



## Delivering DNA from photocrosslinked, surface eroding polyanhydrides

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### Abstract

Sustained delivery of DNA has the potential to enhance long-term gene therapy; however, precise control of a wide range of DNA release profiles may be needed. In this work, multifunctional anhydride monomers were photocrosslinked to produce hydrophobic, highly crosslinked polymer networks that degrade by surface erosion. Surface-eroding polymers can deliver molecules of a wide range of sizes at sustained, steady rates, which is advantageous for DNA delivery, where the high molecular weight may complicate control of the release profiles. When plasmid DNA was released from photocrosslinked polyanhydride matrices, DNA recovery was low (~25%). Electrophoresis indicated that the plasmid DNA was released primarily in the relaxed and supercoiled forms, yet the relative fraction of released DNA in the supercoiled form decreased over time. To improve DNA recovery and reduce the damaging effects of polymer degradation, DNA was pre-encapsulated in alginate microparticles, which served as a temporary coating that quickly dissolved upon microparticle release from the polyanhydride matrix. As photocrosslinked polyanhydrides have highly predictable drug release profiles that depend on the polymer erosion rate and implant geometry and not on the entrapped molecule size, they can serve dual purposes in many biomaterial applications where structural support and drug release would be beneficial.

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### 1. Introduction

Localized and sustained DNA delivery from polymers has the potential to provide long-term gene therapy and researchers have already shown enhanced expression of the foreign protein [1–3], protection of the DNA from enzymatic degradation [1,4,5] and

prolonged foreign protein expression [2,3,6,7] using polymer delivery vehicles. One of the most commonly studied synthetic polymers for gene delivery, poly(lactic-co-glycolic acid) (PLGA) [1,3,4,8–12], has historically been used for a wide range of medical applications [13–16]. PLGA matrices [3], microspheres [8,10–12] and nanospheres [1,9] have been used to deliver DNA and research has shown high levels of foreign protein expression despite an observed decline in plasmid integrity during release [3,11,17,18].

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Over the past decade, there has been emerging interest in polyanhydrides as degradable drug delivery materials. Polyanhydrides are advantageous for drug delivery because they degrade by surface erosion, and thus the erosion profile and drug release can be manipulated by changes in the polymer chemistry, mainly hydrophobicity, as well as changes in the physical properties of the final matrix, including fabrication method, geometry, and addition of hydrophobic and hydrophilic components [19,20]. Surface-eroding polymers can deliver entrapped molecules at sustained, steady rates and such rates are achievable for a wide range of molecular weights. For high molecular weight molecules, steady release can be difficult to achieve with other types of polymer systems, such as systems with bulk degradation, where extensive polymer degradation must occur before the encapsulated drug is released. Thus, surface erosion enables tailoring the release of even high molecular weight molecules like DNA. Polyanhydrides have been studied for the controlled release of a variety of drugs, including antibiotics [21], anesthetics [22], insulin [23], and many types of small molecules to treat cancer [24–26]. Polyanhydrides containing BCNU (carmustin) have been approved for clinical use worldwide for the treatment of brain tumors [26]. However, there has been little exploration of polyanhydrides to deliver DNA. Mathiowitz et al. [27] evaluated *in vivo* transfection resulting from the release of plasmid DNA from poly(fumaric-*co*-sebacic acid) microspheres. Five days after an oral dose of DNA-loaded microspheres, higher  $\beta$ -galactosidase activity was observed in the small intestine and liver than with controls (naked plasmid DNA and empty microspheres). Plasmid DNA extracted from

the microspheres indicated no damage as a result of encapsulation; however, results were not discussed regarding any evidence of DNA damage as a result of polymer degradation or encapsulation efficiency. This initial work thus demonstrated that polyanhydrides have potential for use in gene delivery.

For many drug delivery applications, linear polyanhydrides provide desirable characteristics, but in order to combine drug delivery with other biomedical applications, such as tissue engineering and orthopedic biomaterials, higher strength polymers are useful. Towards this goal, multifunctional anhydride monomers have been developed with methacrylate endgroups that react to form highly crosslinked polyanhydride networks with improved structural properties [28] (structures are shown in Fig. 1). Polymerization of anhydrides functionalized with methacrylate endgroups can be initiated with light and a photoactive initiator [29], which allows crosslinked degradable networks to be formed *in situ* [30]. In addition, drug delivery from photocrosslinked anhydrides has been evaluated for model compounds, where the surface erosion mechanism provided easily tailored linear release profile and photopolymerization of laminated structures allowed pulsatile or more complex delivery patterns [31]. In general, photoencapsulation also allows for high encapsulation efficiencies as the drug is mixed directly into solution prior to polymerization and thus the final network formed requires no further processing after polymerization.

