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ARTICLE

One-Step Syntheses of Photocurable Polyesters Based on a Renewable Resource

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ABSTRACT: The facile design of polyester biomaterials has emerged as an important aspect of polymer synthesis. As opposed to thermoplastics, thermosets are especially attractive for applications in the biomedical fields because they retain their geometry and experience a linear loss of both mass and mechanical properties during degradation. Herein, we report the design of several polyester thermosets based on photocurable prepolymers composed of itaconic acid and various polyols. Itaconic acid is a renewable resource and a component of known biomaterials that is demonstrated to be compatible with thermal polyesterification. This polymerization strategy results in photocurable branched polyester prepolymers in a single and facile step. The cross-linking density and, therefore, the rigidity of the photocured thermosets can be controlled by the addition of a comonomer, such as adipic acid or succinic acid. Additionally, dimethyl itaconate is an ideal monomer for enzymatic polymerization, as demonstrated by the synthesis of linear poly(1,4-cyclohexanedimethanol itaconate), poly(PEG itaconate), and poly(3-methyl-1,5-pentanediol itaconate-*co*-3-methyl-1,5-pentanediol adipate). Novel polyester thermosets designed from these two polymerization strategies achieved Young's modulus, ultimate tensile stress, and rupture strain values of 0.17−398.14 MPa, 0.11−18.20 MPa, and 5−198%, respectively. As all of the monomers used in these materials have previously been utilized in other biocompatible polymers, cytotoxicity was expected to be minimal. In order to verify this hypothesis, an ATP-luminescence assay was conducted with Swiss albino 3T3 fibroblasts. On the basis of preliminary data, we believe that itaconate-based polyesters are versatile, making them excellent candidates as future biomaterials.

Introduction

The need for novel biodegradable polymers in biomedical fields, such as drug delivery and tissue engineering, has motivated the biomaterials community to develop new polymers.[1−3] Aliphatic polyesters are of particular interest due to their biodegradable and nontoxic properties. Currently, a tremendous portion of polyester research for biomedical applications has focused on polymers derived from lactide, glycolide, and ε-caprolactone.[4] However, their utility is limited because the polymerizations require extremely dry conditions, and the resulting polymers are hard and brittle at physiological conditions. Therefore, the evolution of polyester design must continue in order to develop facile syntheses of elastomeric materials.

Many examples of both thermosets and thermoplastics have been described in the literature.[1−3,5] When compared to thermoplastic materials, amorphous thermosets offer a number of advantages for biomedical applications. Thermoplastic elastomers often contain crystalline regions, which result in heterogeneous degradation and a nonlinear loss of mechanical strength.[1,6] Additionally, the three-dimensional (3D) geometry of thermoplastic materials is commonly altered throughout the course of hydrolysis.[1,6] In contrast to thermoplastics, thermosets can be synthesized from precursors that are completely amorphous, enabling a predictable loss of mechanical properties and linear degradation.[1,6] In terms of biomaterials, the ability of a thermoset to retain its 3D structure is critical for various implants, including stents, tissue scaffolds, grafts, and sutures.

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Although both photocurable and thermally cured thermosets continue to be the focus of a significant portion of biomaterial research, the ability to cross-link biodegradable materials with light offers many benefits over thermal gelation. One benefit is that significantly faster curing periods (minutes versus days) and lower curing temperatures (room temperature versus 80−150 °C) are easily accessible. As such, photocuring provides a less harsh route to curing process than thermal cross-linking procedures. Therefore, fragile cargos, which include drugs and proteins, can be encapsulated in thermosets that are cured with light; this characteristic enables the design of drug releasing particles, stents, and sutures.[7,8]

We believe that the ideal polymer for biomedical applications should possess several characteristics. Biocompatibility and biodegradation are requisites in order to minimize the stress that is imposed upon living systems by the material. The polymer should also be a completely amorphous thermoset with no crystalline regions present, minimizing heterogeneous degradation. A wide range of macromolecular properties should also be accessible from a facile and inexpensive strategy. This would allow for the ability to tailor a material's properties to best suit a particular application. Finally and most importantly, the resulting polyesters should be photocurable after a single step while maintaining a simple synthetic scheme. This requirement would allow for curing to take place in several minutes and eliminate the need for postpolymerization modifications that can degrade or alter the labile polyester backbone.

Herein, we describe the synthesis and characterization of several photocurable polyesters based on itaconic acid (IA), a photoactive, biocompatible, and renewable monomer.[9] IA is

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Table 1. Prepolymer Synthetic Conditions

prepolymer	polymerization	reaction time (h)	reaction temp (°C)	topology	polyol[a]	other monomer	IA:DA[b]
PP1	thermal	10.5	145	branched	TMP	—[c]	1.0
PP2	thermal	9.5	145	branched	TMP	adipic acid	0.25
PP3	thermal	8.5	145	branched	TMP	adipic acid	0.1
PP4	thermal	7	150	branched	Sorb	succinic acid	0.4
PP5	enzymatic	48	90	linear	CHDM	—[c]	1.0
PP6	enzymatic	48	90	linear	PEG	—[c]	1.0
PP7	enzymatic	48	90	linear	MPD	adipic acid	0.25

[a] TMP = trimethylolpropane; Sorb = sorbitol; CHDM = 1,4-cyclohexanedimethanol; PEG = poly(ethylene glycol); MPD = 3-methyl-1, 5-pentanediol. [b] Molar ratio of itaconate in total diacid/diester content in monomer feed. [c] Homopolymer.

