A Functionalizable Biomaterial Based on Dihydroxyacetone, an Intermediate of Glucose Metabolism

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A biomaterial and its potential degradation products should be biocompatible, nontoxic, and removed by the body upon expiration of its functional lifetime. One historically successful approach is to create new materials from biomolecules that naturally occur in the human body. Herein, we report the synthesis and characterization of a polycarbonate based on dihydroxyacetone, a 3-carbon ketose, and an intermediate in the glucose metabolic pathway. The polymer was synthesized in a range of molecular weights (∼8000 to ∼37 500) by ring-opening polymerization. The C2 carbonyl of dihydroxyacetone is reactive to amines, and this reactivity was used to functionalize the polymer's surface in a one-step reaction by reductive amination. Additionally, contact angle measurements show the surface of poly(2-oxypropylene carbonate) is hydrophilic even though it is insoluble in water. Mechanical analysis of the polymer revealed it is exceptionally strong for an aliphatic polycarbonate. Specifically, poly(2-oxypropylene carbonate), M_w 37 500, yielded a Young's modulus of 0.5 GPa and a compressive yield stress of 50 MPa. These values equal or exceed those of cancellous bone with similar dimensions.

Introduction

The development of new polymeric biomaterials is a priority for the biomedical industry. Polymeric biomaterials are used medically as integral components of engineered tissues, medical devices, and drug delivery systems.[1−3] The design criteria for new medical biomaterials are relatively straightforward: the materials should maintain their physical and chemical performance over a specified time frame, after which the polymer and its degradation products should remain nontoxic and be removed from the body. However, creating new biomaterials that meet these criteria is nontrivial.

It is difficult to engineer biocompatibility and low toxicity into a new biomaterial. One historically successful strategy is to derive new biomaterials from naturally occurring biomolecules that upon polymer degradation are eliminated via their normal metabolic pathways. This approach led to a number of prominent biomaterials, in particular poly(lactic-*co*-glycolic acid) and poly(ϵ-caprolactone). Another approach toward increasing biocompatibility is to manage the biomaterial−tissue interface by selective functionalization of the material surface.[4−6] Functionalized biomaterials have a host of potential applications ranging from cell-selective drug delivery,[7] tissue engineering,[8] and micropatterned systems for cell-based high throughput screening.[9,10]

With this framework as our motivation, we have sought to synthesize new functionalizable biomaterials derived from natural human metabolites. Potentially useful metabolic synthons are represented in the carbohydrate metabolic pathway because their hydroxyl groups may allow the synthesis of a wide range of polymer types. Inspection of the glucose metabolic pathway led us to investigate dihydroxyacetone as a potential polymer building block.

Dihydroxyacetone (DHA), or 1,3-dihydroxypropane-2-one (I), is a glucolytic intermediate that is accepted by the FDA for human ingestion as well as the active ingredient in sunless tanning lotions.[11,12] It is also readily manufactured as a fermentative product from corn syrup and methanol.[13,14] These characteristics, in addition to its two hydroxyl functional groups, make DHA an attractive building block for a range of polymeric biomaterials such as polycarbonates, polyesters, or polyurethanes. However, the chemistry of DHA is surprisingly challenging because in solution it is in equilibrium with its dimer (II), and it is susceptible to nucleophilic addition at its C2 carbonyl.[15,16] Previously, we reported the synthesis of poly(carbonate-acetal)s from a stabilized form of the DHA dimer.[17] However, there are no literature or patent examples of characterized polymers based on the monomeric form of DHA (I), although some noncharacterized, surmised, and derivative structures have been reported.[18−20] Our interest in the polymeric form of DHA resides not only in its potential to be favorably accepted by the human body, but also in its potential to be readily surface-modified by nucleophilic addition at the C2 carbonyl without the use of condensation reagents.

Herein, we focus on the synthesis of a functionalizable polycarbonate of DHA. Polycarbonate-based biomaterials, particularly those amenable to functionalization and derived from naturally occurring biomolecules, have been reported recently.[21−23] However, functionalization of these materials requires the synthesis of specialized monomers and/or hydrolysis of the polymer backbone followed by treatment with condensation reagents. With a polycarbonate based on DHA, we sought a biomaterial amenable to one-step functionalization through reductive amination at the C2 carbonyl.