For gene delivery, the predictable and flexible drug release photocrosslinked polyanhydrides provide could allow repeated transfections with an appropriate amount of DNA for the rate of local cell division and

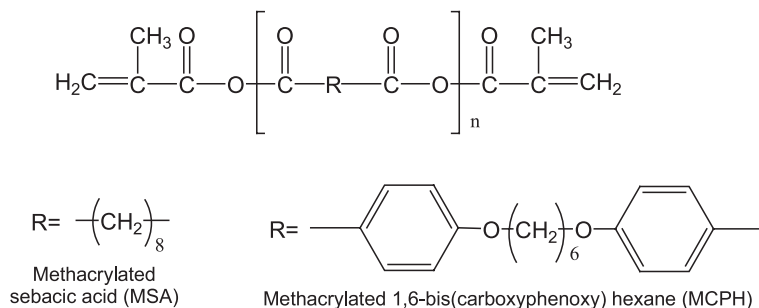


Fig. 1. General structure of multifunctional anhydride monomers that can be photocrosslinked to form high-strength, surface-eroding materials and the structures of the R groups for the monomers used in this work.

the cell's capacity for DNA uptake. In addition, with the high mechanical strength of photocrosslinked polyanhydrides, there is the potential for developing polymers to function both as a mechanical support structure and as a controlled release device. This work addresses the suitability of photocrosslinked polyanhydrides for DNA encapsulation and delivery. Ultimately, the goal is to provide a high-strength polymer that forms under mild conditions, does not damage encapsulated DNA, and can provide easily tailored release kinetics. Such a system might be beneficial as an orthopedic biomaterial, where released DNA could enhance healing by providing a sustained production of osteoinductive stimulants, while the polymer scaffold provides mechanical support. Bonadio et al. [43] showed the potential for the plasmid DNA pMat-1, which encodes for the production of a secreted peptide fragment of human parathyroid hormone (hPTH 1-34) encapsulated in a collagen sponge to induce new bone formation in canine critical tibia defects. Previous reports on DNA delivery from photopolymerized hydrogels [32] showed that photopolymerization was compatible with DNA encapsulation, and through the use of protective agents, the damaging effects of photoinitiated radicals could be reduced. In addition, the integrity of plasmid DNA is maintained when exposed to UV light under the photopolymerization conditions employed in this study [35]. Photocrosslinked polyanhydrides form a much more hydrophobic and glassy polymer environment compared to hydrogels, and instead of swelling in water, polyanhydrides are impermeable to water. This paper addresses the effects of photoencapsulation and polyanhydride degradation on DNA quality and encapsulation efficiency *in vitro*. In addition, the ability to control the DNA release profile from photocrosslinked polyanhydrides through the use of different monomer chemistries is demonstrated.

## 2. Materials and methods

### 2.1. Materials

DNA plasmids encoding for secreted alkaline phosphatase (SEAP, ~ 5.1 kb; Clontech, Palo Alto, CA) and green fluorescent protein (GFP, ~ 5.7 kb; Gene Therapy Systems, San Diego, CA) were pur-

chased and subsequently amplified by a commercial vendor (Aldevron, Fargo, ND). Methacrylic acid (Aldrich, Milwaukee, WI), sebacyl chloride (Aldrich), dibromohexane (Aldrich), hydroxybenzoic acid (Aldrich), triethylamine (Aldrich), methacryloyl chloride (Aldrich, 97%), and the photoinitiator 4-(2-hydroxyethoxy)phenyl-(2-propyl)ketone (I-2959; Ciba Geigy, Basel, Switzerland) were used as received without further purification. Solvents (methylene chloride, methanol; Sigma, St. Louis, MO) were of reagent grade.

### 2.2. Synthesis

Dimethacrylated sebacyc acid (methacrylated sebacyc acid or MSA) was synthesized according to previously reported procedures [33]. Methacrylation efficiency ranged from 90% to 100% according to <sup>1</sup>H NMR.

