Poly(triol α-ketoglutarate) as Biodegradable, Chemoselective, and Mechanically Tunable Elastomers

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ABSTRACT: We report the design of several elastomers based on the thermal polycondensation of α-ketoglutaric acid and one of three triols: glycerol, 1,2,4-butanetriol, or 1,2,6-hexanetriol. By varying the curing temperature and the duration of the curing process, a wide range of mechanical properties was achieved. The values of the Young's modulus (0.1−657.4 MPa), ultimate stress (0.2−30.8 MPa), and ultimate strain (22−583%) encompass the mechanical properties of many biological materials, increasing the probability of success for the use of poly(triol α-ketoglutarate) as a biomaterial. Furthermore, the poly(triol α-ketoglutarate) series hydrolytically degraded in as fast as 2 days and as long as 28 days in phosphate-buffered saline solutions. For postpolymerization modifications, the repeat units contain ketones, which are capable of reacting with a variety of oxyamine-terminated molecules to generate stable oxime linkages. Finally, the versatility and utility of these elastomers were demonstrated by creating micropatterned structures and films for biospecific cell scaffold supports.

Introduction

The development of new biomaterials with a range of functions and properties is becoming increasingly important for new and diverse applications in fundamental biological research, including drug delivery and tissue engineering.[1−5] For applications that require soft materials that can mimic biological tissues, much research is directed toward the design of biodegradable and bioreducible polymers that can form biocompatible elastomeric networks.[6−12] Although this is an area of intense research, many of these materials are generated through complex syntheses or difficult engineering processes, which increases the costs of these materials and decreases the general availability to the broader research community. The design of simple, straightforward, yet diverse materials would provide faster access to the new technology and therefore enable rapid progress in evaluating new biomaterials for a variety of applications.

A traditional approach for generating new biomaterials has been to design one polymer for one particular application. In order to streamline the discovery process, a more efficient strategy would be to design new and versatile materials capable of successful performance in multiple diverse applications. With this approach, materials can be versatile in terms of mechanical characteristics and chemical functionality. Mechanical versatility implies that a single technique can achieve a spectrum of structural and strength-related properties. Chemical versatility can be defined as the ability to introduce a wide range of functional moieties into the polymer at various time points in the synthesis—during the prepolymer stage or as a cross-linked film. A material that possessed both mechanical and chemical versatility could potentially allow for the modulation of both sets of properties independently. Mechanical and chemical versatility could enable the properties of a specific material to be tuned in order to achieve a desired result for a particular application.

We believe, therefore, that the ideal polymer for biological or medical applications should possess several key characteristics. First, biocompatibility and biodegradation are key to ensure that there are no adverse cellular responses to the material. Second, a wide range of degradation rates and mechanical properties should be accessible from a single, facile, and inexpensive design strategy. This would allow for the ability to tailor material properties to best fit a particular application, thereby maximizing the probability of success. Third, the inclusion of a chemoselective ligand conjugation strategy would greatly increase the flexibility of a polymer in biological and medical applications.[13−17] A single strategy that incorporates all of these criteria would allow for the rapid development and testing of new biomaterials.

Herein, we describe the syntheses and characterization of poly(triol α-ketoglutarate) (PTK), a novel and chemoselective series of polyketoesters. Because of the unique characteristics of this polymer family, a wide range of mechanical properties and degradation rates are possible. Also, inclusion of ketones in the repeat unit allows for the mild and chemoselective functionalization of these materials. Upon reaction, and therefore conjugation, of the oxyamine-terminated cell attachment peptide glycine−arginine−glycine−aspartic acid−serine (H_2NO-GRGDS), these materials are able to support cell attachment and cellular growth.[18,19] Because of the potential diversity of mechanical and chemical properties, PTK may be employed for a number of biomedical applications, including tissue engineering and drug delivery.

