasive polymerization process, light attenuation through various tissues was studied. For 470 nm light and ~1 mm thick tissue samples, results showed that ~30% of the light is transmitted through muscle, ~22% through fat, and ~11% through skin (unpublished results). Thus, the two initiating light intensities in Fig. 3 illustrate that if skin were exposed to 54 mW/cm^2 of 470 nm light, then 11% would be transmitted (6 mW/cm^2), and MSA can be polymerized at this intensity in ~20 min.

Finally, a major challenge to utilizing photopolymerizations in orthopaedic applications is the ability to polymerize thick, three-dimensional, complex structures (e.g., filling trabecular bone defects or processing intramedullary pins). While photopolymerizations have been widely used in the fabrication of thin films, the ability to polymerize at depths is challenging due to competitive absorption of light by the initiator, monomer, and polymer, as well as scattering of light in the sample. This work explores one strategy to address these issues through the use of a photobleaching initiation system, camphorquinone. In essence, a self-eliminating light gradient is created in the sample as the absorbance of the initiator changes upon exposure to the initiating light. The photobleaching effect is demonstrated for CQ in Fig. 4a as it is

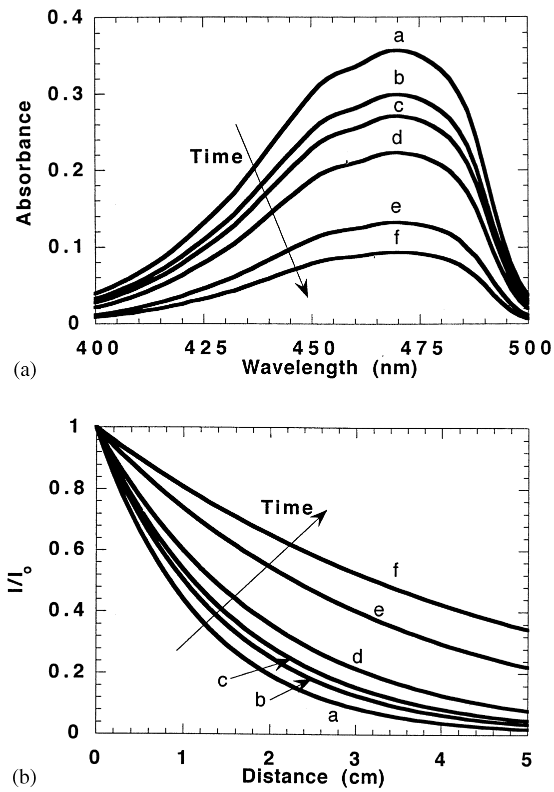

Fig. 4. (a) Absorbance spectra as a function of wavelength and irradiation time for 0.2 wt% CQ in 1-hexanol exposed to 150 mW/cm^2 blue light for: (a) 0 s; (b) 60 s; (c) 120 s; (d) 240 s; (e) 640 s; (f) 720 s. (b) Fraction of light transmitted as a function of depth for 0.2 wt% CQ exposed to 150 mW/cm^2 blue light for: (a) 0 s; (b) 60 s; (c) 120 s; (d) 240 s; (e) 640 s; (f) 720 s.

exposed to 150 mW/cm^2 of 470–490 nm visible light. After only 60 s (curve b), the absorbance is reduced to 84% of its original value; with exposure for 12 min (curve f), the absorbance of CQ declines to 26% of the initial absorbance.

The implication of this decreasing absorbance as a function of exposure time is a greater depth of penetration of the initiating light with irradiation as can be calculated from Lambert–Beer's Law

$$\frac{I}{I_0} = 10^{-\varepsilon[\mathrm{In}]b}. \tag{2}$$

Here, I/I_0 is the fraction of incident light at a distance b into the sample, ε is the molar extinction coefficient of the absorbing specie at the wavelength of interest, and [In] is the concentration of the absorbing specie. Fig. 4b plots light attenuation as a function of depth and time for a polymerizing system with 0.2 wt% CQ. Initially, only ~8% of the incident light is transmitted to a depth of 3 cm. With exposure for 240 s, the fraction of light reaching 3 cm increases to more than 21%, and after 12 min, more than 50% of the incident light is reaching depths of 3 cm. Hence, by careful selection of the photoinitiating

Table 2
Degradation kinetic constants and total degradation time for disks ($d = 2$ cm, $t = 2$ mm) as a function of the polymer network composition

Polyanhydride composition	k (mm/h)	Degradation time (days)
Poly(MSA)	1.3×10^{-2}	3
Poly(MCPH)	8.4×10^{-5}	496
50/50 poly(MSA)/poly(CPP : CPH)	5.4×10^{-4}	78
75/25 poly(MSA)/poly(CPH)	1.9×10^{-3}	22
Copoly(MSA : MC) (75 : 25)	3.0×10^{-4}	138
Copoly(MSA : MStA) (75 : 25)	1.9×10^{-3}	22

molecule, photopolymerization may be employed in situ to react thick three-dimensional polymers. For example, screws up to 3 cm in length have been fabricated [14].

