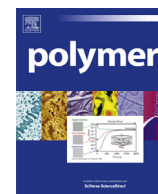




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Time dependence of material properties of polyethylene glycol hydrogels chain extended with short hydroxy acid segments

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ABSTRACT

The objective of this work was to investigate the effect of chemical composition and segment number (n) on gelation, stiffness, and degradation of hydroxy acid-chain-extended star polyethylene glycol acrylate (SPEXA) gels. The hydroxy acids included glycolide (G), L-lactide (L), p-dioxanone (D) and ϵ -caprolactone (C). Chain-extension generated water soluble macromers with faster gelation rates, lower sol fractions, higher compressive moduli, and a wide-ranging degradation times when crosslinked into a hydrogel. SPEGA gels with the highest fraction of inter-molecular crosslinks had the most increase in compressive modulus with n whereas SPELA and SPECA had the lowest increase in modulus. SPEXA gels exhibited a wide range of degradation times from a few days for SPEGA to a few weeks for SPELA, a few months for SPEDA, and many months for SPECA. Marrow stromal cells and endothelial progenitor cells had the highest expression of vasculogenic markers when co-encapsulated in the faster degrading SPELA gel.

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1. Introduction

The delivery of stem cells in a supportive carrier is an exciting approach to the regeneration of damaged biological tissues. In that approach the carrier acts as a temporary matrix for immobilization of the cells in the site of regeneration which is gradually displaced with the extracellular matrix (ECM) secreted by the encapsulated cells. The temporary matrix should provide a three-dimensional hydrophilic and aqueous environment to support complex cell–matrix interactions but gradually degrade concurrent with ECM formation [1–4]. Due to their high water content, hydrogels have high permeability to oxygen and nutrients required for cell survival and function [5]. Further, cell-loaded hydrogel precursor solutions can be injected and gelled *in situ* to fill defects with irregular shapes. Inter-diffusion of hydrogel chains to tissues proximal to the defect leads to superior integration of the carrier with the surrounding tissue after gelation [6].

Due to their non-immunogenic and inert nature, polyethylene glycol (PEG) hydrogels are very attractive as a matrix for stem cell delivery to the site of regeneration [6–8]. Adhesion and differentiation of cells encapsulated in PEG gels can be tuned by

conjugation with integrin-binding ligands and morphogenetic peptides, respectively [9–11]. A major drawback to the wide-spread use of PEG gels is their persistence in the site of regeneration. Although copolymerization of PEG macromers with resorbable monomers can be used to impart degradability to the hydrogel [12,13], degradability is offset by lower macromer solubility in aqueous solution for cell encapsulation and low gel stiffness [14,15].

Recently, we reported that the extension of star PEG macromers with very short hydroxy acid segments imparted degradability to the hydrogel without changing macromer solubility in aqueous solution and hydrogel stiffness [14,16]. Molecular dynamic simulations demonstrated the formation of micellar structures (<3 nm in size) that dramatically increased the local concentration of reactive groups, leading to a potentially higher modulus with little change in the overall water content of the hydrogel [17]. Further, human MSCs encapsulated in the high-stiffness, relatively slow-degrading lactide-chain-extended PEG hydrogels had higher extent of differentiation to the osteogenic lineage than those encapsulated in non-degradable PEG gels [16].

It is well-established that the fate of encapsulated cells is determined by the physical and mechanical properties of their microenvironment including degradation rate and stiffness. For example, marrow stem cells (MSCs) encapsulated in a non-degradable hyaluronic acid (HA) gel had a rounded morphology and underwent adipogenic differentiation whereas those MSCs encapsulated in a degradable HA gel spread and differentiated to the osteogenic lineage [18]. Similarly, the C2C12 mouse myoblast

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cells encapsulated in a soft degradable alginate gel (13 kPa modulus) had a higher extent of differentiation to myotubes and lower proliferation than those encapsulated in a non-degradable stiff gel (45 kPa modulus) [19]. Tissue engineered constructs often times require multiphase hydrogels with different but complementary microenvironments. For example, osteogenic differentiation of MSCs requires a supporting matrix with high compressive modulus and slow degradation [20] whereas vasculogenic differentiation of progenitor endothelial cells necessitates a low modulus, relatively fast-degrading matrix [21,22]. Therefore, there is a need to develop synthetic hydrogels with tunable degradation and stiffness for wide-ranging applications in regenerative medicine.

The objective of this work was to investigate the effect of chemical composition and length of the hydroxy acid in hydroxy acid-chain-extended star PEG acrylate macromer on gelation characteristics, water content, compressive modulus, and degradation of the hydrogels with incubation time. The hydroxy acid monomers included the least hydrophobic glycolide, L-lactide, p-dioxanone, and the most hydrophobic ϵ -caprolactone. The findings of this work demonstrate that chain extension of star PEG macromers with the above hydroxy acids produces hydrogels with a wide range of physical and mechanical properties to serve as cell carriers in regenerative medicine from the compliant vascular tissue to the stiff bone tissue.