The motivation for designing PTK was influenced by several criteria. First, the development of materials with a facile postpolymerization modification strategy was critical. The use of α-ketoglutaric acid (αKG) satisfied this particular requirement due to the presence of a ketone group. Oxyamines, hydrazines, and hydrazides can react rapidly and chemoselectively with ketones at physiological conditions without catalysts or coreagents, allowing for easy ligand conjugation by forming stable oxime linkages.[13−15,20−22] While free acids and alcohols can be accessed for subsequent reactions, the use of catalysts or coreagents is usually required. Second, since biomedical applications are targeted for these PTKs, biocompatibility was required and, therefore, at least one natural product was used in each material to minimize potential cytotoxicity. αKG is a ubiquitous metabolite required for the citric acid cycle, while glycerol is a key component in the synthesis of phospholipids.[23,24] Third, the ability to generate a wide range of elastomer properties with a simple and cost efficient synthesis would greatly enhance the versatility of PTK. By thermally curing the polyesters with varying temperatures and/or durations, the extent

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Scheme 1. Synthesis of Poly(triol α-ketoglutarate)

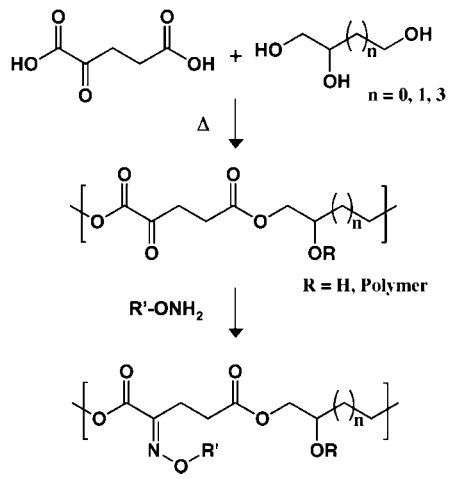

Table 1. Curing Conditions for Poly(triol α-ketoglutarate)

polyketoester	triol	temp (°C)	duration
1A	glycerol	60	7 days
2A	butanetriol	60	7 days
3A	hexanetriol	60	7 days
1B	glycerol	90	2 days
2B	butanetriol	90	2 days
3B	hexanetriol	90	2 days
1C	glycerol	120	1 day
2C	butanetriol	120	1 day
3C	hexanetriol	120	1 day
3D	hexanetriol	120	6 h
3E	hexanetriol	120	12 h
3F	hexanetriol	120	18 h

of cross-linking can be easily controlled for a range of mechanical properties. Although polyketoesters have been synthesized previously, these materials represent linear polymers or hyperbranched materials.[14,25−28] Utilizing random ester cross-linking allows for the design of versatile polyketoester films without the use of a complex synthesis. As the design of PTK is so simple, the larger biotechnological community can take advantage of these materials. Fourth, because of the potential need for consistent degradation profiles, linear degradation of the PTK was desired. By using ester linkages for both polymerization and cross-linking, consistent and uniform degradation could be achieved. With these criteria, αKG was combined with glycerol, 1,2,4-butanetriol, and 1,2,6-hexanetriol to generate poly(glycerol α-ketoglutarate) (PGa), poly(1,2,4-butanetriol α-ketoglutarate) (PBa), and poly(1,2,6-hexanetriol α-ketoglutarate) (PHa), respectively—a series of chemoselective and biodegradable polymers with a range of mechanical and chemical properties. Furthermore, because all four monomers are inexpensive and the prepolymer synthesis can be performed in bulk, PTKs are available for potential large-scale applications.

Experimental Section

Materials. All chemicals were purchased from Sigma-Aldrich (Milwaukee, WI) or Fisher Scientific (Philadelphia, PA), and used without further purification unless otherwise noted. αKG was recrystallized from ethyl acetate.

Poly(triol α-ketoglutarate) Synthesis. Equimolar amounts of αKG and triol (glycerol, 1,2,4-butanetriol, or 1,2,6-hexanetriol) were combined in a round-bottom flask. In an inert environment of N_2, monomers were stirred at 125 °C. After a homogeneous melt formed, the mixture was stirred for 1 h. Polymers were then precipitated in −78 °C methanol (MeOH), concentrated by rotary evaporation, and dried under vacuum at room temperature. Yields for PGa, PBa, and PHa were 93%, 88%, and 81%, respectively. To form elastomeric films, prepolymers were cured at either 60, 90, or 120 °C for times ranging from 6 h to 7 days.

Polyketoester Characterization. Infrared spectra were recorded on an ASI ReactIR 1000 with tetrahydrofuran (THF) as the solvent. Thermal properties were recorded by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). Using a Seiko 220C DSC, prepolymer glass transition temperatures were measured during the second heating cycle (10 °C/min). Thermal degradation was collected with a Perkin-Elmer TGA with a heating rate of 10 °C/min in an atmosphere of N_2.