Dimethacrylated carboxy phenoxy hexane (methacrylated CPH or MCPH) was synthesized by first synthesizing CPH diacid according to published procedures [34]. Residual water was removed by freeze-drying on a LabConco Freeze-Dryer (LabConco, Kansas City, MO). CPH diacid was methacrylated as published for methacrylation of carboxy phenoxy propane (CPP) [33] with slight modification. CPH diacid (~ 0.04 mol) was stirred into methylene chloride (300 ml) (not soluble) under argon for 10 min. Triethylamine (~ 0.1 mol) was added by injection and the mixture was stirred for an additional 30 min at room temperature before transferring to an ice bath for an additional 30 min. Methacryloyl chloride (~ 0.1 mol) was added dropwise to the solution. Stirring on ice was continued for another 4 h, followed by vacuum filtration to remove the precipitated triethylammonium chloride salt. The filtrate was washed twice with saturated sodium bicarbonate (300 ml) and twice with deionized water (300 ml). Sodium sulfate was added to remove water from the solution and subsequently removed by filtration. Vitamin E ( $\alpha$ -tocopherol, Sigma, ~ 50  $\mu$ l) was added to inhibit polymerization prior to removing the methylene chloride under vacuum. The resulting product was a yellow, viscous oil at temperatures >30 °C and a white paste when refrigerated. Methacrylation efficiency ranged from 90% to 100% according to <sup>1</sup>H NMR.

### 2.3. DNA photoencapsulation

I-2959 photoinitiator (Ciba Geigy) was dissolved in the liquid monomer at a concentration of 0.1 wt.% at  $\sim 80^\circ\text{C}$  and cooled to  $<60^\circ\text{C}$ . To incorporate DNA, the DNA was first freeze-dried (LabConco freeze-drier) in the presence of high-purity sucrose (Pfanstiehl, Ferro, Cleveland, OH). The freeze-dried powder was distributed evenly into weigh dishes to provide 20–100  $\mu\text{g}$  of DNA per disk ( $\sim 0.2$ – $1 \mu\text{g}$  DNA per mg of monomer). Alternatively, DNA was encapsulated in alginate prior to photoencapsulation in the polyanyhydrides. Alginate gels were formed by injecting alginate (Sigma) solution (2% (w/v) in water) through a 25-gauge needle into stirring 50 mM calcium chloride (Sigma). DNA was mixed into the alginate solution prior to forming the beads. Alginate-DNA beads were freeze-dried (LabConco) prior to photoencapsulation.

Monomer solutions were polymerized in prepared molds. Glass slides were coated with Rain-X (Blue Coral-Slick 50, Houston, TX) and then a 1-mm thick Teflon sheet with 5-mm-diameter holes was affixed to the glass slides with vacuum grease. Monomer was pipetted into the mold and the DNA powder was mixed into the monomer by pipetting the mixture several times to achieve uniformity. Before covering, filled molds were placed briefly in a  $-20^\circ\text{C}$  freezer to increase the viscosity of the liquid and a second Rain-X-coated glass slide was then placed on top of the filled molds and clipped in place. The monomer-DNA mixture was exposed to 5–11  $\text{mW}/\text{cm}^2$  of 365 nm light (Cole-Parmer, Vernon Hills, IL) for 12 min to produce the DNA-loaded, degradable polyanhydride disks.

### 2.4. Polymer degradation and DNA release studies

Polymerized disks were degraded in 2 ml phosphate-buffered saline (PBS; Gibco) supplemented with 0.05 M 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES; Fisher, Houston, TX; PBS/HEPES, pH=9.0) at  $37^\circ\text{C}$  on an orbital shaker. Buffer was removed periodically (when pH dropped below 7.0, every 1–4 days) and centrifuged at  $12,000 \times g$  for 10 min to pellet any insoluble polymer degradation products. The supernatant was assayed for DNA with the PicoGreen assay (Molecular Probes,

Eugene, OR), while the pellet was resuspended in fresh buffer and added back to the degrading disks to prevent DNA loss by entrapment in the partially degraded/insoluble material. Parallel disks, which contained no DNA, were degraded to account for the effects of the polymer degradation products (i.e., poly(methacrylic acid), sebacic acid and/or CPH) on the PicoGreen assay. Polymer mass loss was determined by freeze-drying disks on selected days throughout the degradation process and comparing the dry mass to the original dry polymer mass.