3.3. Hydrolytic degradation

Table 2 illustrates the wide range of degradation rates attainable in these photocrosslinkable anhydride networks through simple changes in the polymer chemistry. Studies were performed in buffered saline solution at 37°C using disks ($d = 12.0$ mm, $t = 1.4$ mm) to simulate one-dimensional degradation. The surface degradation mechanism in these networks was confirmed by the linear mass loss profile, and degradation kinetic constants, k (mm/h), were calculated from this data as a function of network composition. Since these polymers are surface eroding, k is effectively the speed of the degradation front and is useful in predicting the mass loss and dimensional changes in more complex geometries. In addition to the degradation kinetics, the time for complete degradation of disks 2 cm in diameter and 2 mm in thickness is also shown for several different polymer compositions. For the compositions reported, k varies by three orders of magnitude (from 1.3×10^{-2} to 8.4×10^{-5} mm/h) and the total degradation time ranges from 3 days to nearly 500 days.

A disk of an aliphatic monomer, MSA, is completely degraded in 3 days, whereas a sample of identical geometry, but synthesized from the more hydrophobic monomer, MCPH, is degraded in ~500 days. Thus, the added hydrophobicity imparted by the aromatic rings of MCPH significantly slows the rate of degradation (nearly 170 times), and these monomers can be copolymerized to produce networks with degradation time scales spanning the degradation time for the individual homopolymers. In addition, linear polyanhydrides may be dissolved in the monomers before polymerization, and upon reaction semi-interpenetrating polymer networks (semi-IPNs) are formed. For example, the addition of 50 wt% poly(CPP : CPH) or 25 wt% poly(CPH) to MSA leads to semi-IPNs with increased hydrophobicity and degradation kinetics that are 24 and 7 times lower than that of poly(MSA) networks, respectively. The addition of linear polymer chains to the reaction mixture also provides additional benefits with respect to controlling the initial viscosity and decreasing the reactive group concentration, which minimizes volume shrinkage and the adiabatic temperature rise. Finally, hydrophobic monovinyl monomers can be copolymerized with the dimethacrylate anhydride monomers to alter the hydrophobicity of the network, as well as the reaction kinetics and overall crosslinking density. In this work, 25 wt% methacrylated cholesterol (MC) and 25 wt% methacrylated stearic acid (MStA) were copolymerized with MSA to produce copolymer networks with lifetimes of 138 days and 22 days, respectively.

In addition to characterizing the degradation rate of these crosslinked anhydride networks, the degradation products were also examined. Recall that the degradation products of these networks are the original low molecular weight diacid molecules, as well as a distribution of hydrophilic linear poly(methacrylic acid) (PMAA) chains. For the chosen monomers (MSA, MCPH, and MCPP), the diacid degradation products have been extensively studied, since these diacids are also employed to synthesize linear polyanhydrides used to deliver BCNU to patients with brain tumors, and were evaluated to be neither mutagenic nor cytotoxic [29]. In contrast, the biocompatibility of PMAA chains will be linked to their molecular weight and resorbability. In particular, Murakami et al. [30] followed the intravenous injection of poly(vinyl alcohol), poly(ethylene glycol), and dextran in mice, and their results suggest that the accumulation of polymers at an inflammatory site is based solely on the polymer molecular weight. A maximum accumulation of polymers in the blood circulation was seen for polymers with molecular weights of 200 000 Da.

Thus, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry was used to characterize the molecular weight distribution of the PMAA degradation products. This technique allows for highly resolved measurements of a polymer's MWD, and Fig. 5 shows the distribution of PMMA chains from the degradation of a poly(MSA) network. Specifically, the number fraction of chains with n repeating units, N_n, is plotted as a function of the chain molecular weight. Several features are important to note. First, the molecular weight distribution is relatively narrow, especially for radical polymerizations. Second, all of the PMAA chains in the degradation product are relatively low molecular weight, in the range of 500–3500 Da, and significantly smaller than the molecular weight range that leads to accumulation in the circulatory system for non-charged, hydrophilic polymers.