Tensile tests were conducted on an Instron 5566 at a crosshead speed of 10 mm/min at 25 °C. Samples were cured in a dog-bone-shaped mold with approximate dimensions of 10 mm × 3 mm × 1.5 mm. The Young's modulus (E) was calculated according to the linear segment of the stress/strain curve. Three trials were performed and the average was reported. Using

$$\nu = \frac{E}{3RT}$$

where R is the universal gas constant and T is the temperature in K, the cross-linking density (υ) was calculated.

Sol−gel analysis was conducted by soaking films in THF for 24 h at room temperature. After solvent removal, the films were dried, and the percent soluble fraction (Q_s) was calculated by

$$Q_s = \frac{m_i - m_f}{m_i} \times 100$$

where m_i and m_f represent the initial and final mass of the elastomer films, respectively. Three trials were performed, and the average was reported.

Film Molding. A small amount of prepolymer was placed onto a glass slide and heated to 120 °C in a vacuum oven. After 30 min of reduced pressure (∼50 Torr) to remove any gas or solvent, a polydimethylsiloxane (PDMS) stamp, fabricated by soft lithography, was placed directly on the prepolymer.[25] The material was allowed to cure for 24 h, followed by the removal of the PDMS stamp. The molded elastomeric films were then coated in ∼2 nm of a Pd/Au alloy (Cressington 108 auto sputter coater, Cressington Scientific Instruments Ltd.) and imaged using a Hitachi model 2-4700 scanning electron microscope (SEM).

In Vitro Degradation. To obtain standard degradation rates, ∼75 mg segments of each elastomer were placed in scintillation vials. Vials were filled with phosphate buffered saline (PBS), and samples were then stored in an incubator (37 °C). At predetermined intervals, samples were removed from the incubator, rinsed thoroughly, dried, and weighed again. To prevent saturation, PBS was replaced every 7 days. Each data point was repeated in triplicate, and results were reported as the average percent of the original mass lost.

Film Functionalization. To pattern immobilized ligands, a PDMS microfluidic cassette was placed in direct contact with a PTK film, creating a seal. Then, a 5 mM solution of an oxyamine-modified rhodamine dye (rhodamine-ONH_2) in MeOH was flowed through the channels of a microfluidic cassette. The surface was then rinsed with MeOH and dried with N_2, and fluorescent images were taken using a Nikon Eclipse TE2000-E inverted microscope (Nikon USA, Inc., Melville, NY). To functionalize the elastomer surface with a biospecific cell-adhesive peptide, 100 μL of a solution of H_2NO-GRGDS (1 mM in 1:1 DMSO:H_2O) was added directly to the top of the film and allowed to react for 5 h. The elastomers were then rinsed in PBS and dried in a vacuum chamber at room temperature.

In Vitro Biocompatibility. Polyketoester films were soaked in bovine calf serum-containing Dulbecco's modified eagle medium (Sigma, St. Louis, MO) for 24 h to remove any soluble or degraded portions of the materials. Cytotoxicity was then examined by two methods. First, the serum-containing medium with the elastomer extraction was added to a confluent layer of 3T3 Swiss Albino mouse fibroblasts on tissue culture plastic. After 48 h, cells were examined by light microscopy, and cytotoxicity was determined on the basis of cell morphology, monolayer confluence, and the

Table 2. Mechanical Characteristics of Poly(triol α-ketoglutarate)

	mass loss (°C)[a]							
polyketoester	5%	10%	E^b (MPa)	σ^b (MPa)	ε^b (%)	ν^b (mmol/L)	Q_s^c (%)	complete degradation (days)[d]
1A	NA[e]	NA	NA	NA	NA	NA	NA	NA
2A	259	294	1.95	1.0	583	262.3	48.5	2
3A	267	312	0.1	0.3	395	13.45	59.0	4
1B	239	284	459.4	9.3	121	61790	41.9	3
2B	260	298	161.3	4.7	418	21690	32.5	7
3B	293	332	2.5	2.1	176	336.3	16.3	14
1C	255	300	499.7	16.7	43	67210	28.5	8
2C	285	324	381.9	8.1	95	51370	4.4	17
3C	307	345	657.4	30.8	22	88420	3.5	28
3D	268	313	0.1	0.2	379	13.45	60.9	4
3E	288	331	1.3	1.2	151	174.9	14.8	12
3F	293	332	4.2	2.2	200	566.2	14.5	13

[a] Determined by TGA in N_2, 10 °C/min. [b] Determined by Instron (crosshead speed of 10 mm/min). [c] Extracted in THF for 24 h at 25 °C. [d] Degradation was monitored in PBS at 37 °C. [e] Incomplete curing at given conditions.