### 2.5. Quality of released DNA

Quality of the released DNA was examined with both agarose gel electrophoresis and bacterial transformations. Agarose gel electrophoresis was conducted with 0.8 wt.% agarose (Fisher) gels run at 100 V in Tris-acetate-EDTA (TAE, Fisher) for approximately 1 h. Gel photographs were analyzed with Kodak 1D software (Kodak, Rochester, NY). Samples of released DNA, taken throughout the degradation, were combined and concentrated to provide solutions with high enough DNA concentration for electrophoresis. The combined solutions were filtered through a series of syringe and membrane filters to remove insoluble degradation products: AP syringe prefilters (glass fiber; Millipore, Bedford, MA), 1.2- $\mu\text{m}$  membranes (RTTP; Millipore), 0.8- $\mu\text{m}$  syringe filters (cellulose esters; Millipore), 0.45- $\mu\text{m}$  syringe filters (cellulose acetate; Corning), 0.6- $\mu\text{m}$  membrane (DTTP; Millipore), and finally 0.2- $\mu\text{m}$  syringe filters (cellulose acetate; Corning). After filtration, DNA concentration ranged from 0.1 to 1  $\mu\text{g}/\text{ml}$ . To concentrate the DNA, 15 ml samples were filtered through 100,000 MWCO Centricon filters previously soaked in 1% bovine serum albumin (BSA) in PBS and 5% PEG compound (Sigma) in deionized water. Centricon filtration concentrated the solutions approximately 10-fold. To remove additional polymer degradation products, which generally have molecular weights less than 100,000 g/mol [36], the concentrated solution was diluted in deionized water and filtered through the Centricon filters again. Samples required 2–10 repeats to significantly reduce the concentration of degraded polymer (as determined by PicoGreen assay, which is sensitive to poly(methacrylic acid)).

With some samples, the DNA concentration was too low after Centricon filtration to conduct electrophoresis. In these cases, DNA was further concentrated by ethanol precipitation, which co-precipitates polymer degradation products with DNA. Unfortunately, the methods for concentrating and purifying DNA released from photocrosslinked polyanhydrides were not always effective at removing the polymer degradation products. Thus, electrophoresis could not be conducted with all samples.

As an alternative measure of DNA activity and quality, bacteria were transformed with the polyanhydride-released DNA. Bacterial transformations were conducted with transformation-competent *Escherichia coli*, DH5a (prepared by S. Langer according to standard protocols [37]) to compare the quality of DNA released under various conditions. Polyanhydride-released DNA at  $\sim 0.5 \mu\text{g}/\text{ml}$  was used to transform *E. coli*. Bacterial growth after transformation with released plasmid was compared to bacterial growth with non-encapsulated plasmid DNA spiked into a solution of degraded polymer to give a relative assessment of the quality of the DNA. Transformations were conducted according to standard procedures [37] using 0.5–1 ng of plasmid DNA collected throughout release studies (0.2- $\mu\text{m}$ -filtered or purified as described above for electrophoresis). Briefly, after sample addition the bacteria were incubated on ice for 30 min, heat shocked at 37 °C for 2 min, then returned to ice. Lennox L broth (LB, Invitrogen) was added to each sample to bring the total volume to 300  $\mu\text{l}$  and the samples were shaken at 200 rpm in a 37 °C warm room for 45–60 min. Samples were then

diluted in LB at values ranging from 1:2 to 1:50 dilution. A 100- $\mu\text{l}$  portion of the diluted samples was spread over LB-agar (Gibco) plates containing 50  $\mu\text{g}/\text{ml}$  ampicillin (Sigma). Plated cultures were incubated inverted in a warm room (37 °C) for 15–20 h at which point bacterial colonies were manually counted to give a relative assessment of the biological activity of the released DNA. As a control, 0.5–1 ng of non-encapsulated plasmid diluted in a solution of degraded polymer was also given to bacteria.

### 3. Results and discussion

#### 3.1. Release of naked plasmid DNA from photocrosslinked polyanhydrides

Given the surface erosion mechanism of photocrosslinked polyanhydrides, release of entrapped molecules typically follows the polymer mass loss profile. Indeed, DNA was coupled to polymer erosion as shown in Fig. 2. The disk-shaped geometry leads to a nearly linear mass loss and DNA release profile. This predictability of entrapped molecule release makes photocrosslinked polyanhydrides attractive for controlled DNA release.

In designing a system for gene delivery, the polymer should provide a wide range of release profiles in order to be adaptable to several applications. In general, photocrosslinked polyanhydrides show great versatility in drug release profiles [31]. As DNA release from polyanhydrides is controlled by the surface erosion of the polymer disk, the release profile