Experimental Section

Materials. Dihydroxyacetone dimer (DHA), triethylorthoformate, ethyl chloroformate, *p*-toluenesulfonic acid, tin(II) ethylhexanoate (stannous octoate, $Sn(Oct)_2$), trifluoroacetic acid, triphosgene, and phenethylamine were purchased from Aldrich; triethylamine was purchased from Fluka. Chemicals were used as received without further purification. Polylysine-FITC, albumin-FITC, and fluorescein were purchased from Sigma-Aldrich and used as received.

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Equipment. NMR spectra were recorded on Mercury 300 MHz, Inova 400 MHz, and Inova 500 MHz spectrometers. Gel permeation chromatography (GPC) was carried out using PSS SDV columns 500A, 50A, and linear M (in series) with a THF mobile phase (1 mL/min) and polystyrene standards with UV (Waters 486) and RI (Waters 2410) detection. DSC measurements were performed on DSC Q1000 equipment at a heating/cooling rate of 10 °C per minute and nitrogen flow rate of 50 mL/min. TGA experiments were conducted on TGA Q500 equipment with a heating rate of 10 °C per minute and nitrogen flow rate of 50 mL/min. Uniaxial compression experiments were conducted on an Instron instrument with a crosshead speed of 0.02 in./min at 20 °C and 50% humidity.

Synthetic Methods and Characterization. *2,2-Dimethoxy-propane-1,3-diol (III).* DHA dimer (25 g, 0.139 mol), trimethylorthoformate (30.4 mL, 0.278 mol), and *p*-toluenesulfonic acid (100 mg) were combined in 300 mL of methanol and stirred for 12 h. Next, 300 mg of Na_2CO_3 was added, and the reaction mixture was stirred for an additional 12 h, after which the mixture was filtered and solvent was removed in vacuo. The resulting solid was recrystallized from ethyl ether to give 17.5 g of III (47%). ^{1}H NMR (D_2O): δ 3.58 (s; 4H), 3.24 (s; 6H). Anal. Calcd: C, 44.12; H, 8.82. Found: C, 44.50; H, 8.78.

2,2-Dimethoxypropylene Carbonate (IV). (a) A solution of triethylamine (14.7 mL, 0.1 mol) in 30 mL of THF was added dropwise to a solution of III (7.15 g, 52.5 mmol) and ethylchloroformate (10 mL, 0.1 mol) in 100 mL of THF at 0 °C. After the addition was complete, the reaction mixture was stirred at room temperature for 3 h, after which time the mixture was filtered and THF was removed in vacuo. The product was recrystallized from THF−ethyl ether to yield IV (2.5 g, 30%). (b) To a solution of III (5 g, 36.76 mmol) and pyridine (18 mL, 0.2 mol) in 100 mL of CH_2Cl_2 at −70 °C was added a solution of triphosgene (5.5 g, 18.5 mmol) in 70 mL of CH_2Cl_2. After the addition was complete, the mixture was allowed to warm to room temperature and stirred overnight, after which time the reaction mixture was subsequently washed with ammonium acetate, aqueous HCl, aqueous $NaHCO_3$, and brine and dried over sodium sulfate. After removal of the solvent, the product was isolated by flash chromatography on silica gel (eluent−ethyl acetate) and recrystallized from ethanol−hexanes to yield IV (4.5 g, 75%). ^{1}H NMR ($CDCl_3$): δ 4.28 (s; 4H), 3.30 (s; 6H). Anal. Calcd: C, 44.44; H, 6.17. Found: C, 44.58; H, 6.29.

Poly(2,2-dimethoxypropylene carbonate) (Poly IV). IV and $Sn(Oct)_2$ at various monomer-to-initiator ratios were placed into a glass vessel and evacuated. The reaction was carried out at 100 °C for as long as efficient magnetic stirring was possible (1−2 h), after which time the mixture was dissolved in methylene chloride and the polymer was obtained by precipitation into methanol. Relative molecular weights were determined by GPC with THF as mobile phase using polystyrene standards. ^{1}H NMR ($CDCl_3$): δ 4.28 (s; 4H), 3.30 (s; 6H). ^{13}C NMR ($CDCl_3$): δ 154 (−O−CO−O−), 99 ($-CH_2-$), 63 (acetal carbon), 49 ($-OCH_3$). Anal. Calcd: C, 44.44; H, 6.17. Found: C, 44.26; H, 6.04.