an ideal monomer and/or comonomer for thermal polyesterification, a polymerization strategy that has recently gained popularity in biomaterial synthesis.[1−3,5] IA was combined with adipic acid (AA) and trimethylolpropane (TMP) in order to obtain branched, photocurable polyesters. A similar prepolymer was synthesized by the polyesterification of IA, succinic acid (SA), and sorbitol. Additionally, dimethyl itaconate (DMI) is compatible with enzymatic polyester synthesis, catalyzed by Novozyme 435−Lipase B from *Candida antarctica* (CALB). In order to demonstrate versatility, linear polyesters were generated by combining DMI with various diols. The rigidity and strength of the resulting materials can be tuned by varying the cross-linking density through the initial monomer feed ratio, allowing for control over mechanical properties. Additionally, the noncured prepolymers are easily melted and/or dissolved for easy processing, demonstrated by micrometer-scale particles, embossed films, and porous scaffolds. Finally, *in vitro* cytotoxicity of the materials toward Swiss albino 3T3 fibroblasts (SAFs) was studied due to future biomedical applications.

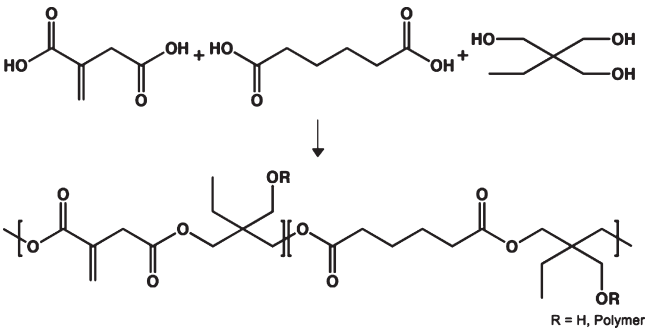
Results and Discussion

Motivation. Aliphatic polyesters are both strengthened and limited by their inherently biodegradable linkages. They are able to hydrolyze under physiological conditions, allowing for the design of medical devices and delivery vectors that do not require surgical removal. However, these relatively fragile linkages are not ideal in some common postpolymerization reaction conditions, including the use acidic and basic reagents. Therefore, including any desired functionality of the final material into the initial polymerization strategy would be beneficial. For example, terminal and pendant hydroxyl units are often converted into acrylates or methacrylates to create photocurable polyesters. The results are often reported to be "without significant degradation", even though the complete absence of degradation is desired. We believe that the ideal photocurable polyester would involve one synthetic step that encompasses both the polycondensation and the introduction of acrylate-like moieties.

The polymers described herein are based on monomers that have been utilized in previously studied biomaterials: IA, DMI, AA, TMP, sorbitol, SA, CHDM, PEG, and MPD (Table 1).[10−17] While all of the starting materials are biocompatible, our novel polyesters are based on IA, which provides an attractive foundation for polymers. IA is a completely renewable resource and can be isolated from bacterial fermentation and the distillation of citric acid.[18−20] Many classes of biomaterials have been based on itaconates, including hydrogels and glass ionomer cements.[10,21,22] These materials are synthesized through radical polymerization of the alkene in the itaconate monomer. As opposed to radical polymerizations, this report focuses on designing itaconate-based materials through polyesterification.

Materials 1−3. Branched polyesters were synthesized by thermal polyesterification. This technique is gaining in popularity due to its simplicity. Polyols and polyacids react to form esters under conditions of heat (120−150 °C) and vacuum without catalysts or coreagents, making this polymerization strategy especially attractive for biomaterials. TMP was combined with IA and AA in various ratios, generating prepolymers with different concentrations of the photocurable moiety (Scheme 1). Prepolymers 1, 2, and 3 (PP1, PP2, and PP3) contained IA, AA, and TMP in ratios of 1:0:1, 1:3:4, and 1:9:10, respectively. The monomers were heated only to the extent of cross-linking that would create oligomeric prepolymers; thermal cross-linking was avoided in order to obtain soluble prepolymers. The molecular weights of these oligomers were relatively small, with PP1, PP2, and PP3 having $\langle M_n \rangle$ values of 1140, 2200, and 1170 g/mol (Table 2). Additionally, all of the prepolymers were amorphous, with glass transition (T_g) temperatures ranging from −28.8 to −7.4 °C. The inclusion of larger amounts of AA caused lower T_g values, most likely due to the resulting decrease in cross-linking density.

Scheme 1. Thermal Synthesis of Poly(trimethylolpropane itaconate-*co*-trimethylolpropane adipate)



In addition to GPC and DSC, PP1, PP2, and PP3 were characterized by ^{1}H NMR. The ^{1}H NMR spectra of PP1, PP2, and PP3 were very similar due to the use of common monomers (Figure S1). The main differences between the prepolymers were caused by different monomer feed ratios. PP1 is a homopolymer, poly(TMP itaconate), whereas PP2 and PP3 are copolymers, poly(TMP itaconate-*co*-TMP adipate). The spectrum associated with PP1 does not display the peaks at 1.6 and 2.3 ppm which correspond to the adipate structure. All three of these prepolymers contained at least 10% itaconic acid in the monomer feed. As such, all of the NMR spectra have alkene peaks at approximately 5.8 and 6.2 ppm. The presence of these peaks is crucial because they support the observation that cross-linking did not occur during the synthesis. The absence of these peaks would imply that the alkene was altered, preventing it from participating in the photocuring process. Previous studies were unable to polymerize IA without the addition of radical inhibitors such as hydroquinone.[23]