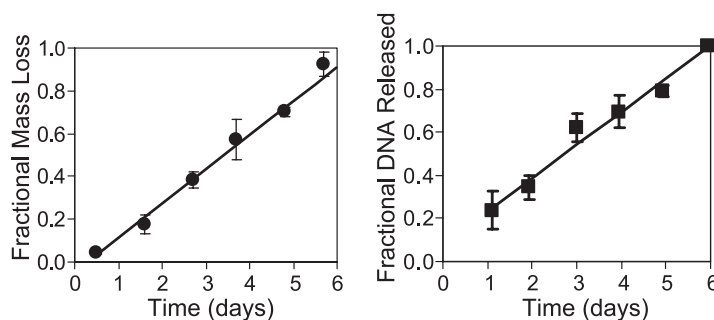


Fig. 2. Fractional mass loss and DNA release from photocrosslinked MSA disks as a function of time. Disks were degraded in PBS/HEPES buffer at 37 °C. Mass loss disks were polymerized with 0.1 wt.% I-2959 and 365 nm light at 11 mW/cm<sup>2</sup> for 12 min; DNA release disks were polymerized with 0.1 wt.% I-2959 and 365 nm light at 5 mW/cm<sup>2</sup> for 12 min. Error bars indicate standard deviation,  $n=4$ .

can be altered by adjusting the geometry or the hydrophobicity of the network. Others have shown how the network geometry affects the degradation and hence the release of entrapped molecules [31]. The work presented here focused on modifying network hydrophobicity through the use of different monomer chemistries, although hydrophobic and hydrophilic constituents could also be added to alter the degradation profiles [38].

To modify the *in vitro* polymer degradation rate and DNA release, multifunctional anhydride monomers with different hydrolytic stability were copolymerized. The faster degrading MSA was copolymerized with the more hydrophobic methacrylated carboxy phenoxy hexane (MCPH) and results indicated that the DNA release rate from MSA could be significantly varied depending on the copolymer composition (Fig. 3). With copolymers containing 65% MSA with 35% MCPH, the release rate was reduced approximately threefold relative to the release rate from MSA homopolymers (0.022/day–65/35-copolymer vs. 0.068/day–MSA), according to slopes taken from linear fits of the data (Fig. 3). Homopolymers of MCPH degraded and released DNA approximately seven times slower than pure MSA (0.009/day). Polymers formed with these two monomers thus provided controlled DNA delivery for periods of 7–42 days. It should be noted the rate at which the networks degraded was

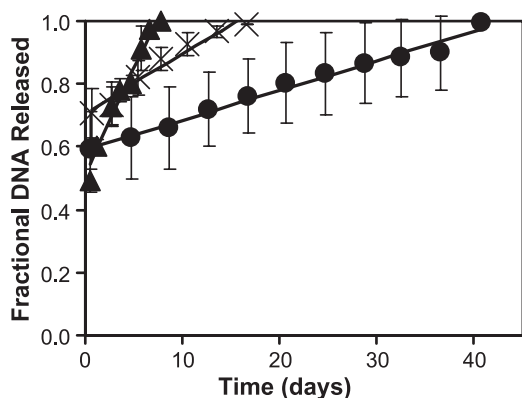


Fig. 3. Fractional DNA release from MSA, MCPH, and a copolymer of the two monomers. MSA (▲), 65% MSA/35% MCPH (×), and MCPH (●). Photopolymerizations conducted with 0.1 wt.% I-2959 and 365 nm light at 11 mW/cm<sup>2</sup> for 12 min. Error bars indicate standard deviation,  $n=3$ .

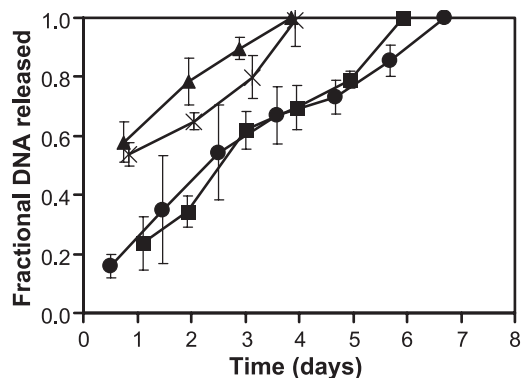


Fig. 4. Fractional DNA release from MSA in the presence of different sugar concentrations, (▲ and ×) ~0.1 mg sugar/mg MSA, (● and ■) ~0.05 mg sugar/mg MSA. Photopolymerization conducted with 0.1 wt.% I-2959 and 365 nm light at 5 mW/cm<sup>2</sup> for 12 min.

accelerated by using a basic buffer (pH 9.0). Under physiological conditions (pH 7.4), the rate of hydrolytic cleavage of the anhydride blocks would be slowed down, but the degradation profile would remain the same. The initial burst is high in this particular experiment with approximately 50% of the total DNA release occurring on day 1. That initial burst depends on the amount of excipient present during freeze-drying of the DNA (in preparation for encapsulation, as described in Materials and methods). After the initial burst, DNA release from the MSA networks proceeded at roughly the same rate, which suggests the encapsulation of freeze-dried powders did not significantly alter the network structure.