Poly(2-oxypropylene carbonate) (V). Deacetylation of poly(IV) was carried out in trifluoroacetic acid−water (4:1 v/v) at a polymer concentration of 0.1 mg/mL for 15 min, during which time the deprotected polycarbonate precipitated. The solid product was washed several times with methanol and dried under vacuum. ^{1}H NMR (DMSO-d_6): δ 3.18 (s; 6H, minor peak), 3.18 (s; 4H, minor peak), 4.90 (s; 4H, major peak), polymer was 85% deprotected to maintain solubility. ^{13}C NMR (solid state): δ 38 ($-OCH_3$), 71 ($-CH_2-$), 156 (−O−CO−O−), 205 (−CO−). Anal. Calcd: C, 41.39; H, 3.47. Found: C, 41.34; H, 3.33.

1,3-Diethyl Carbonate-2-propanone (VI). To a solution of DHA dimer (10 g, 55.5 mmol) in 100 mL of pyridine was added ethyl chloroformate (21.5 mL, 225 mmol) at 0 °C. After complete addition, the solution was stirred for 3 h at room temperature. The reaction mixture was poured into 250 mL of cold water, acidified with HCl to pH 3, and thoroughly extracted with methylene chloride. Combined organic extracts were washed with aqueous HCl, aqueous $NaHCO_3$, and aqueous NaCl and dried over sodium sulfate. Removal of the solvent and recrystallization from ethyl ether afforded 1,3-ethyl carbonate-2-propanone (14.7 g; 60%). ^{1}H NMR ($CDCl_3$): δ 4.82 (s; 4H), 4.26 (q; 4H), 1.34 (t; 6H). ^{13}C NMR ($CDCl_3$): δ 199 (−CO−), 155 (−O−CO−O−), 69 ($O-CH_2-CO-$), 65 (CH_3-CH_2-O-), 15 ($-CH_3$). Anal. Calcd: C, 44.44; H, 6.17. Found: C, 44.26; H, 6.04.

1,3-Diethyl Carbonate-2-(phenethylamino)-propane (VII). To a solution of VI (500 mg; 2.14 mmol) in 5 mL of THF were added phenethylamine (280 μL; 2.14 mmol) and $NaCNBH_3$ (500 mg; 7.96 mmol), and the mixture was stirred overnight. The reaction mixture was poured into 50 mL of cold water and extracted with methylene chloride (5 × 5 mL). Combined organic extracts were washed with aqueous HCl, aqueous $NaHCO_3$, and aqueous NaCl and dried over sodium sulfate. Removal of the solvent was followed by extraction with hot hexanes. The residue was dried under vacuum and directly analyzed by NMR spectroscopy.

Surface Modification. *Polylysine Conjugation.* Thin films of polymer IV were prepared by spin coating from methylene chloride (25% w/v) onto glass slides. Islands of deprotected polymer V were created by spot deprotection with TFA/H_2O (4:1, v:v) and allowing the TFA/H_2O to evaporate over 30 min. The slides were washed with deionized water and dried, then covered with the desired solutions (in phosphate buffered saline) for 30 min, washed with deionized water again, and dried under N_2 gas. Polylysine-FITC concentration, 0.0038 mM (fluorescein mM equivalent); fluorescein concentration, 0.0038 mM.

Albumin Conjugation. Thin films of polymer IV were prepared by spin coating from methylene chloride (25% w/v) onto glass slides. Islands of deprotected polymer V were created by spot deprotection with TFA/H_2O (4:1, v:v) and allowing the TFA/H_2O to evaporate over 30 min. The slides were washed with deionized water and dried, then covered with the desired solutions (in phosphate buffered saline) for 30 min, washed with deionized water again, and dried under N_2 gas. Albumin-FITC concentration, 0.0038 mM; fluorescein concentration, 0.038 mM.