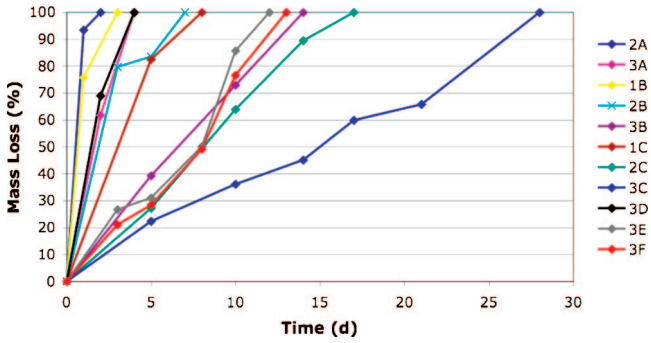

Figure 1. Degradation rate profiles for polyketoester materials 2A−3F found in Table 2. Because of the ease with which the extent of cross-linking and the hydrophobicity of the triol could be controlled, PTK films were able to hydrolytically degrade at varying rates. Complete mass loss was observed in as rapidly as 2 days (2A) and as long as 28 days (3C).

ability to continue subculturing cells (compared to cells cultured on tissue culture plastic). Second, small fragments of the materials were added to a confluent layer of fibroblasts on tissue culture plastic. After 48 h, cells were examined by light microscopy, and cytotoxicity was determined as compared to cells not treated on tissue culture plastic.

Fibroblasts were next added directly to GRGDS-presenting films and incubated in Dulbecco's modified Eagle's medium with 10% bovine calf serum and 1% penicillin/streptomycin at 37 °C with 5% CO_2. The cells were added at a density of ~80 000 cells/mL. Phase-contrast images were taken 24 h after seeding using a Nikon Eclipse TE2000-E inverted microscope.

Results and Discussion

Polyketoester Prepolymer Synthesis and Characterization. The synthesis of PTK was designed for biological applications, and therefore, the elastomers were generated without any potential contaminating catalysts or coreagents (Scheme 1). After forming a homogeneous melt at 125 °C, monomers were stirred while heating for 1 h. Random cross-linking proceeds rapidly without the use of reduced pressure or a catalyst, allowing for purification by precipitation in −78 °C MeOH. The prepolymers were heated only to the extent of cross-linking that would enable purification—the majority of the heating and cross-linking was intended to occur during a second heating phase, allowing for the molding and shaping of elastomeric films.

To determine the influence on mechanical effects of a ketone adjacent to one of the acids in αKG, two control prepolymers were synthesized based on glutaric acid and diethyl β-ketoglutarate. The use of glutaric acid would allow for the determination of the effects of the presence of a ketone while diethyl β-ketoglutarate, which contains a ketone in the β-position