All three of the prepolymers were cured upon exposure to UV light in the presence of DEAP, a photoinitiator that is commonly used in biomaterials.[7] Thermosets were formed

by exposing PP1, PP2, or PP3 with 0.1 wt % DEAP to 365 nm light for 10 min. As the concentration of the cross-linking moiety was controlled by the ratio of IA:AA in the monomer feed, the mechanical properties of these polymers could be controlled (Table 3). Material 1, poly(TMP itaconate), was a strong and brittle polymer, with a Young's modulus (YM), ultimate tensile stress (UTS), and rupture strain (RS) of approximately 200 MPa, 18 MPa, and 23%, respectively. However, by increasing the amount of AA in the monomer feed, the resulting polyesters were weaker and much more flexible. Material 2 (IA:AA = 1:3) displayed YM, UTS, and RS values of 2 MPa, 1 MPa, and 65%, respectively; material 3 (IA:AA = 1:9) was far more elastic, with a YM of 0.2 MPA, an UTS of 0.2 MPa, and a RS of 200%. Between materials 1−3, the YM varied over 3 orders of magnitude, the UTS varied over 2 orders of magnitude, and the RS varied over 1 order of magnitude.

Table 2. Prepolymer Characterizations

prepolymer	T_g (°C)[a]	M_n (g/mol)[b]	PDI[b]
PP1	−7.4	1140	1.3
PP2	−23.2	2200	1.7
PP3	−28.8	1170	1.3
PP4	39.8	940	1.1
PP5	−25.4	2030	1.3
PP6	−50.8	6650	1.3
PP7	−57.6	11900	1.6

[a] Determined by DSC on second heating cycle (10 °C/min). [b] Determined by GPC.

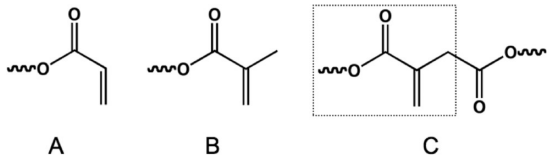


Figure 1. Common photocurable functional groups. The structural similarities in (A) acrylates and (B) methacrylates are also found in (C) itaconates. Itaconic acid is commonly polymerized through radical activation of the alkene. However, it is also a suitable monomer for the condensation polymerization of polyesters.

The hydrophobicity of these polyesters was also investigated by determining the contact angle of water on thin polymeric films (Table 3). The contact angles increased by including larger amounts of AA in the starting formulations due to the longer hydrocarbon portion of AA relative to IA. The contact angle values for materials 1, 2, and 3 were 42.7°, 52.7°, and 64.5°, respectively. The soluble fraction of the thermosets was also calculated (Figure 2). This characteristic followed a logical pattern, with the soluble fraction increasing as the relative concentration of IA decreased; comparable trends have been observed with similar materials.[24] The results concerning the degree of swelling in water were unexpected (Figure 2). Although materials 1−3 are branched and contain free carboxylic acids, the water swelling values were low. Of these polyesters, polymer 1 exhibited the largest degree of swelling in water at 7.5%. As the amount of AA in the monomer feed increased, the amount of swelling in water decreased. This trend is logical as AA contains more hydrophobic methylene units than IA.

Material 4. In order to demonstrate that the macromolecular properties of the cross-linked materials could be further tuned, a branched prepolymer was synthesized from

Table 3. Physical and Mechanical Characteristics of Photocured Itaconate-Based Polyesters

material	prepolymer	Young's modulus[a] (MPa)	UTS[a] (MPa)	rupture strain[a] (%)	n[b] (mmol/L)	T_g[c] (°C)	contact angle (deg)
1	PP1	201.66 ± 37.14	18.20 ± 0.10	23 ± 6	27130 ± 5000	18.4	42.7 ± 1.2
2	PP2	2.03 ± 0.24	1.14 ± 0.32	65 ± 14[e]	270 ± 30	−18.4	52.7 ± 1.3
3	PP3	0.19 ± 0.02	0.22 ± 0.02	198 ± 28[e]	30 ± 3	−25.6	64.5 ± 0.9
4	PP4	11.22 ± 1.22	2.05 ± 0.33	119 ± 19	1510 ± 160	4.0	29.4 ± 0.7
5	PP5	378.82 ± 53.03	16.59 ± 4.52	6 ± 2	50960 ± 7130	8.1	79.5 ± 0.8
6	PP6	7.49 ± 0.55	2.70 ± 0.80	45 ± 16	1010 ± 70	−36.7	87.4 ± 0.5
7	PP7	2.45 ± 0.17	2.04 ± 0.33	99 ± 11[e]	330 ± 20	−62.4	96.0 ± 0.9
1[d]	PP1	186.32 ± 20.11	7.64 ± 2.02	5 ± 1	25070 ± 2710		
2[d]	PP2	1.40 ± 0.17	0.46 ± 0.09	40 ± 10[e]	190 ± 20		
3[d]	PP3	0.17 ± 0.02	0.12 ± 0.03	111 ± 32[e]	20 ± 3		
4[d]	PP4	0.88 ± 0.25	0.11 ± 0.05	23 ± 6	120 ± 30		
5[d]	PP5	398.14 ± 6.62	15.04 ± 3.00	6 ± 1	53560 ± 890		
6[d]	PP6	13.85 ± 0.43	0.97 ± 0.38	12 ± 1	1860 ± 60		
7[d]	PP7	2.40 ± 0.14	1.89 ± 0.64	99 ± 11[e]	320 ± 20		

[a] Determined by Instron (crosshead speed of 10 mm/min). [b] Equation 1. [c] Determined by DSC on second heating cycle (10 °C/min). [d] Samples were hydrated in H_2O at room temperature for 24 h before testing. [e] Samples were cut by Instron clamps before tearing due to strain.