2. Experimental

2.1. Materials

Lactide, glycolide and p-dioxanone monomers (L, G, D; >99.5% purity) were purchased from Ortec (Easley, SC) and ϵ -Caprolactone (C) was purchased from Alfa Aesar (UK). The monomers were dried under vacuum at 40 °C for at least 12 h prior to polymerization. Calcium hydride, tetrahydrofuran (THF), deuterated chloroform (99.8% deuterated), trimethylsilane (TMS), triethylamine (TEA), tin(II) 2-ethylhexanoate (TOC), acryloyl chloride, and dimethylsulfoxide (DMSO) were purchased from Sigma–Aldrich (St. Louis, MO). 4-arm PEG (SPEG, $M_w = 5000$) was purchased from Sigma–Aldrich. The protected amino acids and Rink Amide Nova-Gel resin for the synthesis of acrylamide-terminated GRGD peptide were purchased from EMD Biosciences (San Diego, CA). Dichloromethane (DCM, Acros Organics, Pittsburg, PA) was dried by distillation over calcium hydride. Diethyl ether and hexane were obtained from VWR (Bristol, CT). The dialysis tube (molecular weight cutoff 3.5 kDa) was purchased from Spectrum Laboratories (Rancho Dominguez, CA). EBM-2 medium, vascular endothelial growth factor (VEGF), human fibroblast growth factor-B (hFGF-B), R³-insulin-like growth factor (R³-IGF-1), human epidermal growth factor (hEGF), ascorbic acid hydrocortisone, gentamycin, and amphotericin-B were purchased from Lonza (Hopkinton, MA). Dulbecco's Modified Eagle Medium (DMEM; 4.5 g/L glucose with L-glutamine without sodium pyruvate) was purchased from Mediatech (Herndon, VA). Medium 199 with L-glutamine was purchased from Sigma–Aldrich. 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was purchased from Calbiochem (EMD Millipore, Billerica, MA).

2.2. Macromer synthesis

The 4-arm poly(ethylene glycol) (SPEG) macromer was extended with D, C, G and L monomers via Ring Opening Polymerization (ROP) to synthesize SPED, SPEC, SPEG and SPEL macromers, respectively, as we previously described [15,16]. Briefly, for the synthesis of SPEC, SPEG and SPEL macromers, the PEG and

monomer were added to a three-neck reaction flask equipped with a stirrer and immersed in an oil bath. The feed molar ratio of the PEG to monomer was based on the desired length of the degradable segment on each macromer arm. Next, the flask was heated to 120 °C under nitrogen flow and maintained at that temperature for 1 h before the addition of TOC catalyst. The polymerization reaction was allowed to proceed for 12 h at 140 °C, 160 °C and 140 °C for SPEG, SPEG and SPEL, respectively. For the synthesis of SPED, the PEG and catalyst mixture was heated to 130 °C to remove moisture, cooled to 85 °C for addition of D monomer, and the reaction was allowed to proceed for 48 h at 85 °C. The reaction product was precipitated in ice-cold hexane to remove the unreacted monomer and catalyst.

Next, the hydroxy acid chain-extended PEG macromer was functionalized with acrylate groups. The product of the first reaction was dissolved in DCM and dried by azeotropic distillation from toluene. In a reaction flask placed in an ice bath, equimolar amounts of acryloyl chloride (Ac) and triethylamine (TEA) were added dropwise to the solution of chain-extended macromer in DCM and the reaction was allowed to proceed for 12 h under dry nitrogen atmosphere. After the reaction, the product was dried by rotary evaporation and dissolved in ethyl acetate to precipitate the triethylamine hydrochloride salt. The product was then dried and precipitated in ice-cold ethyl ether twice. Next, the product was dissolved in DMSO and dialyzed against water (3.5 kDa MW cutoff Spectro/Por dialysis membrane) to remove any remaining impurities. Acrylate-terminated SPEXA macromers, where “X” is G, L, D or C (see Fig. 1), were dried in vacuum to remove the residual solvent and stored at –20 °C. The notations nXa is used to identify the number of degradable monomers on SPEXA macromers, where X is type of the hydroxy acid monomer and “a” is the number of hydroxy acid monomers per macromer.

2.3. Macromer characterization

The chemical structure of the macromers was characterized by a Varian Mercury-300 H NMR (Varian, Palo Alto, CA) at ambient conditions with a resolution of 0.17 Hz [23]. The samples were dissolved in deuterated chloroform at a concentration of 50 mg/mL and 1% TMS v/v was the internal standard.

2.4. Macromer gelation and rheological characterization

The SPEXA macromers were crosslinked in aqueous solution by ultraviolet-initiated polymerization as we described previously [16]. Briefly, the photoinitiator solution (Irgacure 2959; CIBA, Tarrytown, NY) was mixed with the macromer solution by vortexing

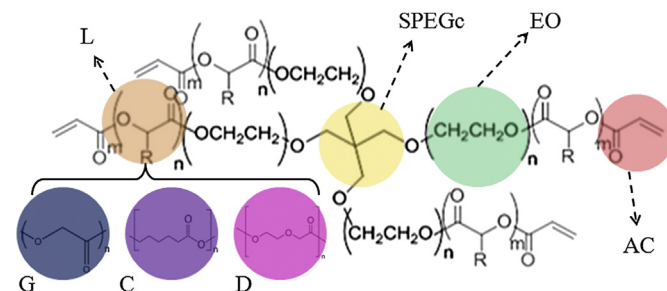


Fig. 1. Schematic representation for the chemical composition of SPEXA macromers. Ethylene oxide (EO) repeat units, SPEG core (SPEGc) and acrylate (AC) functional groups are shown by green, yellow and light red, respectively. Chain extensions with short lactide (L), glycolide (G), p-dioxanone (D) and ϵ -caprolactone (C) are shown by brown, blue, pink and purple colors, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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