Contact Angle Measurements. Contact angle goniometry was performed under ambient conditions with a Ram6-Hart telescopic goniometer with a Gilmont syringe equipped with a 24-gauge flat-tipped needle. The probe fluids used were water (Milli-Q, 10^{18} Ω/cm) and diiodomethane purified by vacuum distillation. Dynamic advancing (θ_A) and receding contact angles (θ_R) were recorded while the probe fluid was added to and withdrawn from the drop, respectively. Contact angles were measured on polymer wafers fabricated by mold compression. The surface energy analysis was conducted according to eq 1:

$$(1 + \cos \Theta) \times \gamma_L = 2 \times [(\gamma_L{}^d \times \gamma_S{}^d)^{1/2} + (\gamma_L{}^p \times \gamma_S{}^p)^{1/2}] \tag{1}$$

where Θ is the contact angle, γ is the surface energy, L and S are liquid and solid, respectively, d and p are dispersive and polar components, respectively, and $\gamma_i = \gamma_i{}^d + \gamma_i{}^p$. Contact angles were measured on pellets of poly IV and poly V with water ($\gamma_d = 22.1$ mJ/m^2; $\gamma_p = 50.7$ mJ/m^2) and diiodomethane ($\gamma_d = 49.5$ mJ/m^2; $\gamma_p = 1.3$ mJ/ m^2) as probing liquids, and the system of equations was solved to produce γ_i for each of the polymers.

Results and Discussion

Our synthetic route to polymer V is shown in Scheme 1. The initial challenge was to devise a simple synthetic approach to lock DHA in a monomeric form that was amenable to polymerization, yet allow deprotection to yield the parent DHA in a polymeric form, a step not yet achieved in the current DHA polymer literature. A review of the DHA literature revealed a C2 dimethyl acetal derivative (2,2-dimethoxy-1,3-propane diol, III) that reverts to the parent ketone in acid.[24,25] Using III as a starting point, a DHA-based polycarbonate was synthesized by ring-opening polymerization of the corresponding six-membered cyclic carbonate, IV.

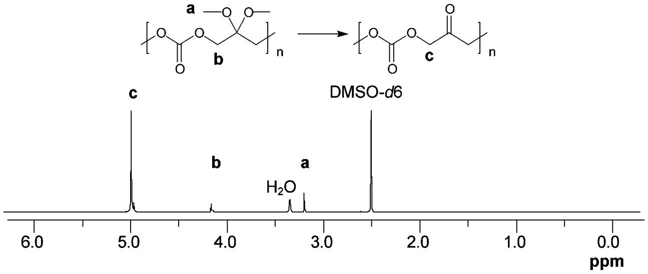
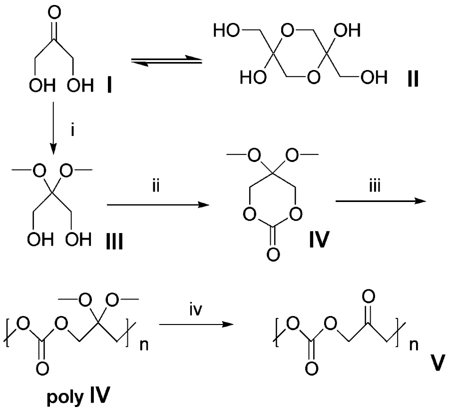


Figure 1. ^1H NMR spectrum of partially deprotected poly(2-oxypropylene carbonate).

Scheme 1. Synthetic Route to Poly(2-oxypropylene carbonate)^a



^{a} (i) Trimethyl orthoformate, MeOH, 24 h; Na_2CO_3, 12 h; (ii) ethyl chloroformate, triethylamine, THF, 3 h or triphosgene, pyridine, −70 °C; (iii) $Sn(Oct)_2$, 100 °C, 1−2 h; (iv) trifluoroacetic acid:water (4:1); 15 min.