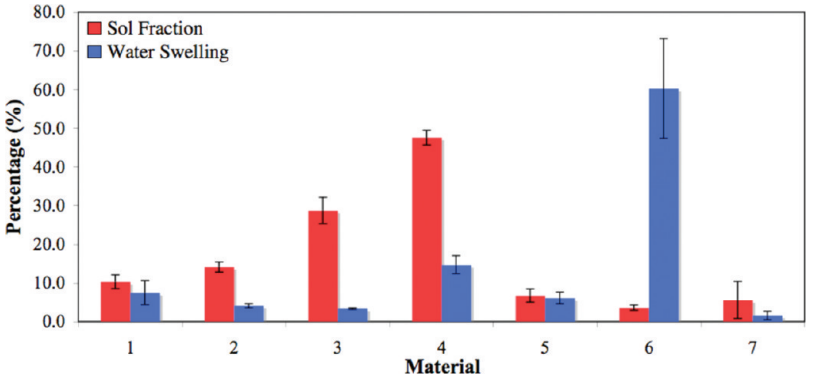
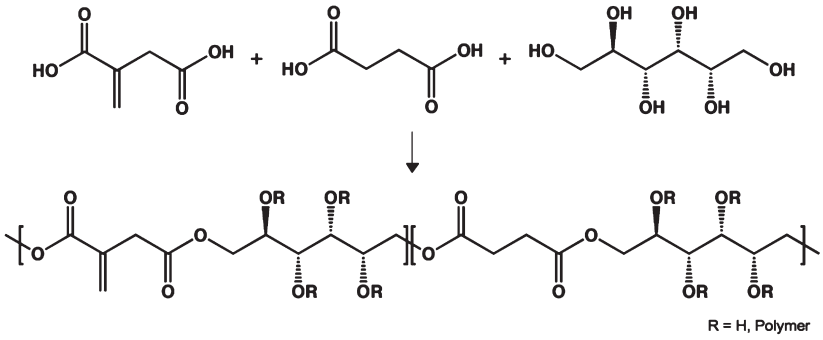


Figure 2. Sol fraction and water swelling of UV-cured itaconate-based polyesters.

Scheme 2. Thermal Synthesis of Hydrophilic Poly(sorbitol itaconate-*co*-sorbitol succinate)



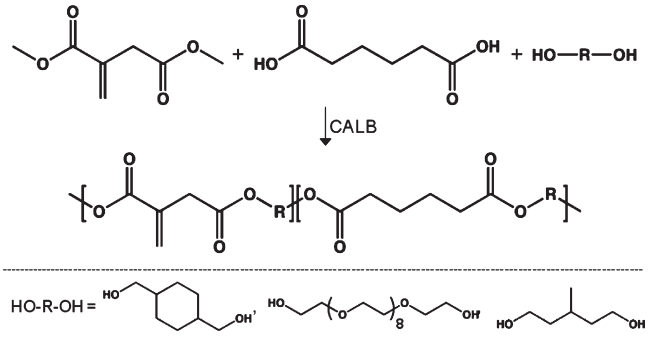
the thermal polyesterification of IA, SA, and sorbitol. The synthesis of prepolymer 4 (PP4) was very similar to those of the TMP-based materials (Scheme 2). Again, the monomers were heated only to the extent of cross-linking that would create oligomeric prepolymers. PP4 was amorphous (T_g = 39.8 °C) with $\langle M_n \rangle$ = 940 g/mol (Table 2). Additionally, as sorbitol contains six hydroxyl groups, this prepolymer should be very hydrophilic when compared to PP1−PP3. This hypothesis was supported by the fact that PP4 was soluble in water, along with other polar organic solvents (DMSO, DMF, methanol, ethanol, etc.); PP1−PP3 were soluble in organic solvents that were much more nonpolar, such as chloroform and methylene chloride.

The ^{1}H NMR spectrum of PP4 is similar to that of other prepolymers based on sugar alcohols (Figure S2).[25] The peaks from esters and alcohols corresponding to sugar alcohols are broad, spanning from 3.3 to 6.3 ppm. The signals that represent esters formed from secondary alcohols are located at higher end of this range (3.3−4.3 ppm). The esters formed by primary alcohols and the remaining unreacted alcohols produce peaks from 4.3 to 6.3 ppm. Common to PP1−PP3, the peaks associated with the pendant alkene are found at approximately 5.8 and 6.2 ppm, indicating that cross-linking did not occur during the reaction. Also, the spectrum contains peaks at 2.5 ppm that result from the use of succinic acid as a comonomer.

Prepolymer 4 was cured based on the process described for materials 1−3. Succinic acid was included in the monomer feed of material 4 in an attempt to dilute the cross-linking density and design a flexible polyester. This goal was realized, as polymer 4 displayed a YM, UTS, and RS of approximately 11 MPa, 2 MPa, and 120%, respectively (Table 3). In the hydrated state, these values changed to 0.88 MPa (YM), 0.11 MPa (UTS), and 23% (RS). Material 4 experienced the greatest loss of mechanical properties as a result of hydration. We believe that these results are due to the fact that polymer 4 is best able to interact with water because of the large number of free hydroxyl groups from sorbitol and the presence of free acids; in a very hydrated state, this polymer becomes much softer and more flexible. In comparison, materials 1−3 do not experience as great a loss in their mechanical properties due to their more hydrophobic repeat units.