For all polyanhydride encapsulations, DNA was freeze-dried prior to encapsulation as the anhydride monomers are immiscible with and quickly degraded by water. Typically, DNA was freeze-dried in a sugar excipient, sucrose. The amount of sugar sucrose loaded into the polyanhydride disks significantly affected the initial DNA release (Fig. 4). At high sucrose concentrations (0.2 mg/mg monomer), nearly 100% of the DNA was released on the first day. With 0.05 mg sucrose/mg monomer, about 20% of the encapsulated DNA was released on the first day (as shown in Figs. 2 and 3). The large initial burst with high sugar concentrations may be due to the formation of a fairly interconnected sugar network throughout the polymer disk, which may allow water to penetrate the polyanhydride disks. Thus, large initial bursts in

DNA release were most likely to be a result of a significant portion of the encapsulated freeze-dried powders being readily dissolved when the disks were placed in buffer. After the initial burst, DNA release from the residual MSA disks prepared in the presence of different sugar concentrations preceded at roughly the same rate, which suggests that encapsulation of the freeze-dried powders did not significantly alter the polymer structure.

### 3.2. Recovery of naked plasmid DNA from photocrosslinked polyanhydrides

Based on previous results [35], the photoinitiation conditions used for these photoencapsulations should damage more than 99% of the naked plasmid DNA beyond assay detection (0.1 wt.% I-2959, 10 min exposure to 365 nm light at 5 mW/cm<sup>2</sup>). However, about 25% of the loaded DNA was recovered from these polyanhydrides. As shown before [32,35], the presence of methacrylated monomer provides a competing reaction site for the radicals and with a high concentration of double bonds, the probability that a radical reacts with a double bond being much higher than reacting with DNA during photopolymerization. The level of protection provided by the vinyl groups depends on the relative concentrations of radicals, DNA, and methacrylated groups.

Although the methacrylated monomer decreases DNA damage in the presence of radicals, the 25% DNA recovery from photocrosslinked polyanhydrides is about half that previously reported for photocrosslinked hydrogels (~60% [32]) and lower than that reported for the most commonly used polymer for gene delivery, PLGA (20–100%, depending on the processing conditions [1,8,9,11,18,39]). The low recovery from photocrosslinked polyanhydrides compared to photocrosslinked hydrogels may be partly a result of the different initiator concentrations used in these studies (0.1 and 0.05 wt.% I-2959). With twice the initiator concentration, twice as many initiator radicals should be generated during photopolymerization. Assuming that the majority of DNA damage is induced by the initiator radicals, half as much DNA would be expected to be recovered after encapsulation due to the presence of twice as many

initiator radicals, which agrees well with experimental observations. Another factor possibly contributing to the low recovery is that the polymerizing radical concentration is much higher in the polyanhydride system than in the previously reported hydrogels, as polyanhydrides are formed by bulk polymerization (pure monomer) while hydrogels are formed by solution polymerization (low monomer concentration). Polyanhydrides form a more densely crosslinked network, which leads to earlier onset of diffusion limitations during polymerization [40], which, in turn, increases the propagating radical concentration.

### 3.3. Conformation and activity of naked plasmid DNA released from photocrosslinked polyanhydrides

The biological activity of released plasmid DNA, which is related to the conformation of the DNA, is critical to the success of a gene delivery system. In the few cases where agarose gel electrophoresis resulted in distinguishable DNA bands, the released plasmid DNA appeared to be entirely in the relaxed and supercoiled conformations (see Fig. 5). Interestingly, negligible linear DNA was observed in DNA released from polyanhydrides. The lack of this damaged form of plasmid DNA is surprising given the low recovery of DNA, which suggests a highly damaging environment. The lack of a linear band may be partly due to the purification methods, which included filtration through 100,000 molecular weight cut-off filters

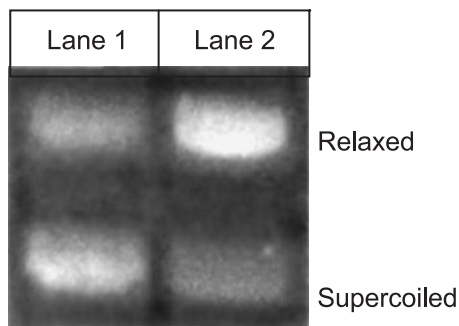


Fig. 5. Agarose gel electrophoresis of plasmid DNA released from MSA disks, first lane shows non-encapsulated DNA in a solution of degraded MSA, second lane shows released naked DNA. Photopolymerization conducted with 0.1 wt.% I-2959 and 365 nm light at 5 mW/cm<sup>2</sup> for 12 min.

