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Poly(carbonate ester)s Based on Units of 6-Hydroxyhexanoic Acid and Glycerol

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Biodegradable polymers such as polyesters and polycarbonates formed by ring-opening polymerizations, including poly(ϵ-caprolactone), poly(*p*-dioxanone), poly(trimethylene carbonate), and most notably poly(glycolic acid) and poly(lactic acid), have met wide acceptance for medical uses as a consequence of their low toxicity, degradation properties, and ease of synthesis.[1−4] As medical device materials, biodegradable polymers do not require removal after implantation, thereby eliminating a second surgical procedure; furthermore, the chronic immune response often associated with permanently implanted synthetic materials is reduced or eliminated as the macromolecules degrade after performing their intended function. These polymers are used in medical applications in various roles including sutures, staples, and stent coatings, as orthopedic cell scaffolds, and as micro- and nanoparticles for drug delivery applications.[5−8] When a polymer does not meet the requirements for an intended application, two monomers are often copolymerized or two or more polymers are blended with each other to alter properties such as degradation rate, flexibility, and strength. A representative example is Vicryl, a commercially available poly(lactide-*co*-glycolide) suture produced by Ethicon. In general, these aforementioned polymers are distinctly limited by the range of properties attainable and lack of chemical side groups for further functionalization, potentially hindering the development and synthesis of more tailored materials.

In recent years, a number of new polymers have been introduced to address the need for functionalizable materials. These include linear polyesters based on amino acids,[9,10] sugars,[11,12] or modified hydroxy acids,[13,14] linear polycarbonates based on sugars,[12] glycerol,[15] and dihydroxyacetone,[16] modified trimethylene carbonate monomers,[17,18] and others.[19] Polyester dendrimers composed of glycerol and lactic or succinic acids have also been reported.[20]

Poly(ϵ-caprolactone) is one polymer that has been used widely in a variety of medical devices. Currently, poly(ϵ-caprolactone) is incorporated in materials for tissue scaffolding including bone,[21] blood vessels,[22] and nerves,[23] as well as drug delivery systems[24] and suture materials,[25] but it is limited by a lack of functional side groups. An advantage of using poly(ϵ-caprolactone) is because of its slow degradation rate which does not create acidic microenvironments like poly(glycolic acid) and poly(lactic acid).[26] Herein we report new copolymers based on 6-hydroxyhexanoic acid and glycerol which contain pendant side chains with varying common reactive groups. The utility of these functionalizable polymers was further demonstrated through the covalent attachment of coumarin, a fluorescent dye molecule. Nanoparticles can be formed from the poly(carbonate ester)s and these particles are internalized by cells.

ϵ-Caprolactone was copolymerized with 5-benzyloxy-1,3-dioxan-2-one to form a copolymer, which, after the removal of the benzyl side chains from the glycerol carbonate units via a mild hydrogenolysis reaction afforded a secondary hydroxyl pendant side groups with the capacity for subsequent functionalization. The carbonate of glycerol monomer, 5-benzyloxy-1,3-dioxan-2-one (**1**), was prepared in three steps, starting from 2-phenyl-1,3-dioxan-5-ol (see synthesis in Supporting Information).[15] Scheme 1 depicts the copolymerization of the carbonate monomer, **1**, with ϵ-caprolactone, **2**, via a ring-opening polymerization using tin(II) 2-ethylhexanoate ($Sn(oct)_2$). Deprotection of the secondary hydroxyl group on the glycerol was achieved using a Pd/H_2 catalyzed reaction to remove the benzyl-protecting group.

Copolymers were synthesized from varying mole ratios of the two monomers, ϵ-caprolactone and 5-benzyloxy-1,3-dioxan-2-one; molecular weight measurements were obtained using size exclusion chromatography and thermal data were recorded using differential scanning calorimetry. Copolymer composition, molecular weights, and thermal data are summarized in Table 1. It should be noted that the molecular weights of aliphatic polyesters and polycarbonates tend to be overestimated by up to 50% by polystyrene-calibrated SEC measurements.[27−29] Both benzyl-protected and -deprotected copolymers demonstrated similar solubility. All copolymers were soluble in dichloromethane, dimethylformamide, and toluene, with limited solubility in tetrahydrofuran. Conversely, all copolymers were insoluble in more polar solvents such as methanol, ethanol, dimethyl sulfoxide, water, and ethyl acetate. Copolymers with melting temperatures above room temperature—protected copolymers with $\geq$70 mol % CG and deprotected copolymers with $\geq$80 mol % CG—formed tough, opaque films upon drying. All other copolymers were viscous liquids at room temperature.

