Linear and star-shaped block copolymers of hydrolytically labile polyNIPAAm and PEG to form thermally responsive hydrogels: Synthesis, characterization and controlled release

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Statement of Purpose: With their ease of minimally invasive delivery, thermally responsive hydrogels represent promising biomaterials for injectable scaffolds, drug carriers and tissue bulking agents.[1] We have previously reported bioabsorbable thermally responsive hydrogels based on random copolymers of poly(isopropylacrylamide-co-hydroxyethylmethacrylate-co-polylactide-methacrylate) (NIPAAm-co-HEMA-co-MAPLA).[2] While these copolymers are attractive for their mechanical properties, they are generally not optimal for drug delivery in that they experience a high degree shrinkage and water exclusion upon gelation at 37°C, leading to poor drug loading efficiency (22%).

The objective of this study was to synthesize a family of thermally responsive hydrogels possessing higher water content upon gelation that would be compatible with extended drug release while still providing adequate mechanical properties and bioabsorbability.

Methods: Tri-block (ABA) or 4-arm star-shaped block (BA_4) copolymers were synthesized by atom transfer radical polymerization (ATRP), where A=poly(NIPAAm-co-HEMA-co-MAPLA) and B=PEG, pluronic F127 or 4-arm PEG (**Fig.**). First, α-bromoisobutyryl bromide was reacted with PEG (Mw=20k, 6k, 3.4k, or 1k), Pluronic F127 (Mw=12.6k) or 4-arm PEG (Mw=10k) to obtain ATRP macro-initiators. In ATRP, NIPAAm, HEMA and MAPLA were copolymerized for 24h in ethanol to which the macro-initiators, CuCl and 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane were added at a molar ratio of 1:2:4. Block copolymers were characterized with NMR, FTIR and GPC. Thermally induced sol-gel transitions were studied with optical absorption and rheometry. ^{125}I-labeled bovine serum albumin (BSA) was used as a model drug to study controlled release behavior.

Results and Discussion: The ABA copolymers where B=PEG20k, PEG6k, PEG3.4k, PEG1k, or Pluronic F127 had molecular weights (Mn) of 37k, 26k, 24k, 19k, 31k respectively, while the 4-arm block copolymer BA_4 was 48k. PDIs ranged from 1.3-1.5. All the copolymers were soluble in PBS at 4°C, and formed stiff gels at 37°C. The ABA hydrogel with B=PEG1k showed similar properties as polymer A, while increasing the length of the PEG blocks from 3.4k to 6k to 20k resulted in increased hydrophilicity compared to polymer A, and a lack of water exclusion and volume shrinkage during gel formation at 37°C. Rheometry of these three hydrogels showed gradual changes of the shear modulus (G′ and G″) over a wider temperature range (10 to 45°C) without the sharp transition of polymer A. The shear modulus (G′) of the three hydrogels (37 °C,1Hz) was 600, 450, and 200 Pa respectively. Optically, the ABA polymers with B=PEG6k or 20k remained transparent after gelation at 37°C, while for B=PEG3.4k the hydrogel became opaque on warming, with an LCST of 25°C. The ABA hydrogel with B=Pluronic F127 experienced volume shrinkage and water exclusion at 37°C, but to a lesser degree than polymer A, and thus had a higher final water content (70 wt% vs. 45wt %). An LCST of 27°C and a G′ of 7kPa (37 °C,1Hz) were determined for this hydrogel. The 4-arm BA_4 hydrogel showed negligible water exclusion when gelled at 37°C. The hydrogel had a G′ of 900Pa (37 °C, 1Hz) and an LCST of 20°C.

Protein (BSA) release from the ABA (B=PEG3.4k) and BA_4 hydrogels was studied with polymer A as control. The loading efficiency, defined as the amount of protein remaining in the gel at 3h after initial gel formation, was 22% in the control, but increased to 89% and 82% in the ABA and BA_4 hydrogel, respectively. Release of 33% and 39% of the loaded BSA over the first day, and 66% and 76% by day 7 was observed for the two hydrogels, respectively.

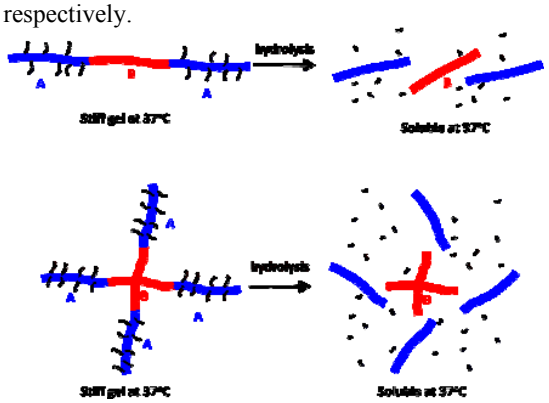

Fig. Macromolecular structure and the degradation process of (**Upper**) ABA and (**Lower**) BA_4 hydrogels, where **A**= Poly(NIPAAm-co-HEMA-co-MAPLA), **B**=PEG20k, PEG6k, PEG3.4k, PEG1k, Pluronic F127 or 4-arm PEG and the short lines represent the PLA side chains in A. Hydrolysis of the ester bonds in the PLA side chains and between the A and B blocks make the polymer soluble in water at 37°C.

Conclusions: A family of linear and star-shaped block copolymers were generated by combining hydrolytically labile and thermally responsive poly(NIPAAm-co-HEMA-co-MAPLA) blocks with PEG-based hydrophilic blocks. The synthesized hydrogels exhibited thermally-induced gelation below body temperature, but excluded minimal water and showed the ability for extended protein delivery. These materials would be attractive for application as injectable temporary soft tissue mechanical support where controlled release (e.g. of a growth factor) might also be desirable.

References:

1. Fujimoto KL, et al. *Biomaterials*, 2009;30:4357.
2. Ma Z, et al. *Biomacromolecules*, 2010;11:1871.