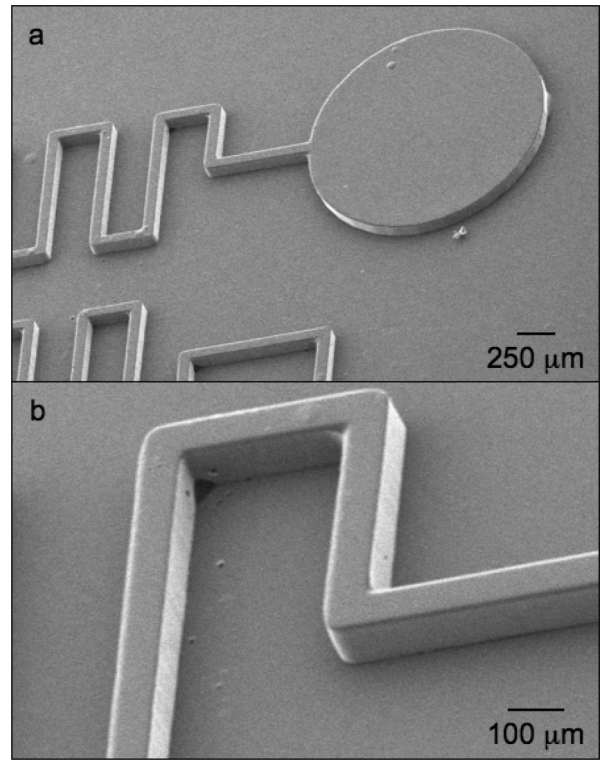

Figure 2. Scanning electron microscopy (SEM) images of thermally cured PHa. After partially curing PHa on a glass slide, a PDMS stamp was applied in order to pattern the elastomeric film. After the curing process ended, the stamp was removed, revealing the molded polyketoester film. The pattern was (a) transferred with high fidelity, (b) creating features with an approximate height of 70 μm.

relative to both carbonyl groups, would help to determine the role of the location of a ketone. However, under identical reaction conditions as were used with αKG, these controls did not sufficiently polymerize in 1 h due to the lower reactivity of the two control molecules. This was determined by attempting to precipitate the control prepolymers in −78 °C MeOH. Furthermore, prepolymers were unable to form from glutaric acid or diethyl β-ketoglutarate after ~3 h of reaction at 125 °C. These results suggest that the rapid cross-linking of PTK is due to the presence of a ketone moiety in the α-position to the acid group in αKG.

PTK prepolymers were then characterized by IR and DSC. Molecular weight determination was unsuccessful using GPC because the prepolymers were too small. Falling under the minimum weight requirement of the GPC instrument implies that PTK prepolymers have molecular weights less than 1000 g/mol. NMR spectra confirmed the low molecular weights, with

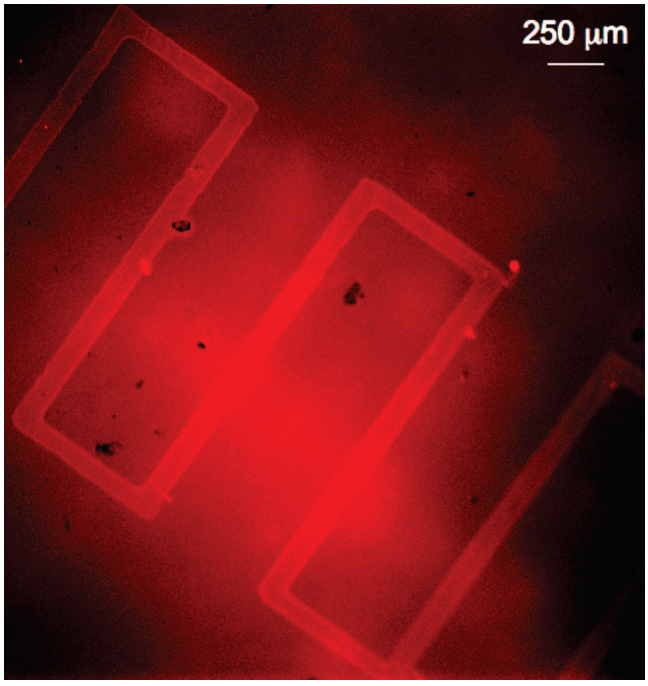

Figure 3. PHa patterned with a fluorescent dye. Using microfluidics, a solution of an oxyamine-modified rhodamine in MeOH was patterned on a flat PHa surface. After rinsing the surface, the fluorescent pattern is clearly visible against the nonmodified PHa background.

end groups analysis offering approximate values of 400, 400, and 600 g/mol for PGa, PBa, and PHa, respectively. The IR spectra of all three prepolymers show an intense carbonyl stretch at ~1740 cm^{-1} due to the formation of multiple ester linkages (Figures S1−S3). DSC was used to calculate the glass transition temperatures (T_g) of PGa, PBa, and PHa (−22.5, −33.4, and −38.4 °C, respectively). With all T_g values less than 0 °C, PGa, PBa, and PHa are completely amorphous at room temperature. Furthermore, the three prepolymers are soluble in several common organic solvents, including acetone, chloroform, dimethyl sulfoxide (DMSO), *N,N*-dimethylformamide, dioxane, ethanol, MeOH, methylene chloride, and THF. The range of organic solvents can facilitate a variety of potential functionalization reactions as well as diverse processing techniques.