The stannous octoate ($Sn(oct)_2$) catalyst was chosen for the ring-opening polymerization in part due to its ubiquity for catalyzing numerous other copolymerization reactions between biodegradable cyclic monomers such as ϵ-caprolactone, trimethylene carbonate, glycolide, and lactide. This catalyst is currently used to synthesize materials intended for use in vivo.[30] $Sn(oct)_2$ has a high polymerization activity for both ϵ-caprolactone and cyclic carbonates, leading to linear chains via a coordination−insertion mechanism.[31]

The relative mole fractions of monomers in the resulting copolymers were determined using ^1H NMR analysis. Comparing the integrations of unique CH_2 species in each monomer unit of the protected copolymer—the benzyl CH_2 peak at δ = 4.65 for glycerol and CH_2 peak alpha to the carbonyl at δ = 2.29 for 6-hydroxyhexanoic acid—shows that the polymerization proceeds with expected mole fractions of each monomer incorporated into the copolymer. Also, the methylene α to the carbonyl of the 6-hydroxyhexanoic acid unit splits into two multiplets with integrations indicative of the monomer ratio in the deprotected polymer (Figure 1). A linear trend of relative mole percent in the polymer feed vs mole percent in the copolymer was observed (see figure in Supporting Information).

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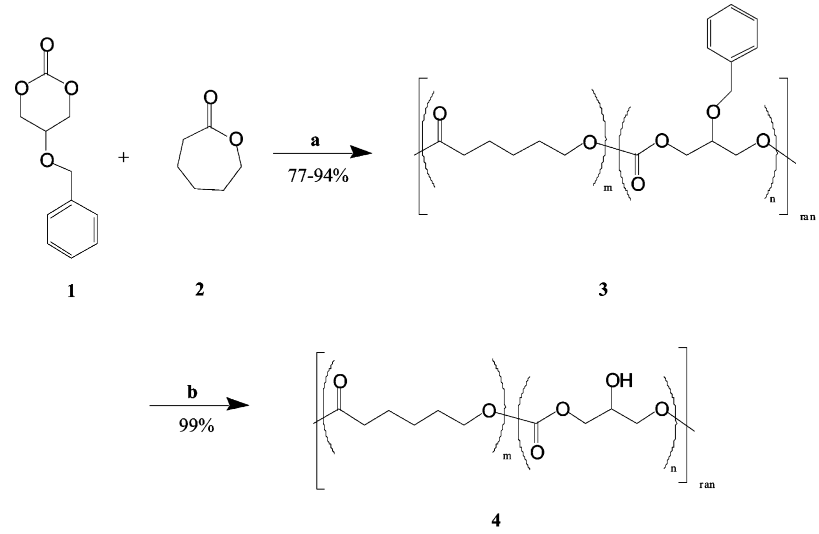
Scheme 1. Synthesis of Poly(ϵ-caprolactone-*co*-glycerol Carbonate)[a]



[a] Key: (a) $Sn(Oct)_2$, 140 °C; (b) H_2, Pd/C, $Pd(OH)_2/C$, DCM.

Table 1. Composition, Molecular Weight, and Thermal Data of Copolymers[a]

				mol wt			thermal properties			
polymer	f_{cg}	F_{cg}	yield (%)	M_n(theor)	M_n(SEC)	M_w/M_n	T_g (°C)	T_c (°C)	T_m (°C)	ΔH_f (J/g)
CL-CG-100−0	0	0	77	57 000	22 700	1.47	−64	36	57	61.5
CL-CG-90−10-Bn	10	11	87	61 700	13 300	1.67	−54	8	40	38.5
CL-CG-90−10-OH	10	11	99	57 200	12 200	1.67	−59	7	35	32.6
CL-CG-80−20-Bn	20	23	92	66 400	10 200	1.96	−49	−4	31	25.4
CL-CG-80−20-OH	20	23	99	57 400	8600	1.96	−56	0	23	8.5
CL-CG-80−20-C_6-OH	20	23	86	68 800	10 100	1.91	−47	−1	43	33
CL-CG-80−20-C_5-CO_2H	20	23	83	70 200	10 400	1.96	−46	−5	40	33
CL-CG-80−20-C_6-NH_2	20	23	85	68 700	10 100	1.94	−44	8	44	35
CL-CG-70−30-Bn	30	30	94	71 100	9300	1.78	−43	3	22	13.4
CL-CG-60−40-Bn	40	42	79	75 800	7900	1.94	−38	none	none	none
CL-CG-0−100-Bn	100	100	42	104 000	3600	3.16	−10	none	none	none

[a] f_{cg} = mole percent carbonate monomer in polymerization feed; F_{cg} = mole percent carbonate monomer in copolymer; M_n = number average molecular weight; M_w/M_n = polydispersity index; T_g = glass transition temperature; T_c = crystallization temperature; T_m = melting temperature; H_f = heat of fusion. The synthesis and characterization of poly(carbonate-ester)s prepared form 5-benzyloxy-1,3-dioxan-2-one and ϵ-caprolactone are reported. These copolymers contain a hydrolyzable backbone and functionalizable heteroatom pendant groups.

