100f Effect of Lactide to Ethylene Glycol Ratio on Material Properties of Novel Biodegradable Poly(Lactide-Ethylene Oxide-Fumarate) Terpolymer Hydrogels

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Hydrogels due to their hydrophilicity and high water content, coupled with minimally invasive arthroscopic techniques are an attractive cell carrier for treating irregularly shaped defects with minimum tissue dissection and retraction. After injection and hardening in-situ, these three dimensional matrices guide the organization, differentiation, proliferation, and development of seeded cells into the desired tissue. Although preliminary results are promising for naturally derived hydrogels, their low mechanical properties, their pathogenicity, and their limited availability has prompted researchers to investigate the use of synthetic biodegradable and injectable hydrogels. An ideal hydrogel as a cell carrier should have controlled swelling ratio, mesh size and crosslink density, and degradation characteristics. In this work, we present synthesis and characterization of a novel degradable terpolymer hydrogel composed of short lactide and ethylene oxide chains linked by unsaturated fumarate units.

Difunctional hydroxyl terminated short lactide chains were first synthesized by melt ring-opening polymerization of L-lactide (LA) monomer with diethylene glycol (DEG) as the initiator and tin II-ethyl hexanoate as the catalyst. The molar ratio of LA to DEG was varied from 10 to 30 to produce low molecular weight PLA (LMWPLA) chains with number average molecular weights (Mn) in the range of 1000 to 4000 Dalton. The synthesized LMWPLA was characterized by ^{1}H-NMR, FTIR, and gel permeation chromatography (GPC). The polydispersity index of PLA was 1.5-1.6 independent of the PLA molecular weight. The degree of crystallinity of PLA was also independent of PLA molecular weight in the Mn range of 1000 to 4000 Dalton. The melting point of the semi-crystalline PLA, measured by DSC, depended on the molecular weight of the LMW PLA.

PLEOF was synthesized by condensation polymerization of low MW PLA, poly(ethylene glycol) (PEG), and fumaryl chloride (FuCl) with triethylamine (TEA) as the catalyst. FuCl was purified by distillation at 161°C and PEG was dried by azeotropic distillation from toluene. The molar ratio of FuCl:PLA+PEG and TEA:PLA+PEG were 0.9:1.0 and 1.8:1.0, respectively. PLEOF macromer was synthesized using PEG with Mn ranging from 1 to 5 kD and PLA with Mn ranging from 1 to 7 kD. The weight ratio of PEG to PLA was varied from 100/0 to 85/15 to produce hydrophilic water-soluble terpolymers. In a typical reaction, the dried PEG and LMW PLA were dissolved in methylene chloride under dry nitrogen atmosphere in a three-neck reaction flask. The reaction vessel was placed in an ice bath to limit the temperature rise of the exothermic reaction. Next, FC and TEA each dissolved were added dropwise to the reaction with stirring. After the addition of FC and TEA, reaction was continued for 6 h under ambient conditions. After completion of the reaction, solvent was removed by and residue was dissolved in anhydrous ethyl acetate. The mixture was kept at 5°C for 12 h for complete precipitation of the by-product triethylamine hydrochloride and the salt was removed by filtration. Ethyl acetate was removed by vacuum distillation at 30°C. The macromer was re-dissolved in methylene chloride, precipitated twice in ice cold ethyl ether, and dried before use. The structure of PLEOF macromer was characterized by ^{1}H-NMR and FTIR. Four singlet chemical shifts with peak positions at 1.6, 3.5, 6.8, and 6.9 ppm, two triplets with peaks positions at 3.6 and 4.2 ppm, and a quartet with peak position at 5.1 ppm were observed in ^{1}H NMR spectrum of the terpolymer. The singlet chemical shift with peak position at 1.6 ppm was attributed to hydrogens of the methyl group (-CH3) of the lactide monomer. The singlet chemical shift at 3.5 ppm was attributed to the methylene hydrogens (CH2-CH2-O-) of the ethylene oxide repeat units. The triplet chemical shifts centered at 3.6 and 4.2 ppm were due to the hydrogens of the methylene groups attached to ether (-CH2-O-CH2-) and the methylene groups attached to the ester group of lactide (CH2-OOC-), respectively, on the initiator DEG. The quartet chemical shift with peak position at 5.1 ppm was due to the hydrogen attached to the methine group of the lactide monomer. The singlet shifts at 6.90 and 6.95 ppm were attributed to the methine hydrogens of the fumarate in the middle of the chain (-OOC-CH=CH-COO-) and at the chain ends (-OOC-CH=CH-COOH), respectively. The presence of peaks at 6.90 ppm in the NMR spectrum attributable to the hydrogens of the fumarate group, and the presence of a band due to the ester carbonyl stretching vibration centered at 1725 cm^{-1} in the FTIR spectra, confirmed the incorporation of fumarate monomers into the PLEOF macromer. The ratio of the peaks in the NMR spectrum of PLEOF due to chemical shifts centered at 5.1 ppm (due to the one hydrogen attached to the methine group of the lactide monomer) and 3.6 ppm (due to the four methylene hydrogens (CH2-CH2-O-) of ethylene oxide repeat units) was related to the molar ratio of the PLA to PEG in the terpolymer. For 10% by weight PLA in the feed, this ratio was 0.055, corresponding to 5.2% by mole and 8.7% by weight of the PLA in the terpolymer. Therefore, the copolymer reactivity of PLA with fumaryl chloride was slightly less than that of PEG. The PLEOF macromer with PLA and PEG molecular weights of 3.3 kD (PI of 1.6) and 3.4 kD (PI of 1.3) had Mn and PI of 6.3 kD and 2.9, respectively, as determined by gel permeation chromatography (GPC).

Hydrogels were prepared using PLEOF as the degradable macromer, methylenebisacrylamide (MBIS) as the crosslinking agent, and a neutral redox initiation system. The redox system consisted of ammonium persulfate (APS) and tetramethylethylenediamine (TMEDA), respectively. In a typical procedure, PLEOF was added to 1.65 ml of a 0.24 M solution of BISAM in PBS and vortexed. To this mixture, 0.21 ml of 0.3 M APS and 0.21 ml of 0.3 M TMEDA were added and vortexed. The mixture was degassed, injected between two glass plates separated by a 0.5 mm gap, and fastened with clips. The assembly was placed in a convection oven at 37°C for 15 min to crosslink. After crosslinking, the gel was removed from the glass plate and disks were cut from the gel. The disk-shaped samples were used for swelling, cell viability, cell function, and degradation studies. Our results demonstrate that the water content, mesh size, and degradation characteristics of these novel terpolymer hydrogels can be controlled independently by the molecular weight of PEG, the weight ratio of PLA to PEG, and the molecular weight of PLA, respectively. These novel degradable Poly(lactide-ethylene oxide-fumarate) terpolymers are potentially useful as injectable in-situ crosslinkable cell carriers in tissue regeneration.