3.4. Histological evaluation

Biocompatibility and in vivo degradation of the photopolymerized anhydride networks was assessed

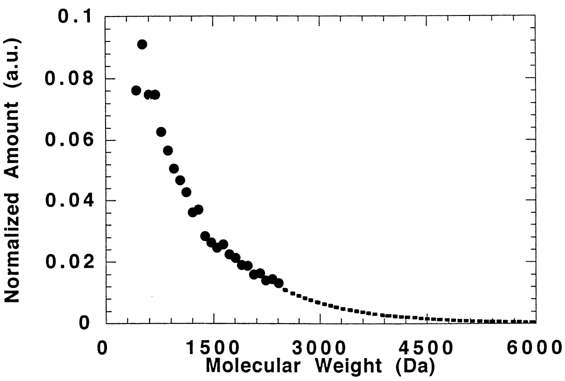

Fig. 5. Molecular weight distribution of poly(methacrylic acid) degradation products from a sample of MSA polymerized with 0.1 wt% 2,2-dimethoxy-2-phenylacetophenone (DMPA) and 100 mW/cm^2 ultraviolet light.

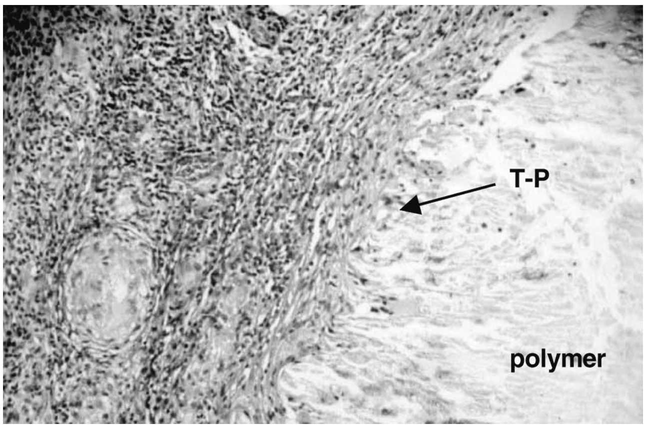

Fig. 6. Hematoxylin- and eosin-stained histological sections of the tissue—polymer interface (T–P) from a subcutaneous implant of 50/50 w/w poly(MSA)/poly(CPP : CPH) at 28 days.

through subcutaneous implants in rats. Fig. 6 presents a micrograph that is representative of the tissue/polymer interface for a photocrosslinked semi-IPN disk of 50 wt% MSA and 50 wt% poly(CPP : CPH). In contrast to a typical foreign body response, whereby the body walls off an implant with a dense connective tissue capsule, the aforementioned anhydride materials exhibit greater integration of tissue into the polymer. After 28 days in subcutaneous tissue, the surrounding tissue looks healthy with many spindle-shaped fibroblasts invading the degrading polymer construct among a loose collagen network. Similarly, the presence of a capillary network and few macrophages demonstrates a mild reaction to the photocrosslinked networks and their degradation products.

Preliminary studies were also performed in a model bone defect to determine the feasibility of placement and in situ photopolymerization of the monomer, as well as to assess any adverse effects of the polymerization reaction on the local tissue. A 2.3 mm bone defect was created in the anteromedial tibial metaphysis of a rat by drilling a hole through the near cortex and underlying trabecular bone. A moldable putty comprised of 25 wt% methacrylated cholesterol and 75 wt% MSA along with the CQ/TEA visible light initiating system was packed into the defect and polymerized in situ with approximately 50 mW/cm^2 of blue light for 8 min. Macroscopic observations indicated that the polymer was well-adhered and filled the defect structure. Following the surgery, there