Next, ^{13}C NMR spectra of the poly(carbonate ester)s were analyzed to determine the monomer sequence in the copolymer. For the case of a copolymer with two constituent monomers, 2^3 triads can theoretically be formed, and their relative intensities in the spectra can suggest a blocky, random, or statistically random chemical structure. There are eight possible triads for the ester-carbonate copolymer system (see Supporting Information for details). Triad peaks that do not overlap the equivalent homopolymer peaks from triads **LLL** (ϵ-capro*l*actone) and **CCC** (5-benzyloxytrimethylene *c*arbonate) indicate a degree of randomness in the sequence. Comparison of relative intensities shows a propensity for randomness as carbonate monomers tend to precede ϵ-caprolactone additions.

Thermal analysis via differential scanning calorimetry (DSC) revealed several property trends as a function of glycerol carbonate mol % in the copolymer. Table 1 shows the glass transition temperature (T_g) of the copolymers increasing, from −64 °C (pure poly(ϵ-caprolactone)) to −10 °C (pure poly(5-benzyloxy-1,3-dioxan-2-one)), as the ratio of 5-benzyloxy-1,3-dioxan-2-one to ϵ-caprolactone monomer increased. Conversely, the melting temperature (T_m) of the copolymer decreased with increasing 5-benzyloxy-1,3-dioxan-2-one monomer, before disappearing completely for CL-CG-60−40-Bn (40 mol % carbonate monomer). Relative amounts of crystallinity were determined from the heat of fusion, ΔH_f. For each additional 10 mol % 5-benzyloxy-1,3-dioxan-2-one monomer in the reaction mixture, the percent crystallinity was approximately halved. When the reaction consisted of less than 70 mol % ϵ-caprolactone, the copolymer became completely amorphous, as determined by the absence of a crystallinity temperature and melting temperature.

Primary hydroxyl-, amine-, and carboxylic acid-derivatized copolymers were synthesized via the addition of 6-benzyloxyhexanoic acid, hexanedioic acid monobenzyl ester, or fmoc-6-aminohexanoic acid, respectively, to poly(carbonate-*co*-ester), CL-CG-80−20-OH (Scheme 2). Side chain deprotection was subsequently performed by Pd-catalyzed hydrogenation to remove the benzyl-protecting group, or a 40% piperidine/dimethylformamide mixture to remove the fmoc-protecting group, and verified by ^1H NMR. The three copolymers including CL-CG-80−20-C6-OH, **5**, CL-CG-80−20-C5-COOH, **6**, and CL-CG-80−20-C6-NH_2, **7**, demonstrated similar thermal trends as CL-CG-80−20-Bn with appropriate changes in molecular weights, with the exception of small increases in melting temperatures (Table 1). Differences in melting temperature are likely due to increased hydrogen bonding from the side chains. The modification of a functionalizable copolymer was demonstrated by conjugating the fluorescent chromophore coumarin to the free secondary hydroxyl units on CL-CG-80−20-OH.

Poly(ϵ-caprolactone) was identified for this study in part due to the wide use of this polymer in drug delivery systems, where an agent, for example, is encapsulated within the interior

