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Photocrosslinkable biodegradable elastomers based on cinnamate-functionalized polyesters

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ABSTRACT

Synthetic biodegradable elastomers are an emerging class of materials that play a critical role in supporting innovations in bioabsorbable medical implants. This paper describes the synthesis and characterization of poly(glycerol-*co*-sebacate)-cinnamate (PGS-CinA), a biodegradable elastomer based on hyperbranched polyesters derivatized with pendant cinnamate groups. PGS-CinA can be prepared via photodimerization in the absence of photoinitiators using monomers that are found in common foods. The resulting network exhibits a Young's modulus of 50.5–152.1 kPa and a projected in vitro degradation half-life time between 90 and 140 days. PGS-CinA elastomers are intrinsically cell-adherent and support rapid proliferation of fibroblasts. Spreading and proliferation of fibroblasts are loosely governed by the substrate stiffness within the range of Young's moduli in PGS-CinA networks that were prepared. The thermo-mechanical properties, biodegradability and intrinsic support of cell attachment and proliferation suggest that PGS-CinA networks are broadly applicable for use in next generation bioabsorable materials including temporary medical devices and scaffolds for soft tissue engineering.

1. Introduction

Biodegradable elastomers are an emerging class of biomaterials that show promise for use in temporary medical implants such as scaffolds and surgical materials [1,2]. This family of synthetic polymers includes polyester-based materials such as poly(glycerol-*co*-sebacate) (PGS) [3], poly(1,3-diamino-2-hydroxypropane-*co*-polyol sebacate) (APS) [4] and poly(1,8-octanediol-*co*-citric acid) (POC) [5]. Other types of biodegradable elastomers include poly(urethane urea) [6], poly(trimethylene carbonate) [7] and their associated co-polymers and derivatives [8]. These polymers are rapidly gaining acceptance as valuable bulk materials in a variety of biomedical applications including surgical meshes [9], controlled release matrices [10,11], synthetic vascular grafts [12,13] and scaffolds for peripheral nerve regeneration [14,15]. The utility of this class of materials in biomedical applications is derived from the combination of mechanical compliance, total macroscopic biodegradation and subsequent metabolism of non-toxic monomers. Furthermore, a wide range of the aforementioned thermo-mechanical properties and bioabsorption kinetics can be accessed by careful selection of monomer stoichiometry and the conditions during network formation [16–18].

Polyester-based biodegradable elastomers are typically synthesized using polycondensation schemes that use in vacuo environments and high temperatures [3–5]. These processing conditions largely prohibit the inclusion of biological materials such as bioactive proteins or viable pre-seeded cell populations [5]. Room temperature free radical crosslinking of polymers in mild environments is a suitable strategy to obviate restrictive processing conditions. There are several prominent examples of photocrosslinkable elastomers, including poly(trimethylene carbonate) [19], poly(octamethylene maleate citrate) [20] and poly(β-amino ester)s [21]. PGS can be modified with pendant acrylate groups to produce poly(glycerol-*co*-sebacate)-acrylate (PGSA) [22], which can be used as a precursor for photocrosslinkable biodegradable elastomeric networks. This strategy has also been applied to natural and synthetic hydrogels including poly(ethylene glycol) [23] and hyaluronic acid networks [24]. In situ photocrosslinking of diene functionalities presents two potential limitations. First, introducing an exogenous toxic photoinitiator can negatively impact the viability of seeded cell populations [25]. Second, free radical polymerization of pendant dienes produces non-degradable high molecular weight aliphatic backbones, which can retard hydrolytic-mediated degradation [22]. Therefore, alternative photocrosslinking schemes that can address these potential limitations are of interest.

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Cinnamates are a class of naturally occurring aromatic compounds that are found in some fruits [26]. Cinnamate derivatives undergo reversible photodimerization upon irradiation of ultraviolet light with wavelengths longer than 260 nm [27]. The aforementioned dimerization forms a cyclobutane ring by [2+2] photocycloaddition [28,29]. The resulting α-truxillic acid derivatives, which are the products of photodimerization of cinnamates, are one kind of naturally occurring compounds [30]. Photocleavage of the cyclobutane ring is possible by irradiation at wavelengths of less than 260 nm [28]. Derivatizing hyperbranched polyester pre-polymers with pendant cinnamate can therefore produce photocrosslinkable elastomeric networks that are hydrolytically labile, intrinsically cell adherent and composed of simple monomers.

2. Experimental section

2.1. Synthesis of poly(glycerol-co-sebacate)-cinnamate

PGS pre-polymer was synthesized according to a previously reported method [3]. Briefly, equimolar amounts of sebacic acid and glycerol were combined by melting at 130 °C under a blanket of nitrogen. Polycondensation was accelerated through a gradual reduction in pressure from atmospheric pressure to 50 mTorr over 1 h. After 48 h, a transparent viscous product was recovered. PGS-CinA pre-polymer was prepared as follows: 5 g of PGS pre-polymer was dehydrated at 100 °C under vacuum for 2 h and dissolved in 30 ml anhydrous chloroform in a three-neck round bottom flask. The solution was purged under nitrogen for 3 h before adding 20 mg of 4-dimethylaminopyridine. The reaction mixture was cooled to 0 °C and charged with cinnamoyl chloride (1.62 g, 50% mol mol^{-1} on a basis of pendant hydroxyl groups) and an equimolar amount of triethylamine (1.36 ml). The reaction proceeded for 24 h as the temperature gradually increased from 0 °C to room temperature. Chloroform was removed by rotary evaporation and 30 ml ethyl acetate was added to precipitate the salt, which was removed by filtration. Ethyl acetate was removed by evaporation to produce a viscous, transparent, light yellow product. PGS-CinA pre-polymer was purified by re-crystallization in ethanol.

