Amphiphilic PEG-4 Arm Maleimide—Pluronic-Dithiol Click-Linked Hydrogels

Stephen W. Linderman[1,2], Andrés J. García[2]
[1]Emory University Division of Cardiology, [2]Georgia Institute of Technology School of Mechanical Engineering.

Statement of Purpose:
Hydrogels are widely used for drug delivery and tissue engineering applications, but extreme swelling especially upon degradation limits clinical applications. When implanted in tight surgical spaces, gel swelling creates pressure on surrounding tissue that can cause pain and tissue necrosis.[1] Hydrogel stiffness also decreases with swelling, limiting utility. Amphiphilic hydrogel networks can minimize swelling.[2] Here, we evaluate the effects of thiolated PEG-PPG-PEG (Pluronic) crosslinkers in well characterized PEG-4 arm maleimide (PEG-4Mal) networks on swelling and hydrogel stiffness across PEG-4Mal molecular weights (MW) and concentrations.

Methods and Materials:
A thiolated version of Pluronic L64 (Plu2900DT; PEG-PPG-PEG, 40 wt % PEG, molar mass = 2900 g mol^{-1}, Sigma-Aldrich) was custom synthesized (Nanocs, Boston, MA), directly replacing terminal hydroxyl groups with sulfhydryl groups. This amphiphilic molecule is not soluble in aqueous solutions, but dissolves readily in dimethyl sulfoxide (DMSO). PEG-4Mal 5 kDa and 10 kDa at 5 or 10% w/v in 25 mM HEPES buffer were crosslinked with Plu2900DT in DMSO (60:40 mixing fraction, 20 µL total), at 1:1 maleimide:thiol ratios. A variety of casting temperatures and aqueous component pH ranges were evaluated, with most consistent gels at 24 hours at pH 7.0, room temperature. Serial gel weights were obtained daily (n = 4, after casting, swelling in PBS, lyophilizing, and reswelling), followed by rheology in each test state (1% shear strain, linear range at angular frequency 3-6 rad/s).

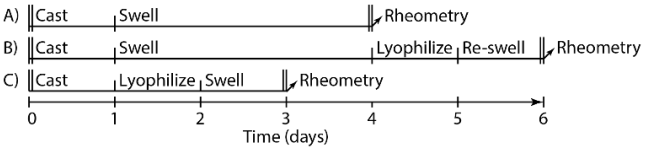

Figure 1: Study design for gel swelling and rheology.

Results:
Unlike PEG-4Mal with hydrophilic thiolated crosslinkers, which crosslink within minutes, amphiphilic hydrogels with Plu2900DT required >16 hours to crosslink at room temperature and pH 7. There was not a significant difference in gelation timing with different pH ranges, and gelation was slower at 37 °C or 4 °C than room temperature.

Amphiphilic hydrogels of 5 kDa PEG-4Mal crosslinked with Plu2900DT swelled 40-fold and 24-fold compared to lyophilized weight for 5 and 10% w/v gels, respectively (Figure 2). After lyophilization and reswelling, gels swelled 12- and 10-fold for 5% and 10% w/v, 5kDa gels, respectively. Storage modulus increased 1 to 2 orders of magnitude from the swollen state to the lyophilized and reswollen state (Figure 3). Gels with 5 kDa PEG-4Mal were stiffer than gels with longer polymer arms (10 kDa), especially after lyophilization.

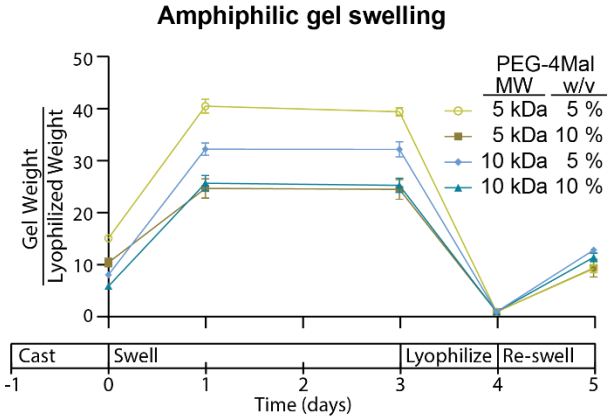

Figure 2: Gel swelling for 5 and 10% w/v, 5 and 10 kDa PEG-4arm maleimide crosslinked with amphiphilic, dithiolated Pluronic L-64.

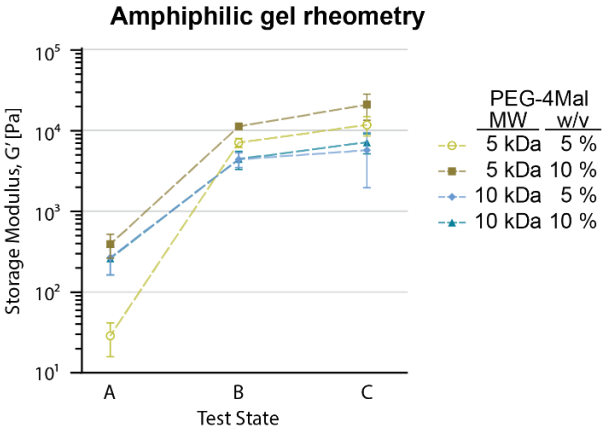

Figure 3: Storage and loss modulus from rheological testing in each gel test state (as defined in Figure 1).

Conclusions:
PEG-4Mal and Plu2900DT form amphiphilic hydrogel networks with tunable mechanical properties based on PEG-4Mal polymer size and weight percent. Lyophilization leads to increased gel stiffness and decreased swelling, possibly from annealing of the polymer arms and tighter clustering of hydrophobic segments. After lyophilization and reswelling, amphiphilic gels swell much less than typical hydrophilic gels, which swell ~20- to 30-fold. Future work evaluating long-term degradation is expected to show reduced swelling with degradation compared to hydrophilic gels due to the incorporated hydrophobic PPG moiety.

References:
1. Kamata H. Adv Healthc Mater. 2015; 4(16):2360-74.
2. Truong VX. Biomacromolecules. 2017; 18(3):757-766.