(MWCO) with pores that might allow linear DNA to pass through.

As Fig. 5 shows, the plasmid DNA bands are slightly distorted. In the majority of cases, polymer degradation products completely inhibited gel electrophoresis as they dissolved the agarose. Poly(methacrylic acid) (poly(MA)) is the most likely polymer degradation product interfering with electrophoresis. Due to difficulties in purifying released DNA for electrophoresis, alternate methods for evaluating the integrity of the plasmid DNA were investigated. Bacterial transformation has previously been explored as an indicator of plasmid quality [41] and bacteria provide an ideal host for testing the quality of DNA released from polyanhydrides as they do not require high concentrations of plasmid in order to be transformed.

In all cases, bacterial transformations indicated significantly reduced transformation capacity of released DNA relative to non-encapsulated DNA. It is unclear why unprotected DNA typically transformed ~ 50% as many bacteria as non-encapsulated DNA as only linear plasmids have been shown to have reduced transformation efficiency [41] and electrophoresis shows minimal linear plasmid DNA. Perhaps damage other than conformational change occurs during photoencapsulation and/or polymer degradation that is not clear with the electrophoresis results, such as depurination.

One of the most important factors affecting the bacterial transformation efficiency of released naked DNA was the time spent in the polymer. DNA released in the first day had much higher activity than DNA released at any later time point (Fig. 6A), indicating that DNA damage may occur as a result of polymer degradation. Polymer degradation produces acidic products that can damage DNA; however, unlike PLGA systems where degradation occurs throughout the polymer and all of the entrapped DNA may be exposed to an acidic environment, with polyanhydrides, low pH should only occur on the surface of degrading polyanhydrides [19,20]. Thus, in theory DNA is only exposed to an acidic environment for a short time as the degradation front passes by. The low activity of DNA released at later time points suggests that DNA spends a significant time in the presence of high acid concentrations or that the anhydride polymer might be damaging the DNA.

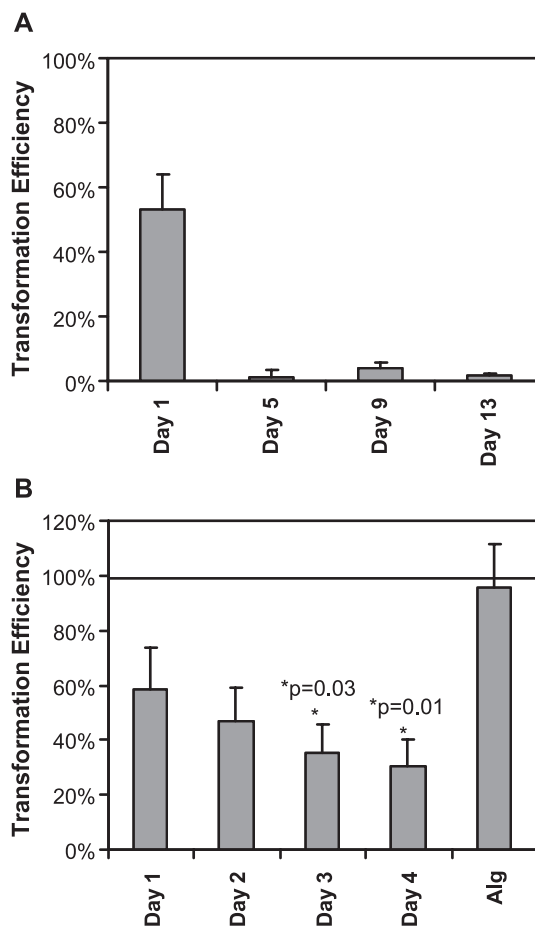


Fig. 6. Normalized counts of bacterial colonies transformed with (A) naked DNA released from copolymers containing 65% MSA with 35% MCPH as a function of release time; (B) alginate-encapsulated DNA released from MSA matrices as a function of release time and alginate-encapsulated DNA (not encapsulated in MSA). Colony counts were normalized to control (non-encapsulated) DNA in an appropriate solution of degraded polymer or degraded alginate. Error bars indicate standard deviation,  $n=3$ . \*Statistically significant difference from day 1 counts (sample  $t$ -test).