The hydrophobicity of material 4 was determined by contact angle measurements (Table 3). Polymer 4 was expected to display significant hydrophilic character due to the presence of six hydroxyl groups in sorbitol. This hypothesis was verified when the contact angle for material 4 was determined to be 29.4° (Table 3). The soluble portion of this polyester was surprisingly large at 47.6% (Figure 2). This result may be due to the reactivity of the hydroxyl groups in sorbitol relative to those in TMP. Although sorbitol contains six alcohol moieties, four are secondary. TMP contains three primary hydroxyl groups, which are more reactive than their secondary counterparts. The soluble fraction may be higher in polymers dependent upon esterification involving secondary alcohols because fewer ester bonds are formed. This conclusion is also supported by the fact that PP4 achieved the lowest molecular weight out of all of the branched prepolymers (Table 2). The degree of swelling in water for polyester 4 was 14.7%. The use of such a hydrophilic monomer facilitated the design of a polymer with many free acids and alcohols that experienced at least twice as much swelling in water as materials 1−3.

Scheme 3. Enzymatic Synthesis of Photocurable Linear Poly(diol itaconate-*co*-diol adipate)



Materials 5 and 6. The versatility of itaconic acid would be increased greatly if linear polymers could also be synthesized. Initially, tin(II) 2-ethylhexanoate was employed as the catalyst in order to obtain poly(CHDM itaconate); however, the reaction gelled within hours. We therefore catalyzed all linear polymerizations with CALB, an enzyme isolated from a species of thermophilic bacteria (Scheme 3).[26] PP5 and PP6, both homopolymers, were designed by combining DMI with CHDM and PEG, respectively. In the presence of 10 wt % CALB, polymerizations occurred at 90 °C for 48 h under a partial vacuum for the final 46 h. PP5 and PP6 achieved molecular weights of 2030 and 6650 g/mol, respectively (Table 2). The choice of diol had a large impact on the thermal properties. PP5, composed of the cyclic diol CHDM, had the largest T_g of all linear materials (−25.4 °C). The T_g of PP6 was −50.8 °C, much lower due to the use of PEG as the diol.[27]

The ^{1}H NMR spectra of PP5 and PP6 are much easier to distinguish than the spectra of branched PP1−PP3 (Figures S3 and S4). The peaks representing the pendant alkene of the itaconate group were easily distinguished at 5.8 and 6.2 ppm. The methylene from the itaconate structure produces a singlet at 3.3 ppm. In Figure S3, the ester peaks are present at ~4.0 ppm, with the rest of the spectrum resulting from the

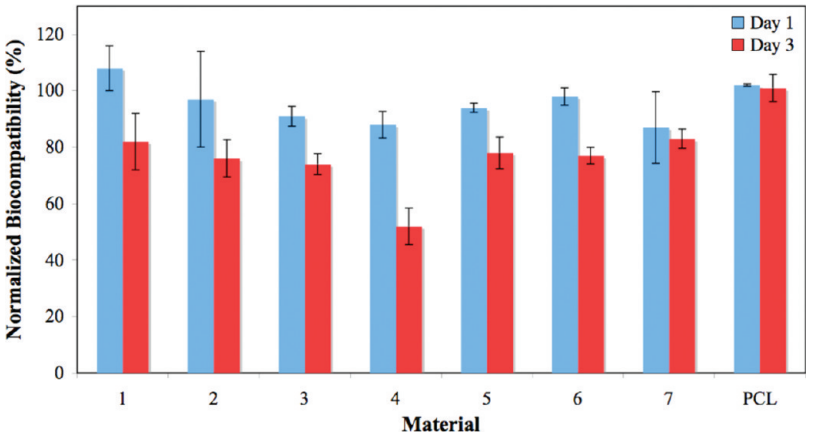


Figure 3. *In vitro* cytotoxicity based on an ATP luminescence assay. SAFs were cultured on tissue culture plastic in the presence of itaconate-based polyesters. As a positive material control, cells were cultured in the presence of PCL. The viability of the cells exposed to polymer samples was expressed as a percentage of the viability of cells grown in the absence of polymers.

hydrophobic protons of the cyclic CHDM group. The peaks corresponding to the protons adjacent to the ester groups in PP6 are located at 4.2 ppm. The major peak at 3.6 ppm represents the large number of ethylene glycol units in the PEG diol.

Although materials 5 and 6 were designed from DMI and a diol, the cross-linking density of these two polymers is very different due to the size of those diols. Prepolymers 5 and 6 were cured based on the process described for materials 1−3. Polyester 5 was composed of DMI and CHDM, a small and cyclic diol. As such, thermoset 5 is very strong and very brittle; its YM, UTS, and RS values are 380 MPa, 16.5 MPa, and 6%, respectively (Table 3). Since this polymer is linear and hydrophobic, it displays a very similar mechanical profile when it is hydrated. However, polyester 6 is based on a PEG macromonomer, which provides more space between cross-linking sites. The result is a material that is more flexible than the other homopolymers. The YM, UTS, and RS values for polymer 6 are approximately 7.5 MPa, 2.7 MPa, and 45%, respectively. This polymer was unique in terms of its hydrated mechanical profile; the YM increased by 85% (13.85 MPa), the UTS decreased by 64% (1 MPa), and the RS decreased by 73% (12%). Current studies are attempting to understand the difference between the mechanical properties of the dry state and those of the hydrated state.