Monomer IV undergoes ring-opening polymerization using stannous octoate as the coordination catalyst. Three molecular weights were synthesized in a bulk melt (100 °C) under vacuum by altering the monomer:catalyst (m:c) mole ratios (m:c 100 − $M_w = 8000$, PDI = 1.5; m:c 200 − $M_w = 19\,500$, PDI = 1.35; m:c 400 − $M_w = 37\,500$, PDI = 1.5). These molecular weight profiles were reproducible across multiple reactions. Deprotection of the dimethoxy acetal group by treatment with trifluoroacetic acid/H_2O (4:1, v:v) afforded poly(2-oxypropylene carbonate), V, in greater than 80% material yield and with over 95% deprotection yield as determined by elemental analysis and corresponding spectra. Complete deprotection of the homopolymer is hindered by precipitation in the TFA:water mixture, but, as reported in the accompanying manuscript, ^1H NMR analysis of diblock copolymers of V and poly(ethylene glycol), which remain soluble throughout the deprotection process, shows quantitative removal of the dimethoxy acetal group with acid treatment.[26] Additionally, the results also show that the deprotection method has no effect on the degree of polymerization of V. All structures in Scheme 1 were confirmed via ^1H NMR, elemental analysis, and/or ^{13}C NMR. Figure 1 shows the ^1H NMR spectrum of V (85% deprotected) in DMSO-d_6. Partial deprotection was used to maintain solubility. A solid-state ^{13}C NMR spectrum, consistent with the proposed structure of V, is shown in Figure 2.

