Influence of Cross-linker Chemistry on Hydrogel Properties for DNA Delivery

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Statement of Purpose:
Hydrogels produced by photopolymerization of polymers have been extensively used for a number of biomedical applications.[1] The photopolymerization process allows the hydrogel to be generated *in vitro* or *in vivo* from a low viscosity of low molecular mass (MM) macromer solution via a free radical pathway at any desired shape of the site of injection capable of adhering tissue surfaces together.
Several types of biodegradable hydrogels, including those derived from poly(ethylene glycol) (PEG) copolymerized with poly(α-hydroxy esters) derivatives have been investigated for use as bioerodible materials.[2] The use of PEG increases the hydrophilicity, and Poly(α-hydroxy esters) such as Poly(glycolic acid) (PGA) produce polymers that would degrade upon hydrolysis into biocompatible substances.
Significant work has been done on investigating the effects of macromer composition and cross-link density on hydrogel mechanical and degradation characteristics.[3] The focus of this work is to describe that by simply varying the macromer en group chemistry, it is possible to tune the mechanical properties and degradation rate of hydrogels.
Methods: PEG-co-PGA were synthesized based on a water-soluble central block of PEG that was extended with PGA and terminated with different cross-linkable end-groups such as diacrylates (DA), dimethacrylates (DM), and urethane dimethacrylates (UDM). These non-toxic, water-soluble macromers were photocross-linked under long-wave ultraviolet illumination in the presence of Irgacure 2959 of aqueous solution (10% and 20% by mass fraction) for 10 min to obtain hydrogels. RGD peptide was covalently incorporated to hydrogels to promote cell adhesion, spreading, and organization of cells for in-situ polymerization. The prepolymers were characterized by proton nuclear magnetic resonance (^{1}H NMR) and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Rheology testing, rate of degradation, cell viability and adhesion studies have been conducted on these hydrogels.
Results / Discussion: The central bifunctional PEG block has a MM of 4 kDa that forms the basis for the hydrated matrix. Blocks of PGA with a degree of polymerization (DP ≈ 5) were copolymerized from both ends of PEG to provide hydrolysable linkages. Finally, each end of the macromer (4KG5) was reacted with acryloyl chloride, methacrylic anhydride, or 2-isocyanatoethyl methacrylate (Figure 1). These vinyl groups provide rapid gelation by containing four sites for cross-linking on each macromer. The gels degrade upon hydrolysis through a bulk mechanism into PEG, α-hydroxy acid, and oligo(acrylic acid).
Combined analyses of ^{1}H NMR and MALDI-TOF MS confirmed the formation of prepolymers of high conversion (> 81%), high degree of purity, and narrow mass distribution (PD < 1.03). The shear moduli of hydrogels were measured using a stress controlled rheometer.

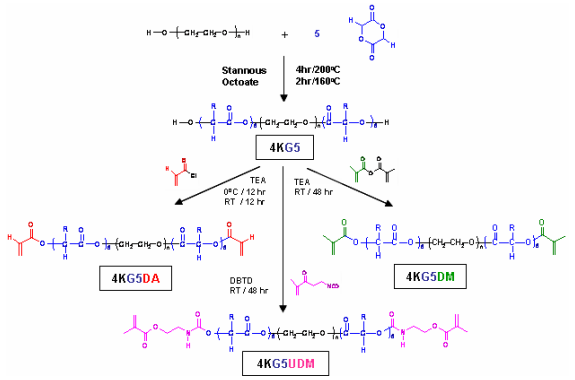

Figure 1. Synthesis of 4KG5DA, 4KG5DM, and 4KG5UDM macromers.

As shown in figure 2, shear modulus of hydrogels prepared using various end group chemistries and mass fractions, exhibit a greater mechanical strength for the 20% weight hydrogels compared to the 10% weight hydrogels due to higher degree of cross-linking.

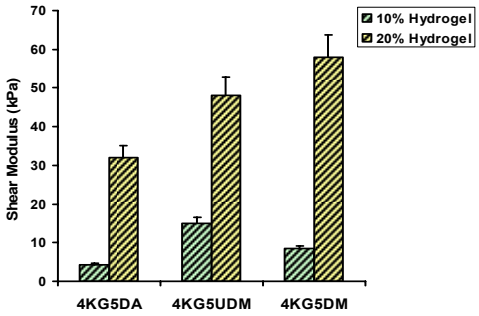

Figure 2. Shear Modulus of hydrogels prepared using various 4KG5 end groups and at various mass fractions.

The shear modulus is also affected by the nature of the vinyl groups. We notice significant differences between the 4KG5AC, 4KG5DM, and 4KG5UDM for both mass fractions. Time for total degradation of these gels at pH ≈ 7.4 varied from 1 day up to 3 weeks, depending on the polymer concentration and the terminal cross-linker used. Mouse fibroblast cells seeded in hydrogels showed that cells were completely viable, proliferating, and adhering on the three type hydrogels after 5 days.
Conclusions: Water-soluble and bioerodible PEG-based macromers were synthesized by initiating ring opening polymerization of PGA using α- and ω- hydroxyl terminal groups of PEG. Materials undergo rapid gelation under mild conditions. Physical properties and degradation rates of the hydrogels can be controlled by choosing appropriate photopolymerizable end groups and by varying macromers mass fractions. These types of hydrogels can be used for DNA delivery in a sustained and controlled manner.
References:
[1] D.S. Muggli et al. *Macromolecules* **1998**, 31, 4120-4125.
[2] K.A. Davis et al. *Biomaterials* **2003**, 24, 2485-2495.
[3] A.S. Sawhney et al. *Macromolecules* **1993**, 26, 581-587.