Polyketoester Cross-Linking and Mechanical Properties. The ability to design elastomers with a wide variety of mechanical properties was directly related to the ease with which the extent of cross-linking could be controlled. Therefore, several curing conditions were used to create materials with a range of structural properties (Table 1). Cross-linking the prepolymers at lower temperatures for longer time periods (60 °C, 7 days) led to very soft, elastic materials while shorter curing periods at higher temperatures (120 °C, 1 day) produced hard, glassy materials. Interestingly, certain combinations of temperature and curing duration led to materials with similar mechanical properties. Materials 3A and 3D exhibit nearly identical properties, ranging from thermal degradation to stress/strain to hydrolytic degradation. This relationship can be advantageous for two reasons: (1) curing for shorter periods at higher temperatures obviously reduces the amount of time needed for film preparation, and (2) cross-linking over longer periods at lower temperatures may allow for the encapsulation of materials that degrade at high temperatures.

TGA gave insight into the thermal stability of the 11 elastomers that were produced (Table 2). All 11 elastomers exhibited 5% mass loss at temperatures of at least 239 °C. In addition, eight of the materials experienced 10% mass loss at temperatures above 300 °C. A general trend became apparent in that the triol used, not the curing conditions, influenced the thermal stability of the elastomeric films. For example, material 3D, which was only cured for 6 h, exhibited higher thermal stability than four other materials (2A, 1B, 2B, 1C) that were cured for at least 24 h. The main difference between these materials is that 3D is derived from PHa, while the other four are derived from PGa (1B, 1C) or PBa (2A, 2B).

The mechanical properties exhibited by various PTK elastomers were dispersed over a wide range (Table 2). Several unique combinations of Young's modulus (E), ultimate stress (σ), and ultimate strain (ε) were present in these materials. For example, materials 1B and 2B were fairly rigid with correspondingly large Young's modulus values (E = 459.4 and 161.4 MPa, respectively) and ultimate stress values (σ = 9.3 and 4.7 MPa, respectively) when compared to the other materials. However, both were also capable of extreme elongation, at least doubling in length (ε = 121% and 418%, respectively). In addition, typical soft and elastic materials were also produced. Materials 3A and 3D both had a Young's modulus value of 0.1 MPa, ultimate stress values under 0.5 MPa (0.3 and 0.2 MPa, respectively), and ultimate strain values over 350% (395% and 379%, respectively). Overall, the Young's modulus varied by 3 orders of magnitude (0.1 MPa < E < 657 MPa), the ultimate stress varied by 2 orders of magnitude (0.2 MPa < σ < 30 MPa), and the ultimate strain varied by 1 order of magnitude (22% < ε < 583%).

These prepolymers could also be cured at physiological temperature (37 °C). However, the duration of the cross-linking process was dramatically increased (2−4 weeks). Temperature-sensitive cargo could therefore potentially be entrapped in the branched polyketoester network. Biopolymers such as proteins and nucleic acids, which can denature upon heating, may be able to survive the curing process at 37 °C, allowing for the potential delivery and release of natural therapeutics. Experiments are being pursued to fully determine the flexibility of curing materials at physiological temperatures.

Extending the potential for the success of PTK as a biomaterial is the fact that the mechanical properties of many biological materials fall within the ranges achieved by these materials. For example, elastin from bovine ligaments exhibits a Young's modulus of 2 MPa, an ultimate stress of 1.1 MPa, and an ultimate elongation of 150%.[26] All of these values fall within the spectrum of material properties of PTK due to the ease with which the cross-linking density can be controlled. Also, collagen can recover from deformations of up to 20%, while arteries and veins can achieve ultimate strains of up to 260%.[27,28] Furthermore, because of the ease with which the mechanical properties can be finely tuned, PTK could potentially be used for applications requiring a combination of flexibility and strength or for applications necessitating strength and rigidity.