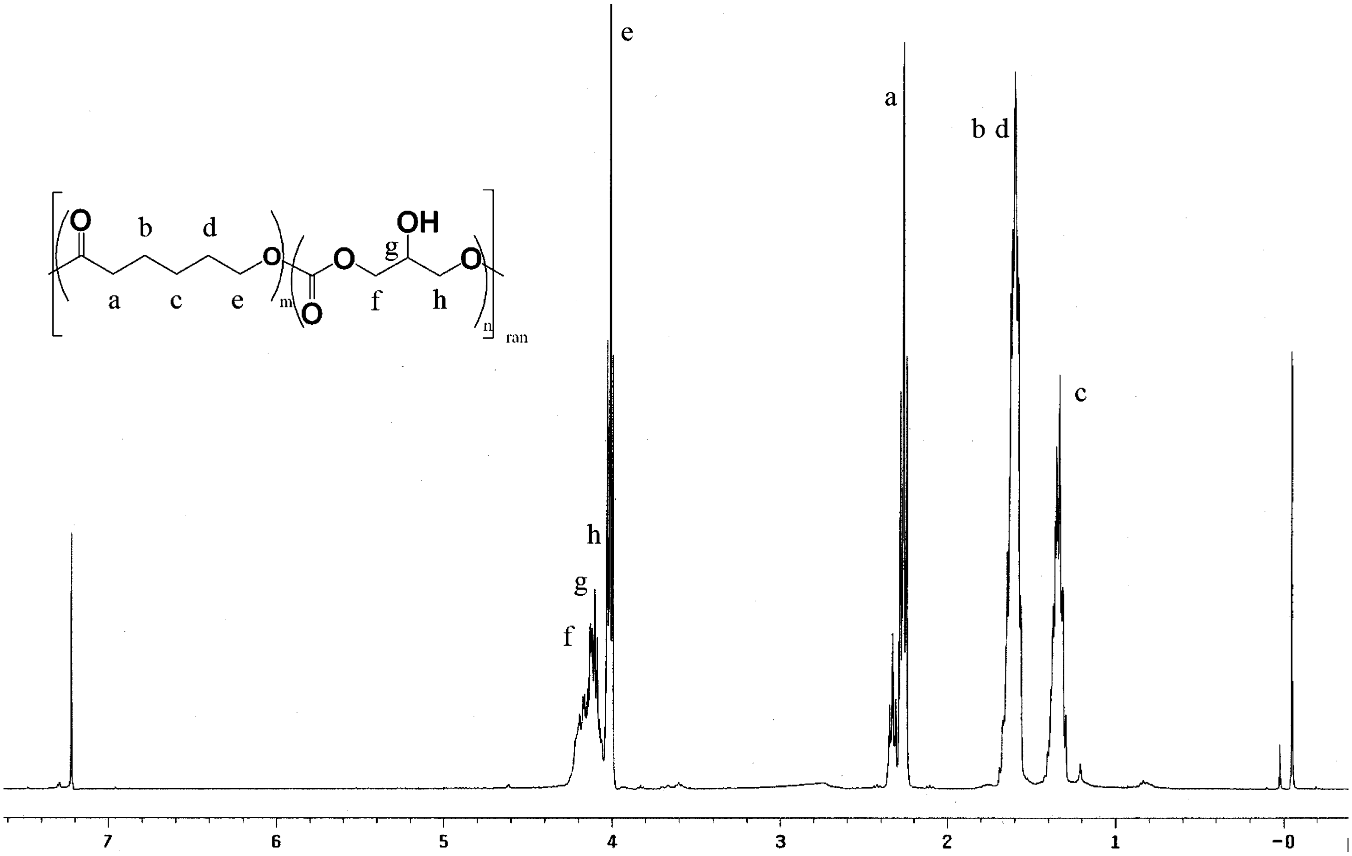
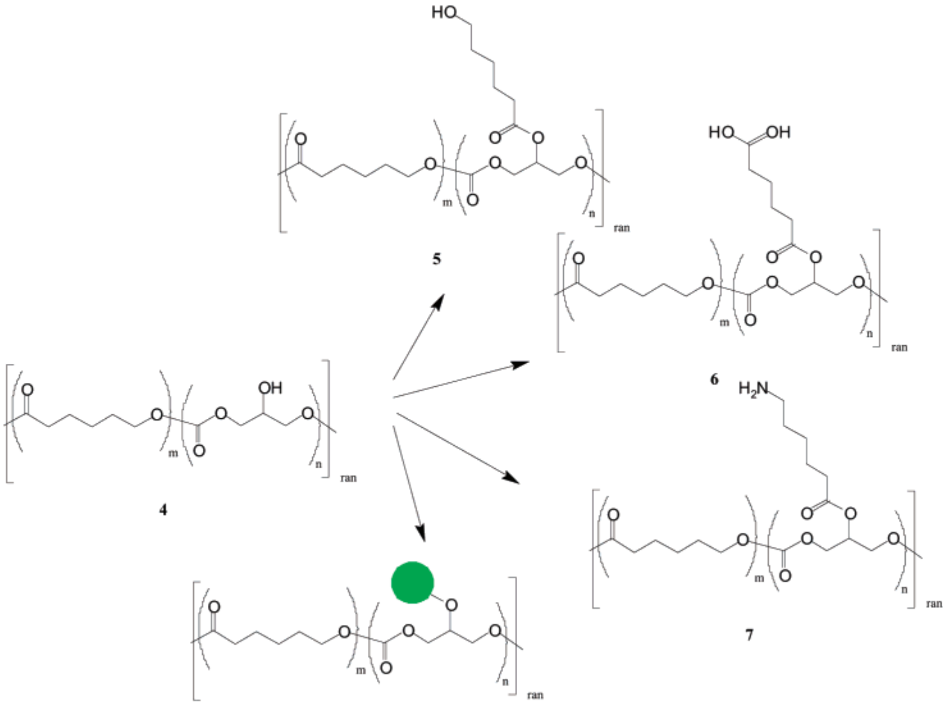


Figure 1. ^{1}H NMR spectra of the CL-CG-80−20-OH copolymer.