Materials 5 and 6 are both homopolymers based on DMI. However, the choice of extremely different diols (CHDM versus PEG) allowed for the synthesis of dissimilar polymers. Polyester 5 was expected to be very hydrophobic due to the cyclic hydrocarbon interior of CHDM. This hypothesis was validated by the contact angle (79.5°) and water swelling results (6.1%) (Table 3 and Figure 2). Also, because polymer 5 was a homopolymer, the prevalence of itaconate motifs resulted in a highly cross-linked network with a low soluble fraction (6.7%). Polyester 6 was thought to be very hydrophilic since a large PEG diol (400 g/mol) was employed as a macromonomer. The degree of swelling in water, 60%, supported this expectation; material 6 swelled at least 4 times as much as any of the other polyesters in this study. However, the contact angle implied that this polymer was surprisingly hydrophobic (87.4°). The interactions between material 6 and water potentially required a large amount of time; contact angle measurements occur on the order of minutes while water swelling takes place over a 24 h period. Additionally, due to the fact that material 6 is a homopolymer, the soluble fraction was very low (3.6%).

Material 7. When PP5 and PP6 were cured, the resulting polymers were brittle due to the high-cross-linking density which is due to each repeat unit containing a site for radical cross-linking. We hope to further increase the flexibility of itaconate-based polyesters by producing a copolymer in an attempt to dilute the cross-linking sites. In order to accomplish this goal, AA was included in the monomer feed; DMI:AA:MPD = 1:3:4. The synthesis was identical to the procedure used for PP5 and PP6. The inclusion of AA caused a large increase in the molecular weight of the prepolymer, which obtained $\langle M_n \rangle$ = 11 900 g/mol (Table 2). The T_g of PP7, −57.6 °C, was again low due to the choice of the diol. The branched structure of MPD prevented efficient packing, which resulted in a low T_g.[28]

In addition to basic structural characterization, the ^{1}H NMR spectra of PP7 allowed for the determination of the ratio of DMI:AA in the purified prepolymer (Figure S5). The monomer feed contained DMI:AA in a 1:3 molar ratio. By integrating peaks "b" and "g", the ratio of DMI:AA in the final prepolymer was 1:3.07. In terms of the structural components of PP7, peaks "a" and "b" are common to PP5 and PP6 as all three are based on DMI. Again, their presence is critical because the pendant alkene is preserved through the enzymatic polymerization. Peaks "g" and "h" are the methylene groups from the adipate unit in the copolymer. Peaks "c" to "f" are associated with the hydrophobic core of the diol, MPD.

Curing PP7 with UV light led to the design of a tough and flexible polyester, which was intended. Adipic acid was included so as to decrease the relative concentration of cross-linking sites in the resulting thermoset. Polymer 7 displayed a YM, UTS, and RS of approximately 2 MPa, 2 MPa, and 100%, respectively (Table 3). The hydrated state of this polyester produces similar mechanical characteristics; the new values are 2.4 MPa (YM), 1.9 MPa (UTS), and 100% (RS).

Polyester 7 is the most hydrophobic material that was designed for this study. Three factors led to this result: (i) PP7 was linear; (ii) the diol used in the polymerization, MPD, was hydrophobic; (iii) a significant portion of AA was employed as a comonomer. As stated previously, AA contains a longer hydrocarbon segment than IA. Because of this hydrophobic nature, the water swelling value was low (1.6%) while the contact angle measurement was high (96.0°) (Figure 2 and Table 3). Finally, the soluble fraction of this thermoset was similar to that of the three homopolymers studied here (5.6%).

***In Vitro* Cytotoxicity.** These polyesters were designed for drug delivery, tissue engineering, and other biomedical applications. As such, the cytotoxicity of these thermosets