In Vitro Degradation. On the basis of the α location of the ketone relative to the acid in PTK, electron-withdrawing effects should increase the electrophilic character of the carbonyl carbon in the adjacent ester. Accordingly, we expected these polyketoesters, which are also hydrophilic, to have rapid in vitro degradation rates.[29] As expected, PGa, PBa, and PHa exhibited 100% mass loss in relatively short periods of time (<28 days). However, since the curing conditions led to varying extents of cross-linking, a range of degradation rates were obtained (Table 2, Figure 1). The rates seem to be controlled by two factors: the extent of cross-linking within the polymer network and hydrophobicity of the elastomer. Therefore, the fastest degrading material should be soft and hydrophilic (1A) while the slowest degradation rate should be associated with a material that is hard and hydrophobic (3C). The only exception to this general

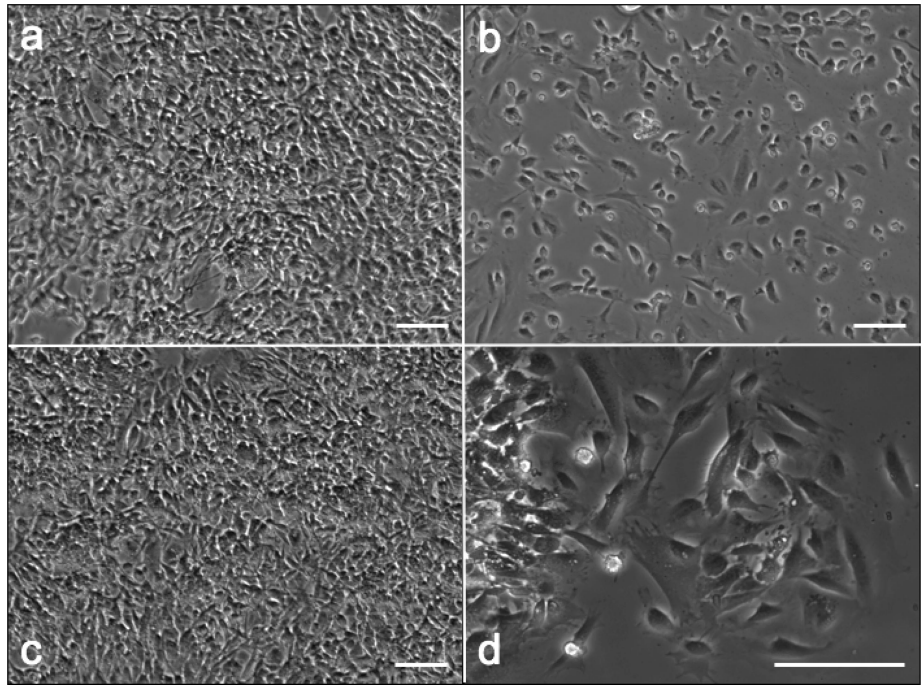

Figure 4. Poly(triol α-ketoglutarate) as cell scaffolds. 3T3 Swiss Albino fibroblasts were seeded onto elastomer films to determine their ability to act as cell scaffolds. Films were functionalized with H_2NO-GRGDS. On material 2C, cells were able to create a lawn (a) as well as take extended morphologies when space was available (b). Cells were also able to form a lawn (c) and take extended morphologies (d) on material 3C. The scale bars represent 50 μm.

trend was material 1A—curing for 7 days at 60 °C led to insufficient cross-linking, producing a pseudoamorphous material. For the remaining 11 materials, complete hydrolytic degradation occurred in as little as 2 days (elastomer 2A) and as many as 28 days (elastomer 3C). The other nine materials were dispersed between these two, allowing for the potential to tune the desired degradation rate or release profile of a particular material.

Film Micromolding. One way to enhance the versatility of a material is to allow for the patterning of topologies and microstructures. Imprint lithography was used to easily generate features in PTK. After placing a small amount of prepolymer on a glass slide in a vacuum oven at 120 °C, the pressure was reduced for 30 min to remove any residual solvent or gas. Using a patterned PDMS stamp, designed features were inversely transferred to polyketoester films by placing the stamp directly in contact with the prepolymer. The pressure was reduced for an additional 20 min to remove trapped air. After a total curing period of 24 h, the PDMS stamp was removed. The elastomeric film was able to receive the inverse pattern of the PDMS stamp with high fidelity (Figure 2). This micromolding strategy has the potential to create microfluidic devices or complex 3D microstructures for cell scaffolds.