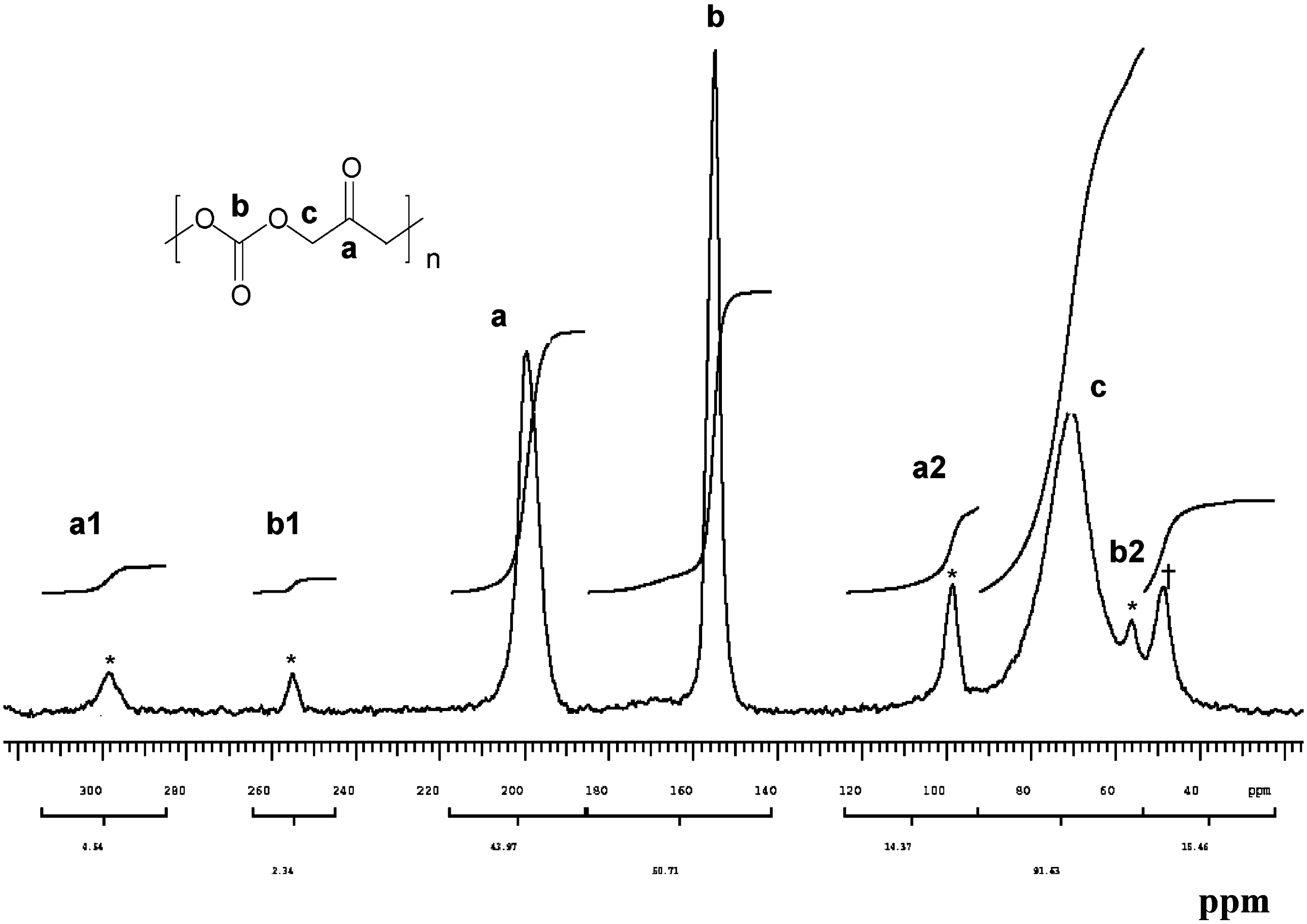


Figure 2. ^{13}C CPMAS spectrum of solid poly(2-oxypropylene carbonate). Relaxation delay = 12 s, pw = 90°, gated ^1H decoupling, contact time = 2 ms. *: Magic angle spinning sidebands. Mass spinning sidebands a1 and a2 belong to peak a, and b1 and b2 belong to peak b. The remaining peak's mass spinning sidebands are not visible. †: CH_3O^- peak.

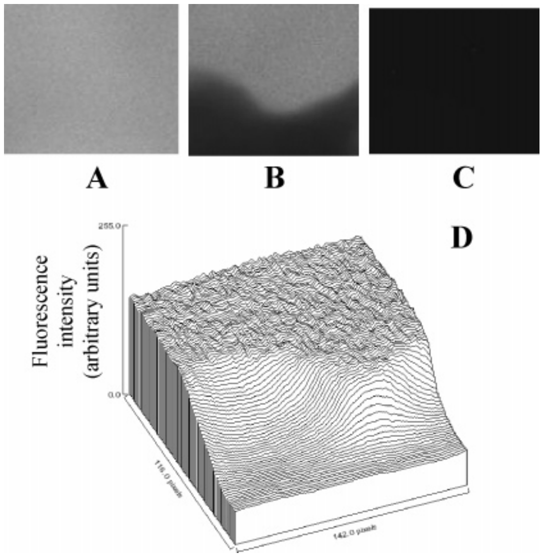


Figure 3. Polylysine surface conjugation. Fluorescent micrographs of polymer IV and V thin films following incubation with fluorescently labeled amine-containing ligands. Panel A shows a region of polymer V alone incubated with polylysine-FITC. Panel B shows the interface between polymer V (light region) and polymer IV (dark region) following incubation with polylysine-FITC. Panel C shows regions of polymer V incubated with fluorescein alone. Panel D is a three-dimensional representation of the polymer V (high fluorescence) and polymer IV (low fluorescence) interface following incubation with polylysine-FITC.

Control over a biomaterial's surface correlates to control over its material−tissue interface, and consequently to greater control over its biocompatibility and interaction with a host organism at the molecular level. Both inert[27] and biomimetic[28] materials are desired depending upon the specific purpose. As previously mentioned, the C2 carbonyl of monomeric DHA forms a Schiff base with primary amines. We were interested to determine if this reactivity was retained when DHA was in the polymeric state because it could provide a facile means to functionalize its surface.

Fluorescently labeled polylysine was used to initially characterize the surface reactivity of polymer V. Islands of V were formed by acid spot-treatment of spin-cast thin films of IV. These protected and deprotected regions were determined independent of fluorescence by visual inspection. The spun-coat polymer IV films were transparent, whereas upon deprotection polymer V became opaque. A solution of polylysine conjugated with fluorescein was added to the surface, covering both the islands of polymer V and the surrounding polymer IV. Fluorescent micrographs of the surface revealed strong fluorescence correlating to regions of V, but not in the surrounding vicinities occupied by IV (Figure 3A−D). Because the attachment of protein ligands to surfaces is more apropos to controlling the material−cell interface, the attachment of a model protein (bovine serum albumin) to both IV and V was also investigated. Figure 4A−C shows micrographs of the homogeneously fluorescent surface of V, the interface between V and IV, and the control of V incubated with fluorescein alone (at 10× the concentration relative to the protein experiments), respectively. Figure 4D shows the three-dimensional representation of the polymer IV (low fluorescence) and V (high fluoresence) regions. These results reveal a strong affinity of V to both polylysine and albumin, and a corresponding low affinity of polymer IV.