2.2. Pre-polymer characterization

The molecular weight distribution of PGS pre-polymer was measured by gel permeation chromatography (Polymer Standards Services, Amherst, MA) using a poly(methyl methacrylate) (PMMA) standard in N,N-dimethylformamide (DMF) as the eluent. ^{1}H nuclear magnetic resonance (Bruker Avance 300 MHz, Billerica, MA) was performed in $CDCl_3$ to determine the degree of substitution (DS) of cinnamate groups on PGS pre-polymer. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.7 (1H, d, −CO−CH=), 7.56 (2H, t, Ar−H), 7.4 (3H, t, Ar−H), 6.5 (1H, d, Ar−CH=), 5.2 (1H, m, −CH−O−), 4.2 (4H, d, $-CH_2-O-$), 2.3 (4H, m, $-CO-CH_2-$), 1.6 (4H, m, $-CH_2-CH_2-$), 1.32 (8H, m, $-CH_2-CH_2-$). Cinnamate incorporation was confirmed by FT-IR (Mattson ATI Affinity 60AR, Madison, WI). Samples used in FT-IR were prepared by drop-casting a dilute solution of PGS-CinA (DS35%)/chloroform on zinc selenide substrates (Alfa Aesar, Ward Hill, MA). The intrinsic viscosity of PGS-CinA pre-polymer was measured using an Ubbelohde viscometer at 25 °C. Briefly, solutions of PGS-CinA in DMSO (3.1–7.5 g dl^{-1}) were filtered and efflux time was measured at least three times.

2.3. Characterization of photocrosslinked PGS-CinA networks

PGS-CinA films (DS = 26%, 29%, 35% and 45%) were melt-pressed between two quartz slides with a spacer of 350 μm. Films were exposed to UV light on both sides each for 2 h using a 600 W UVB lamp (Integrated Dispensing Solution, Agoura Hills, CA). The mass density was measured using a 25 ml pycnometer. The sol content (Sol_{cont}) of crosslinked PGS-CinA was determined by weighing the mass difference of dry films before ($m_{gel+sol}$) and after sol extraction (m_{gel}) and calculated using the following equation:

$$sol\ content = \frac{m_{gel+sol} - m_{gel}}{m_{gel+sol}} \quad (1)$$

Sol content was measured as a function of UV exposure time to elucidate the behavior of the sol–gel transition in PGS-CinA networks during photocrosslinking. The glass transition temperature (T_g) of crosslinked PGS-CinA sol-free films was measured by differential scanning calorimetry (DSC, TA Instrument, Q20, New Castle, DE) at a heating rate of 10 °C min^{-1}. The kinetics of photopolymerzation was measured by UV-vis spectroscopy (Cary 300, Foster city, CA). Briefly, 30 mg PGS-CinA DS35% pre-polymer was dissolved in 1 ml chloroform and spin coated on quartz slides with the speed of 1500 rpm for 30 s followed by solvent evaporation under vacuum for 2 h. UV-vis spectra were recorded after exposure to UVB lamp radiation for various times. Photocrosslinked PGS-CinA elastomers were cut into rectangular coupons with a length:width ratio 2:1 for macroscopic mechanical characterization. Tensile tests ($n = 4$) were conducted (Instron 5943 equipped with Bluehill 3 software, Norwood, MA) using a 10 N load cell. A strain rate of 1 mm min^{-1} was applied to the sample until failure. The Young's modulus was calculated using the stress–strain slope until 5% of strain.

2.4. Replica molding of PGS-CinA

Replica molding of PGS-CinA was demonstrated through photopolymerization of PGS-CinA on microfabricated substrates using a method analogous to soft lithography. Briefly, negative molds composed of silicon were fabricated as previously described [31]. PGS-CinA pre-polymer (DS45%) was applied to the microfabricated silicon substrate and pressed to form a film 100 μm in thickness. Films were exposed to UVB light for 2 h and carefully delaminated by mild thermal expansion. The fidelity of pattern transfer in PGS-CinA films was verified via a scanning electron microscope (SEM, Philips XL30).

2.5. In vitro degradation and cell adhesion

PGS-CinA elastomers (DS26%, DS29%, DS35%, DS45%, $n = 4$) were cut into disks with a diameter of 9.5 mm and a thickness of 350 μm. Sol-free samples were incubated at 37 °C in 20 ml 3 M sodium acetate solution with pH equal to 5.2 with medium exchange after every 48 h. After prescribed intervals of degradation, samples were washed with deionized (DI) water, incubated in ethanol for 12 h, and equilibrated in DI water for 12 h. Hydrated mass was measured after gently removing excess water. Samples were dehydrated for 6 h before the dry mass was recorded.

Elastomeric disks ($n = 6$) 5 mm in diameter prepared from DS26% and DS45% pre-polymer formulations were incubated in ethanol for 12 h to remove sol followed by rinsing in DI water for 12 h for equilibration. PGS-CinA substrates were transferred to 96-well plates and disinfected using a 100 W Black Ray UVA lamp for 1 h. NIH/3T3 fibroblast cells (ATCC, Manassas, VA) were seeded on elastomers at a density of 65,000 cells cm^{-2} using tissue culture polystyrene (TCPS) as a control. Cell metabolism was measured using a tetrazolium salt WST-1 assay (Roche Applied Science, Indianapolis, IN), which can be cleaved to a soluble formazan by viable metabolically active cells. Briefly, after 1, 2, 3, 5 and 7 days of cell incubation, 15 μl WST-1 reagent was added to each well with 150 μl growth medium (Dulbecco's modified Eagle's mdium with 10% concentration of bovine calf serum) and incubated for 4 h at 37 °C in a 5% CO_2 atmosphere. Absorbance measurements were