Film Functionalization. The main factor for developing these PTKs was the desire to include a simple and mild functionalization strategy in a biomaterial. Using monomers that contain ketones accomplishes this through the ability to form stable oximes upon reaction with oxyamine-tethered molecules. Previous work has shown that ketones could survive extended periods of time at up to 170 °C during polymer synthesis.[30,31] Therefore, the ketones in the polyketoesters should be able to survive a curing process at temperatures of 120 °C or less, allowing for mild postpolymerization modifications.

To demonstrate the covalent immobilization of ligands, an oxyamine-modified fluorescent dye was coupled to the surface of a polyketoester film. After placing a PDMS microfludic cassette in direct contact with a film of PHa, a liquid-tight seal was created. By flowing a MeOH solution containing an oxyamine-modified rhodamine, spatial control of ligand immobilization was controlled. As seen in Figure 3, the fluorescent pattern is easily visible through the background fluorescence from the polyester film. Therefore, the ketones are still capable of reacting with oxyamine-containing ligands. As controls, when the oxyamine group was not present in the fluorescent dye, no fluorescent pattern was observed. Since oxyamines are easily incorporated into a wide range of molecules, including peptides and carbohydrates, PTKs have the potential to be modified for biospecific tissue engineering and targeted delivery applications.

In Vitro Biocompatibility. To determine biocompatibility, cells were cultured in the presence of both PTK degradation byproduct and pieces of elastomer films. None of the 11 films produced were deemed cytotoxic, as determined by cell morphology, monolayer confluence, and the ability to continue subculturing cells. A noncytotoxic result was expected, as two of the four monomers are naturally occurring metabolites. The fact that materials 2C and 3C are noncytotoxic may also be due to their low sol−gel fractions (4.4% and 3.5%, respectively).

A second method for determining biocompatibility was conducted by seeding cells on elastomers 1C, 2C, and 3C. Cells were unable to attach or proliferate on any of these three materials. Previous materials that were synthesized similarly were able to support cells without adhesive ligands.[10,11] Most likely, this is due to a much higher hydrophobic content of the polyesters films. We believe that cells cannot attach to the unmodified PTK films due to the hydrophilic nature of PGa, PBa, and PHa. However, when H_2NO-GRGDS peptide was coupled to the surface (1 mM, 5 h), cells were able to recognize the peptide ligand and attach to the elastomer surface (Figure 4). The RGD peptide sequence is the minimal cell adhesive ligand found in the extracellular matrix protein fibronectin.[18,19] After 48 h of division and migration, a lawn of contact inhibited cells was formed on materials 2C and 3C. Interestingly, although cells were able to attach to 1C after immobilization of H_2NO-

GRGDS, PGa degraded too rapidly and did not allow for cellular division and migration.

Conclusions

Poly(triol α-ketoglutarate) was produced from the condensation of α-ketoglutaric acid and either glycerol, 1,2,4-butanetriol, or 1,2,6-hexanetriol. Altering the triol, the curing temperature, or the duration of the curing period allowed for control over the mechanical and degradation properties. A wide range of strengths and elasticities were easily achieved, producing materials that were strong and flexible, strong and inflexible, or weak and flexible. In addition, the ketone in the repeat unit is capable of reacting with oxyamine-terminated ligands, allowing for facile modification of polyester films. By immobilizing the cell adhesive peptide GRGDS, these materials were able to support cell adhesion through a receptor−ligand interaction without any cytotoxicity from the degradation byproducts. We believe this methodology will have broad impact in the field of biodegradable polymers for applications in tissue engineering and drug delivery. The ketone-oxyamine immobilization strategy can be extended to covalently attach a wide range of biomolecules and nanomaterials to polyketoester films. The major advances of this material are the ease of the polymerization technique, the ability to tune the mechanical and degradation properties of the material, the presence of a chemoselective postpolymerization modification strategy, and the inclusion of at least one natural product in each polyketoester. Attempts to extend the versatility of this strategy to include the design of new cell scaffolding materials, such as polyketoamides, are currently being pursued.

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Supporting Information Available: IR spectra for prepolymers and additional image of cells attached to films. This material is available free of charge via the Internet at http://pubs.acs.org.

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