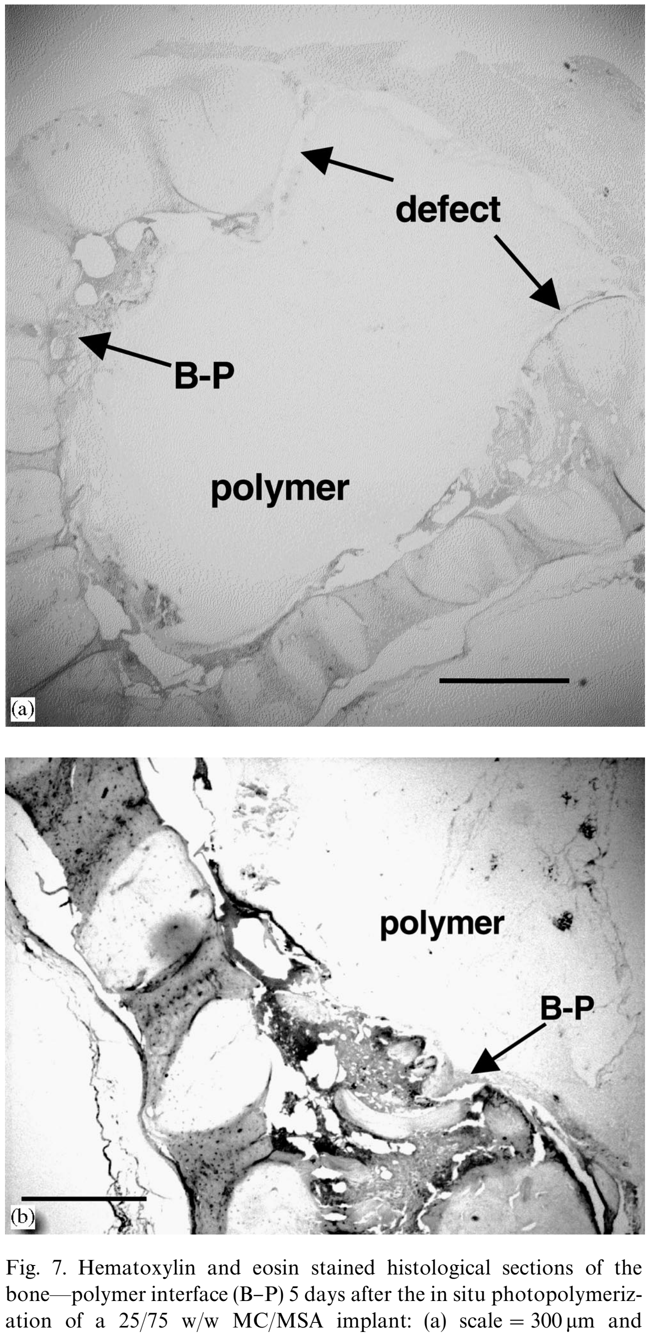

Fig. 7. Hematoxylin and eosin stained histological sections of the bone—polymer interface (B–P) 5 days after the in situ photopolymerization of a 25/75 w/w MC/MSA implant: (a) scale = 300 μm and (b) scale = 600 μm.

were no postoperative complications; incision scars healed normally, there were no cases of swelling, and functional recovery of the limb was immediate. The tibias were retrieved after 5 days, and cross-sectional micrographs of the treated defect are shown in Fig. 7a and b. In Fig. 7a, it is clear that the polymer fills the defect and entire marrow canal; the implant has not drifted; and no obvious damage to the local tissue from the polymerization reaction is observed. In addition, a callus of fibrous tissue surrounding the defect, evidence of initiation of the healing process, is observed. Fig. 7b illustrates the intimate contact of the polymer with the bony tissue (i.e., trabecular bone and marrow cells). While some areas have pulled away during processing of the sample, in general, the interface is tightly adhered to the cellular material, and the absence of inflammatory cells in the regions between the polymer network and cortical bone suggest that the polymer composition is tolerated well by the surrounding tissue components at 5 days. Furthermore, additional studies are in progress to assess the in vivo response to these polyanhydride networks and their acidic degradation products at longer time points to determine their overall biocompatibility in bone.

4. Conclusions

A new class of methacrylate anhydride monomers has been synthesized for orthopaedic applications. The novelty of these materials lies in their ability to form in situ, and we have utilized photopolymerization as a technique for reacting these monomers under physiological conditions. The methacrylated monomers photopolymerize quickly to form densely crosslinked networks, and the rate of polymerization can be readily controlled by the photoinitiation conditions (e.g., incident light intensity or initiator concentration). Thick, three-dimensional devices can be fabricated with photobleaching initiating systems, where the bleaching effect of the initiator allows greater penetration of light into the sample.

The resulting photocrosslinked polyanhydrides degrade from the surface inward, and the degradation rate can be changed simply from application to application by altering the overall hydrophobicity in the polymer network. MALDI-TOF analysis of the degradation products showed that the MWD of PMAA chains was narrow and at low molecular weights (500–3500 Da), suggesting that the PMAA chains may be easily resorbed in vivo. Finally, preliminary studies in rats show good compatibility of the materials in subcutaneous tissue. In addition, we successfully polymerized the network in situ in a tibial bone defect, and preliminary histological evaluation showed good adhesion of the polymer to the cortical bone and medullary cavity, as well as minimal adverse tissue reaction to the photopolymerization reaction.

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