To characterize the reaction between DHA in the polymeric state and amines in greater detail, a low molecular weight analogue of the polymer was synthesized (1,3-diethyl carbonate-2-propane, VI). In solution, VI maintains the electronic character of V and has a clearly interpretable NMR spectra. VI was aminated using phenylethylamine, and the Schiff base was reduced using sodium cyanoborohydride. The ^{13}C NMR spectrum revealed the absence of the C2 carbonyl peak of VI, the presence of phenylethylamine, and spectra consistent with the product 1,3-diethyl carbonate-2-(phenylethylamino)-propane (VII) (Figure 5). The minor peaks in the NMR spectra of VII are expected due to the rapid rearrangement of amine adducts of DHA before reduction to the secondary amine.[29]

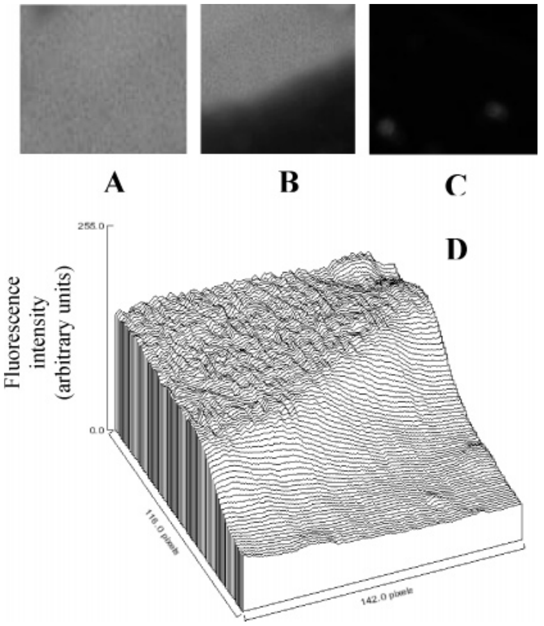


Figure 4. Albumin surface conjugation. Fluorescent micrographs of polymer IV and V thin films following incubation with fluorescently labeled amine-containing ligands. Panel A shows a region of polymer V alone incubated with albumin-FITC. Panel B shows the interface between polymer V (light region) and polymer IV (dark region) following incubation with albumin-FITC. Panel C shows regions of polymer V incubated with fluorescein alone. Panel D is a three-dimensional representation of the polymer V (high fluorescence) and polymer IV (low fluorescence) interface following incubation with albumin-FITC.

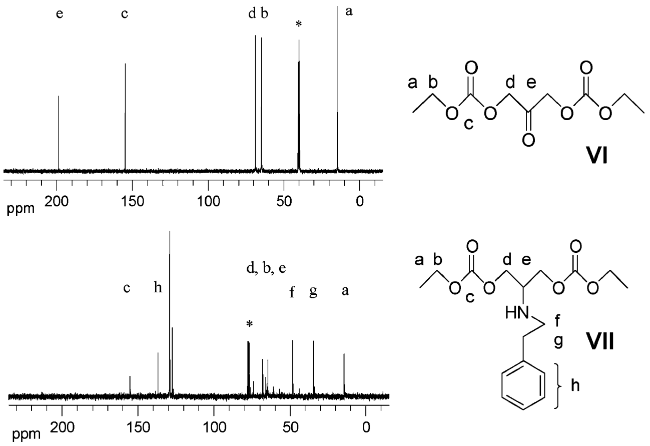


Figure 5. ^{13}C NMR spectra of 1,3-diethyl carbonate-2-propane (VI), the model analogue of V, and its primary reductive amination product (VII).