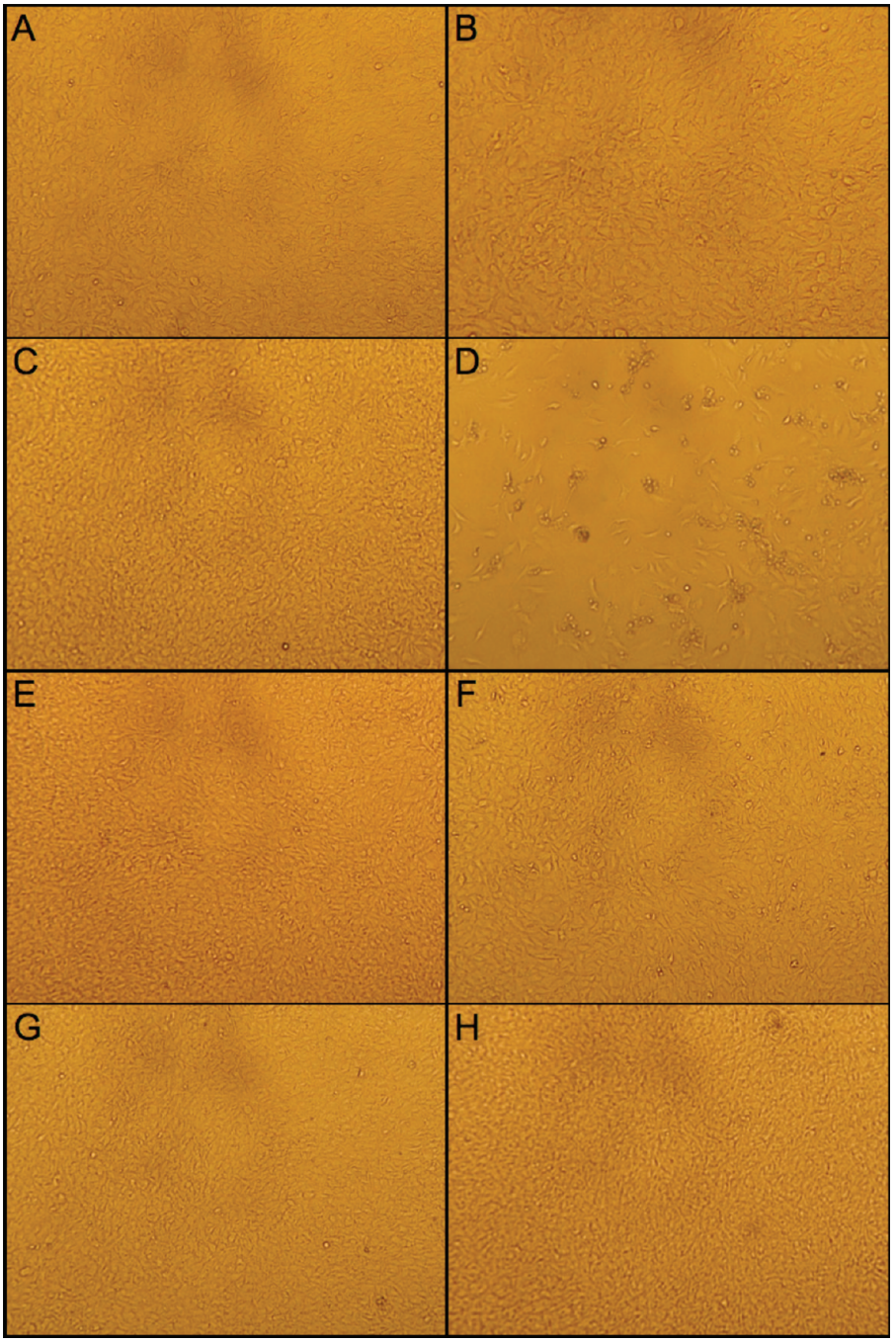


Figure 4. Optical micrographs from *in vitro* cytotoxicity assay. SAFs were imaged after 72 h of culture with materials 1 (A), 2 (B), 3 (C), 4 (D), 5 (E), 6 (F), and 7 (G). As a control, cells were cultured with samples of PCL (H). Only material 4 displayed significant toxicity toward SAFs.

was studied by monitoring the intracellular concentration of ATP. Polyester thermosets were sterilized in ethanol and then soaked in PBS under a germicidal lamp in order to remove any remaining ethanol. SAFs were cultured in a 24-well plate containing transparent and permeable inserts. Because preliminary data suggested that viability could be limited by polymer samples resting directly on cells, thermoset samples were placed in the insets to prevent direct contact between polyesters and cells. During culture, the cells were exposed to the soluble fraction of the polyesters and any degradation products, both of which were able to diffuse through the permeable inserts. Two positive controls were employed during these studies: cells were exposed to no polyester extracts and to PCL as a material control. The cytotoxicity was evaluated after 24 and 72 h. As seen in Figure 3, cultured SAFs showed no negative response to any of the polyesters after the first day. After 3 days, all of the materials showed good toxicity profiles except for material 4, which displayed moderate toxicity (Figure 4). During the study, the culture medium that was exposed to material 4 turned from red to yellow; this change in color indicated a drop in pH. This observation is consistent with the fact that material 4 has the highest soluble fraction and is the most hydrophilic (contact angle of 29.4°), allowing water to interact with the free carboxylic acid. The other materials allowed $\geq 85\%$ and $\geq 75\%$ of the viability associated with cells that were not exposed to polymer extracts after 24 and 72 h, respectively. This preliminary biological data indicate that photocured itaconate-based polyesters warrant further exploration as novel biomaterials.

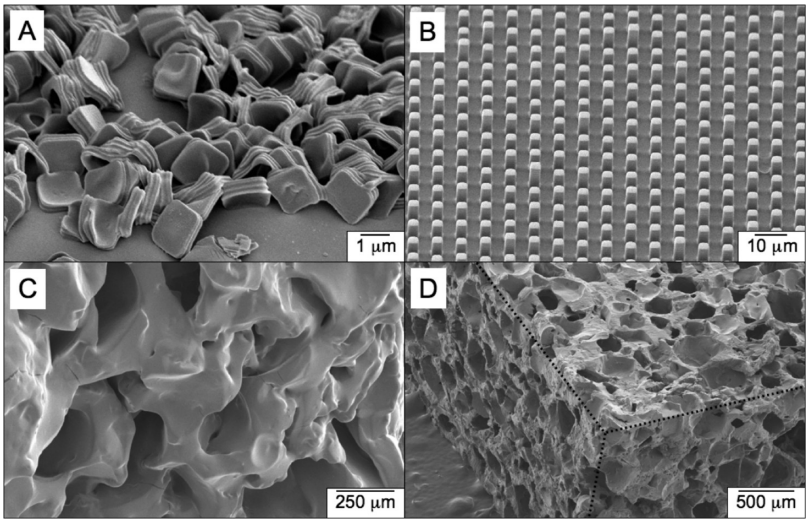


Figure 5. Scanning electron microscopy images of 3-dimensional itaconate-based thermosets. (A) Curing prepolymer 5 in the presence of a patterned perfluoropolyether film enabled the design of micrometer-scale particles (2 μm × 2 μm × 1 μm). (B) On a glass slide, an embossed film of material 1 was designed with features of 2 μm × 2 μm × 6 μm by utilizing a similar perfluoropolyether mold. (C) Prepolymer 3 was used as a cross-linking agent for PEG methyl ether methacrylate. Sieved salt was employed as a porogen, which, after leaching in water, enabled the fabrication of porous scaffolds. (D) Prepolymer 3 was also employed as the cross-linking agent in a porous film of 2-(methacryloyloxy)ethyl acetoacetate. The dotted lines help to display three faces of the scaffold converging at a corner. Sieved salt was again utilized as the porogen.

