Synthesis of NIPAAm-based random and block copolymers as biodegradable thermally responsive hydrogels

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Statement of Purpose: A variety of synthetic and natural polymers have been investigated as injectable materials for cell delivery and tissue bulking applications, for instance with ischemic cardiomyopathy [1]. Design and development of injectable hydrogels with improved elasticity and mechanical strength, proper gelation speed, controllable degradation rate and suitable water content still remain a challenge. Here we report synthesis of the random copolymer poly(n-isopropylacrylamide-co-hydroxyethylmethacrylate-co-polylactide-methacrylate) (NIPAAm-co-HEMA-co-MAPLA) and an ABA type copolymer where A=poly(NIPAAm-co-HEMA-co-MAPLA) and B=PEG. Hydrogels synthesized from 3 different monomer feed ratios for polymer A and 3 different PEG lengths for block copolymer ABA were characterized for phase transition behavior, mechanical properties and basic cytocompatibility.

Methods: Random copolymers poly(NIPAAm-co-HEMA-co-MAPLA) were synthesized by radical copolymerization of NIPAAm, HEMA and MAPLA at 3 monomer feed ratios of 80/10/10 82/10/8 and 84/10/6. ABA type block copolymers were synthesized by atom transfer radical polymerization. First, α-bromisobutyric acid PEG ester (BBPEG) was synthesized by reacting one of three PEGs (1, 6 or 20 kD) and α-bromoisobutyryl bromide. NIPAAm, HEMA and MAPLA were copolymerized for 24 h in methanol containing BBPEG, CuCl and the ligand 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane with a molar ratio of 1:2:2.

Polymer structures were confirmed by NMR and FTIR. Hydrogel thermally induced sol-gel transitions were studied with DSC, optical adsorption, and rheometry. Rheometery and tensile testing characterized hydrogel mechanical properties. Gelation speed was characterized by measuring water content during gelation. Hydrogel degradation rates were characterized by mass loss in PBS at 37°C. Hydrogel morphology was observed with electron microscopy. Degradation product cytocompatibility was evaluated with addition of degradation solution to culture medium for rat vascular smooth muscle cells and quantification of longitudinal cell viability.

Results and Discussion: Each of the 3 A-type hydrogels had sol-gel transition temperatures of ~20°C. At 10°C, the 80/10/10 copolymer solution showed a viscosity change from 3 to 2 Pa·sec over a shear rate range of 0-10 sec^{-1}, while the 82/10/8 and 84/10/6 copolymers showed constant viscosities of 1.2 and 0.14 Pa·sec respectively over the same shear rate range. Since the copolymers all had similar molecular weights (M_n=22k, M_w/M_n=1.6), the viscosity differences were attributed to varying hydrophobicity, with increased viscosity driven by increased inter-molecular hydrophobic interactions. Water exclusion occurred during the gelation process at 37 ºC (**Fig. 1**), and gelation speed increased with MAPLA feed ratio. All three A-type hydrogels had final water contents of 40~45 wt%. Tensile strengths of 80/10/10 82/10/8 and 84/10/6 copolymers at 37°C were 103±24, 95±25 and 37±3 kPa, respectively. The storage modulus (G′) of the 80/10/10 hydrogel was 45 kPa and the loss modulus (G″) 54 kPa at 1 Hz. A frequency sweep from 0.1 to 30 Hz showed that the G″ was close to G′ at low frequencies but was higher than G′ at increased frequency, indicating that the hydrogel was not ideally elastic. Degradation rates of the hydrogel in PBS increased with the feed ratio of biodegradable monomer (MAPLA) in the copolymer. Microstructure observed with SEM showed a uniform structure of the hydrogel at the beginning of the gelation and a collapsed structure at the end (**Fig. 1**). Hydrogel biodegradation solutions were not found to exhibit cytotoxicity.

For ABA type block copolymers, 30 kD A blocks were combined with PEG blocks of 1, 6 and 20 kD. Chemical structures of the copolymers were confirmed by NMR and FTIR. With a 20 kD PEG block, the polymer solution showed increased viscosity and formed gels with negligible water exclusion at 37°C. Copolymers with low B/A mass ratios showed hydrogel formation with water exclusion, behaving similarly to A-type hydrogels.

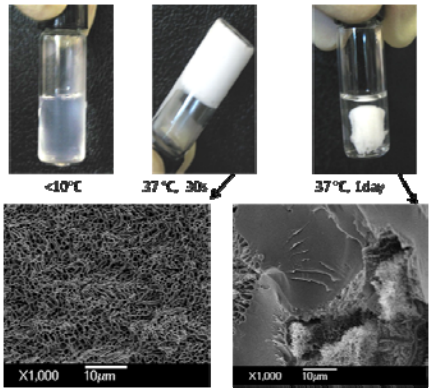

Fig. 1 Sol-gel transition behavior of poly (NIPAAm-co-HEMA-co-MAPLA) (80/10/10) and the microstructure of the hydrogel. The copolymer solution was incubated in a 37°C water bath and quenched in liquid nitrogen for SEM observation.

Conclusions: A family of NIPAAm-based random and block copolymers was synthesized. By manipulating the molecular design, it was possible to vary a number of key parameters important for injectable biomaterial design, including: viscosity below the transition temperature, gelation speed, gel mechanical strength, final water content, and degradation rate. Depending upon the area of application and hypothesized needs, these materials might be applied as temporary tissue bulking agents, injectable carriers for cell therapy, or as local reservoirs for controlled release.

References:

1. Biomaterials, (2009) 30:4357.