Novel Macromers for the Fabrication of Injectable, Calcium-Binding Hydrogels

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Statement of Purpose: The combination of physical gelation and chemical crosslinking mechanisms is a suitable strategy to generate rapidly gelling hydrogels (e.g. via thermal gelation) with an irreversible character (e.g. via covalent bond formation from two acrylic groups) *in vivo* [1]. This study aims at synthesizing and characterizing two novel macromers that both combine functional groups for physical and chemical gelation and contain a lipophilic domain (stearic acid) to strengthen intermolecular interactions and the mechanical stability of the resulting hydrogels. Biodegradable thermogelling macromers (TGMs), that contain poly(*N*-isopropylacrylamide) (PNiPAAm) rich domains for thermogelation, were developed and will finally be acrylated to add moieties that allow for chemical crosslinking (Fig. 1). Calcium-binding macromers (CBMs) were similarly designed using poly(vinylphosphonic acid) (PVA) instead of PNiPAAm as the physical crosslinking functionality to create domains that are capable of complexing Ca^{2+} ions. Ultimately, injectable systems composed of TGMs and CBMs, that thermogel quickly after injection, crosslink chemically *in situ*, and slowly harden through the incorporation of Ca^{2+} ions, will be formulated.

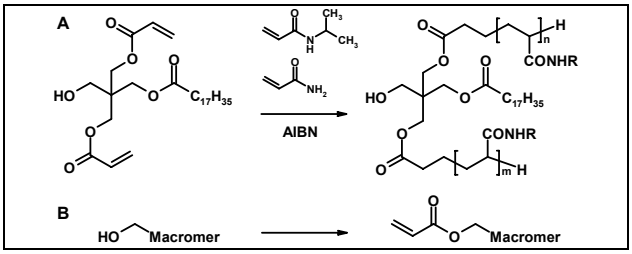

Figure 1: A: Synthesis of TGM (R: -H or $-CH(CH_3)_2$).
B: Acrylation of the macromers.

Methods:

Macromer synthesis: Pentaerythritol monostearate diacrylate (PEDAS), *N*-isopropyl-acrylamide (NiPAAm), and acrylamide (AAm) were dissolved in tetrahydrofurane at the desired ratio under nitrogen at 60°C (Fig. 1). Radical polymerization was initiated by 2,2'-azobisisobutyronitrile, and the system was refluxed over 16h at 60°C. The TGMs were isolated and purified by precipitation in diethyl ether. CBMs were synthesized from PEDAS, vinylphosphonic acid (VPA), AAm, and 2-hydroxyethyl methacrylate (HEMA) as described above.

Macromer characterization: Macromers were characterized by gel permeation chromatography (GPC) and NMR. Rheological characterization was performed on an oscillating rheometer at 1Hz and a displacement of 0.1 mrad. The calcium-binding efficacy of the CBMs was determined using a spectrophotometric calcium assay (Arsenazo III).

Results / Discussion: TGMs of different composition were synthesized from PEDAS, NiPAAm, and AAm by radical polymerization. GPC revealed the conversion of PEDAS with the acrylic monomers. ^{1}H-NMR spectroscopy of the TGMs showed typical signals of the stearic acid ($-CH_3$ at 0.85 ppm and $-CH_2-$ at 1.25 ppm) and PNiPAAm ($-CH(CH_3)_2$ at 1.2 and 4 ppm, polymeric -CHR- at 1.4 – 2 ppm). No acrylic olefins were detectable in the TGM (5.8 – 6.4 ppm) (Fig. 2A). TGM composition and properties were varied by altering the PEDAS to acrylic monomer ratio and by copolymerizing acrylamide (AAm) with NiPAAm. The replacement of NiPAAm with AAm is indicated by a decreased NMR signal at 1.2 ppm (Fig. 2B).

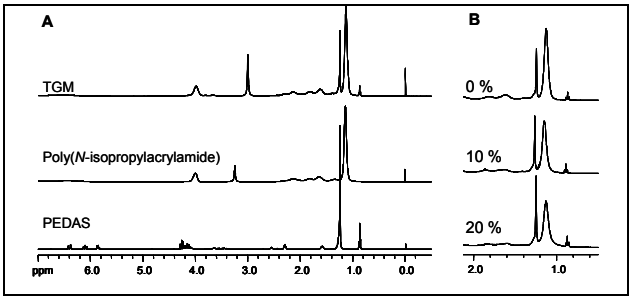

Figure 2: ^{1}H-NMR spectra: A: TGM and structural components.
B: TGMs with different acrylamide contents (0-20%).

Rheological characterization of the different TGMs revealed characteristical changes in the storage (G') and loss modulus (G'') during the temperature sweep. At low temperatures, G'' far exceeded G', a property characteristic of viscous liquids. During heating and thermogelation, G' exceeded G'', which indicated the formation of a viscoelastic hydrogel. Based on a PEDAS to monomer ratio of 1:20, variations of the content of hydrophilic AAm resulted in an increase in transition temperature with the systems remaining stable. A linear correlation was found between the AAm content of the macromer and the onset temperature of the phase transition (Fig. 3). Macroscopic gelation studies at 37°C revealed quick gel formation for all different TGMs (0-20% AAm). After several hours, TGMs with low AAm contents (0-15%) that correspond to transition temperatures below 30°C underwent syneresis, while the 20% AAm TGM formed a stable hydrogel.

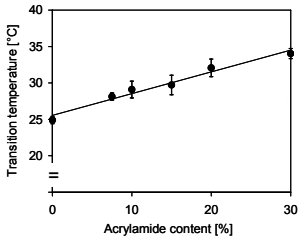

Figure 3: Onset temperatures of the phase transition of TGMs with different AAm contents (n=3).

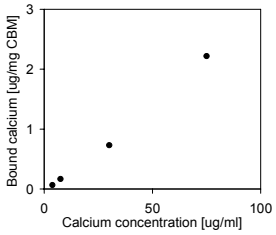

Figure 4: Calcium binding capacity of CBM composed of PEDAS, VPA, and AAm.

CBMs were synthesized from different molar ratios of PEDAS, VPA, AAm, and HEMA. Monomer conversion was shown using GPC and NMR. As a proof of concept, a neutralized aqueous solution of a CBM was added to calcium standard solutions, and the amount of bound calcium was determined indirectly. Figure 4 shows that the CBMs did complex calcium ions as a function of the initial calcium ion concentration.

Conclusions: Thermogelling and calcium-binding macromers were synthesized, and the effect of macromer composition on the thermogelling properties was investigated. The ability of the calcium-binding macromers to complex calcium ions was demonstrated. Ongoing research is focused on the synthesis of acrylated thermogelling and calcium-binding macromers and the characterization of the chemical crosslinking.

References:

1. Cellesi F et al. *Macromol Chem Phys* **203**, 1466 (2002)

Acknowledgements:

Deutsche Forschungsgemeinschaft DFG (HA 4444/1-1), and the National Institutes of Health (R01 DE15164).