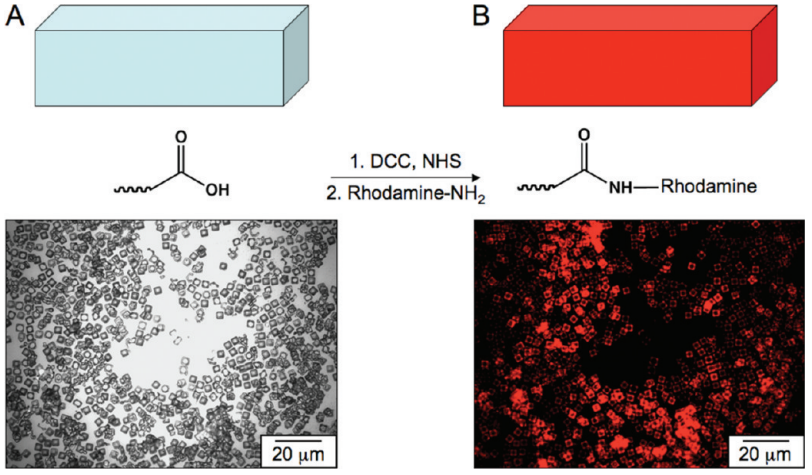


Figure 6. Amidation-based functionalization of acid-containing particles. (A) Material 2, a branched polyester, was cured into particles (3 μm × 3 μm × 1 μm) through PRINT. As material 2 was cross-linked radically, the particles contain free acids that are capable of functionalization. The particles were visualized by optical microscopy. (B) An amine-functionalized rhodamine dye was coupled to the particles through standard DCC/NHS chemistry. As seen by fluorescent microscopy, the resulting particles were fluorescent due to the immobilized dye.

Polymer Molding. Biodegradable materials often need to be molded into complex 3-dimensional structures for specific applications in tissue engineering or drug delivery.[7,25,29] Therefore, itaconate-based materials were engineered and molded with a variety of strategies (Figure 5). Utilizing particle replication in nonwetting templates (PRINT), 2 μm × 2 μm × 1 μm particles of polymer 5 were fabricated in the shape of rectangular prisms (Figure 5A). Additionally, an embossed film of material 1 was designed on a glass slide with 2 μm × 2 μm × 6 μm features by employing a similar perfluoropolyether template (Figure 5B). Also, porous scaffolds were fabricated due to their importance in tissue engineering.[30−33] Prepolymer 3 was dissolved in PEGMEM or MEA so that it could act as a polyfunctional cross-linker; no solvent was added. The solution was combined with DEAP and sieved NaCl, cured, and swelled in water for 4 days. As the water dissolved the salt, the films became porous (Figure 5C,D). Importantly, materials 1−3 can act as cross-linking agents for other acrylate- and methacrylate-based monomers, increasing their versatility in the design of biodegradable materials.

As described above, these itaconate-based polyesters were compatible with PRINT, a fabrication technique that allowed for the design of particles. If one of the branched materials (materials 1−4) were molded, the resulting particles would contain free acids and alcohols, as cross-linking through the alkenes does not involve esterification. This fact implies that functionalization could occur through the unreacted functional groups. In order to demonstrate this, material 2 was cured into particles (3 μm × 3 μm × 1 μm) through the PRINT methodology (Figure 6A). After harvesting the particles, they were resuspended in DMF with excess NHS and DCC for 30 min. An amine-modified rhodamine dye was then added to the reaction, followed by 5 h of agitation. The reaction was halted by centrifuging the particles and discarding the supernatant. Purified fluorescent particles were obtained after repeated rounds of resuspension in acetone, centrifuging, and decanting the solution; the purification

process was ended when the acetone solution containing suspended particles was colorless. The resulting particles were imaged on a fluorescent microscope due to the conjugation of the rhodamine dye (Figure 6B). This amidation strategy is extremely versatile, resulting in the immobilization of hydrolytically stable ligands; labile bonds could also be introduced through esterification. While material 2 was chosen as an example, this functionalization strategy should be compatible with particles composed of any polymer that includes free carboxylic acids.

Conclusions

We have designed several novel photocurable polyesters based on IA, a natural and renewable monomer. Established polyester synthesis strategies are compatible with IA and DMI, allowing for the development of photocurable polyesters in one step without secondary steps or end-capping. Altering the polyol and the cross-linking density of the itaconate-containing family of materials provides control of the mechanical profiles, hydrophobicity, and other macromolecular properties. The mechanical properties of many biological materials fall within the values displayed by these branched itaconate-based polyesters.[1−3] Furthermore, numerous mechanical profiles can be achieved by the design of new materials from unique monomer feeds. The prepolymers are also compatible with imprint lithography and other fabrication techniques; particles and complex scaffolds can be easily designed for potential biomedical applications. To the best of our knowledge, this is the first use of IA in thermally synthesized branched polyesters, the first enzymatic synthesis of itaconate-based polymers, and the first use of itaconate-based materials in combination with the photoinitiator DEAP. Additionally, water-soluble polymers, such as poly(itaconate sorbitol-*co*-succinate sorbitol), offer versatility because they can also be used to design hydrogels. Future work will focus on new polymer formulations and *in vivo* characterization of this class of polyester thermosets in regards to tissue engineering and drug delivery. On the basis of our preliminary results, we believe that the poly(polyol itaconate) series of polyester thermosets should find wide use in the biomedical and biotechnological fields.

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Supporting Information Available: Experimental details; fully labeled ^{1}H spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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