One unique attribute to V is that, although it is poorly soluble in water, its surface is hydrophilic and partially wettable with water. Contact angle measurements with both water and CH_2I_2 are consistent with a hydrophilic surface, counter to surfaces comprised of the polyesters based on lactic and glycolic acids

Table 1. Experimental Contact Angle Values Measured on Pellets of Poly IV and Poly V and Values of Dispersive and Polar Components of Surface Energy of Polymers and Their Total Surface Energy

	contact angle Θ, deg (adv/rec)		γ^d,	γ^p,	γ_s,
polymer	H_2O	CH_2I_2	mJ/m^2	mJ/m^2	mJ/m^2
poly IV	73 ± 3/55 ± 3	35 ± 1/18 ± 2	38.1	6.5	44.6
poly V	57 ± 2/10 ± 2	25 ± 2/<5	39.6	14.0	53.6

(Table 1).[33] Contact angle hysteresis, defined as the difference between the advancing and receding contact angles, is an indicator of surface heterogeneity, roughness, permeation of the liquid into the material, and/or the extent to which the material surface reorients to the drop.[34] Hysteresis is significant for polymer V, but the extent to which each of these characteristics contribute to the contact angle hysteresis is currently unknown and is under investigation. Interestingly, previous work in our lab on poly(carbonate-acetal)s made from a stabilized dimer of DHA showed very little contact angle hysteresis.[17]

The free energies of IV and V were derived using the method of Owens and Wendt.[17,35] Briefly, the free energy was calculated using the contact angles of water and diiodomethane, whose dispersive and polar contributions to surface energy are known. These measurements can be used to calculate the free surface energy of a surface. The free surface energies of IV and V (Table 1) are very different primarily due to the higher polar contribution in V. Additionally, the surface free energies of both polymers are substantially higher than the polyesters based on lactic and glycolic acid (~35 mJ/m^2).

Mechanical and thermal analysis of polymer V revealed some interesting characteristics. First, polymer V is surprisingly strong for an aliphatic polycarbonate. Unconfined uniaxial compression of V (cylindrical pellets, $M_w = 37\,500$) yielded a Young's modulus of 0.5 GPa and a compressive yield stress of 50 MPa (Figure 6). These values equal or exceed those of cancellous bone with similar dimensions analyzed under analogous conditions.[30,31] Second, the thermal characteristics of V are also unusual for an aliphatic polycarbonate. The glass transition temperature ($T_g = 60$ °C) of V is high relative to its non-carbonyl analogue, poly(1,3-trimethylene carbonate) ($T_g = -15$ °C).[32] This is important from a biomaterials perspective because to maintain structural integrity in the body the material should have a T_g above 37 °C. The most probable explanation of the observed high T_g value of polymer V is that a large dipole moment of the C2 carbonyl (2.9 D) causes strong interactions among the polymer chains (VDW, dipole/dipole), which, in turn, results in a higher T_g as compared to that of the aliphatic polycarbonates.

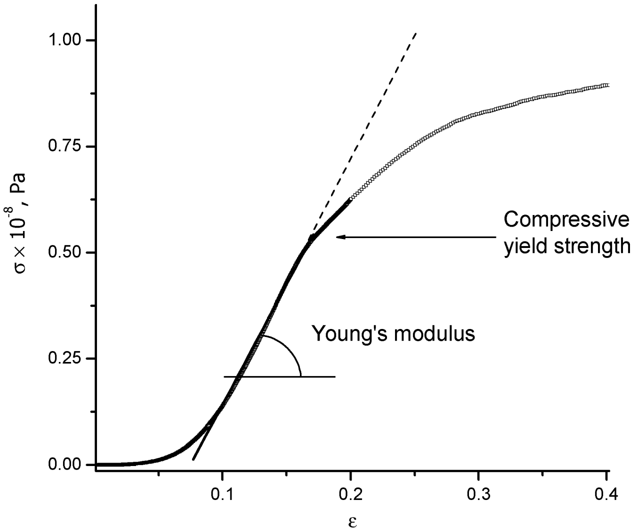


Figure 6. Uniaxial compression stress−strain curve obtained on polymer V pellet (100 mg; compressed at 5000 psi for 2 min; aspect ratio ~0.5).

Conclusions

We report the first synthesis and characterization of a polymeric biomaterial comprised of the glucolytic intermediate, dihydroxyacetone. The material possesses some unique characteristics that make it attractive for its potential use in the human body. Specifically, its surface can be functionalized in a facile one-step manner, it has a glass transition temperature that exceeds body temperature, and its compressive strength is similar to that of human bone. Analysis of the vivo biocompatibility of V, its in vivo degradation kinetics, and its application as an integral component of biomedical devices is ongoing.

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