### 3.4. Improving DNA recovery and quality with protective agents

Although polyanhydrides provide a desirable linear release profile, photocrosslinked networks formed from MSA and MCPH clearly damage DNA. Conformation of the released plasmid DNA suggested minimal damage during photoencapsulation and polymer degradation, yet activity in bacterial transforma-

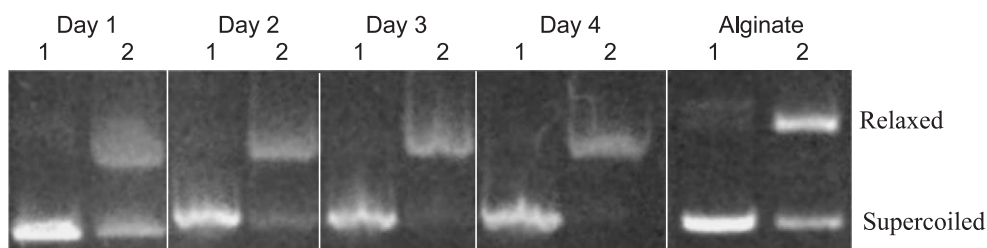
tions was only half that of non-encapsulated DNA and only about 25% of loaded DNA was recovered from the degrading polymers, suggesting significant damage. As shown previously, several agents can reduce radical damage of DNA [35], including molecules that react with radicals and those that surround DNA to physically block radical attack. These molecules may also protect DNA during polymer degradation.

The most successful of these approaches in the polyanhydride system was to put a physical barrier around the DNA. DNA was pre-encapsulated in beads formed with alginate (a naturally occurring polysaccharide that can be ionically crosslinked) and the alginate-DNA beads were subsequently freeze-dried. With the alginate-encapsulated DNA, the total DNA released from MSA disks was  $\sim 70\%$  of loaded DNA. Some of the DNA loss was due to alginate encapsulation (which in general released only 75–80% of the encapsulated DNA) and thus DNA damaged by photoencapsulation and polymer degradation was estimated as 5–10% of that initially loaded.

In addition, alginate-encapsulated DNA released from MSA matrices had slightly higher activity in bacterial transformations, particularly with DNA released at later times. As shown in Fig. 6B, transformation efficiency decreased with time spent in the polymer, a trend also observed with naked DNA

released from MSA/MCPH matrices (Fig. 6A). The decrease in efficiency was considerably reduced with alginate-encapsulated DNA, indicating that alginate partially protected encapsulated DNA from the polymer environment, as might be expected since alginate has been shown to effectively protect pH-sensitive molecules from acidic environments when pores in the alginate matrix shrink [42].

With the higher DNA concentration released from MSA disks containing alginate-encapsulated DNA, samples did not require additional concentration or purification to conduct agarose gel electrophoresis. Electrophoresis results agree well with data from bacterial transformations (Fig. 7). A steady decline in the supercoiled form of the encapsulated plasmid DNA was observed as the polymer degraded, indicating increasing damage with time spent in the polymer. Interestingly, electrophoresis of DNA released from alginate microparticles (not encapsulated in MSA) showed a reduction in the supercoiled form (Fig. 7). However, bacterial transformations indicated no decrease in activity of DNA released from alginate (Fig. 6B). The day 1 MSA-released, alginate pre-encapsulated DNA showed roughly the same ratio of relaxed and supercoiled conformations as alginate-encapsulated DNA ( $\sim 50:50$ ) and yet transformation efficiency was considerably lower than simply alginate released



	Relaxed	Supercoiled
Day 1	60	40
Day 2	70	30
Day 3	82	18
Day 4	100	---
Alginate	57	43

Fig. 7. Agarose gel electrophoresis of MSA-released, alginate-encapsulated plasmid DNA at different times during degradation. The first lane in each section shows control (non-encapsulated) DNA in a solution of the appropriate degraded polymer, while the second lane shows the released DNA. Table shows relative percent in each conformation according to image analysis.

DNA. While conformation of the plasmid DNA generally correlates with activity, these results suggest that plasmid conformation only gives a relative assessment of activity for samples with similar treatment. Thus, alginate pre-encapsulation is an effective means for protecting DNA from damage during photoencapsulation and in the degradation environment of photocrosslinked polyanhydrides.

#### 4. Conclusions

Photocrosslinked polyanhydrides offer a surface-eroding polymer with readily tailored release profiles. These materials provide greater versatility of the *in vitro* release profiles than many of the current polymer systems for gene delivery. However, without protection, little DNA was recovered from photocrosslinked polyanhydrides and the biological activity of the encapsulated DNA decreased with time spent in the polymer. By pre-encapsulating the DNA in alginate microparticles, less than 10% of the DNA was significantly damaged during photoencapsulation and the biological activity of the encapsulated DNA was better maintained. Thus, with DNA protection, photocrosslinked polyanhydrides provide a means for gene delivery with easily manipulated release profiles.

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