Scheme 2. Functionalized Poly(ε-caprolactone-*co*-glycerol carbonate)s, with Hydroxyl (5), Carboxylic Acid (6), Amine (7), and Coumarin-Conjugated (8) Copolymers Synthesized from CL-CG-80−20-OH Copolymer



structure of microparticles or nanoparticles.[24] Nanoparticles were formed from CL-CG-80−20-OH or CL-CG-80−20-coumarin copolymers using an emulsion/solvent evaporation method.[32] Size distribution measurements obtained by dynamic light scattering indicate a mean particle diameter of 125 nm with a narrow polydispersity index (0.128). To determine the potential applicability of these nanospheres as drug delivery vehicles, we performed an initial cell uptake using fluorescent CL-CG-80−20-coumarin nanoparticles and A549 human lung carcinoma cells. As shown in Figure 2, particles were internalized by the cells. The cytotoxicity of nanoparticles (CL-CG-80−20-OH) was then assessed with A549 cells. Cell viability was determined

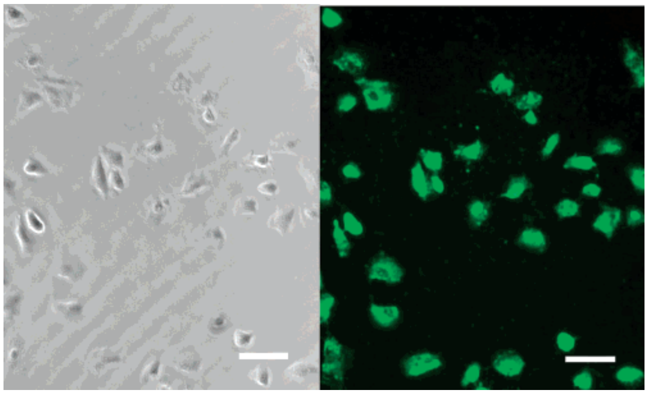


Figure 2. Fluorescence microscopy images of coumarin-conjugated CL-CG-80–20-OH nanoparticles after cellular uptake by A549 non-small cell lung cancer cells (phase contrast, left; fluorescence, right; 10×, scale bar = 100 μm).

using a standard MTT cell proliferation assay. No cytotoxicity was observed with the nanoparticles, as the data was similar to the positive control—untreated cells (see Supporting Information).

New poly(ester-carbonate)s comprised of glycerol and 6-hydroxycaproic acid repeating units have been synthesized via ring-opening polymerization of 5-benzyloxy-1,3-dioxan-2-one and ϵ-caprolactone, followed by catalytic hydrogenolysis. Copolymerizing 5-benzyloxy-1,3-dioxan-2-one with biodegradable aliphatic polyesters such as poly(ϵ-caprolactone) introduces side chains for subsequent modification by incorporating a biocompatible monomer unit of glycerol. Further functionalization via hydroxyl, carboxylic acid, and/or amine side chains will facilitate the attachment of chemically diverse molecules to the polymer chain. The utility of side-group modification was demonstrated through the covalent attachment of the chromophore, coumarin, to the secondary hydroxyl groups of the polymer. Furthermore, the polymer can be processed to give nanoparticles. Application-specific tailoring of the chemical, physical, and mechanical properties of the polymer for medical uses by varying monomer units, composition, degradable linkages, and side group moieties, is highly advantageous given the varied design requirements of a specific application, be it controlled drug delivery or degradable scaffolding. Continued research in this area will lead to increasingly specialized materials that are multifunctional (e.g., delivery, targeting, and imaging), responsive to stimuli from their local environment (e.g., pH), or processable into unique structures (e.g., fibers, particles, and 3D constructs).

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Supporting Information Available: Text giving synthetic procedures, NMR analysis, TEM data, figures showing a plot of relative mole percentages, ^{13}C NMR anaylsis, and cytotoxicity of the nanoparticles. This material is available free of charge via the Internet at http://pubs.acs.org.

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