

In Situ Formation of Copolymeric Hydrogels as Vitreous Substitutes

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Statement of Purpose: The vitreous humor is a natural polymeric hydrogel composed of hyaluronic acid, collagen, and 98-99% water¹. It functions as a viscoelastic damper during eye movements and ensures attachment of the retina to the retinal pigment epithelium. The vitreous gradually liquefies with age, leading to a number of vision-threatening phenomena such as macular holes, retinal detachments, and vitreous hemorrhages². Currently, gases or silicone oil are used as temporary vitreous substitutes during surgery. However, these substitutes require the patients to position themselves face-down for weeks. Additionally, silicone oil is difficult to remove, is toxic to intraocular structures, and is associated with glaucoma and corneal decompensation, which can lead to blindness³. In recent years, the use of polymeric hydrogels as longer term vitreous substitutes has become the focus of research. We have designed water-soluble copolymers that form a hydrogel *in situ* under physiological conditions. The use of a disulfide crosslinker enables gel reduction and purification before regelation. The polymer formulation has been modified and rheological properties and regelation kinetics have been determined.

Methods: Six-month-old porcine eyes were obtained from a local abattoir (Weyhaupt, Belleville, IL). All reagents were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Acrylamide (AAm), N,N'-bis(acryloyl)cystamine (BAC), and N-phenylacrylamide (NPA) were copolymerized at acrylic mole ratios of 92.5/4.5/3.0 (AB4.5N3.0) and 92.5/3.0/4.5 (AB3.0N4.5). The hydrogel preparation, reductive liquefaction with dithiothreitol (DTT), and precipitation in methanol have been previously reported⁴. Both polymer formulations were prepared for regelation in DPBS at concentrations (w/w) of 2% and 3%. The 3% solutions were added to 18 mm Spectra/Por dialysis tubing with molecular weight cutoff 3500 Da and were equilibrated in DPBS solution at controlled oxygen volume fractions of 0%, 5%, and 20% to monitor the rate of gelation. Viscoelastic properties of porcine vitreous and hydrogels were determined using a Vilastic-3 oscillatory capillary rheometer (Austin, TX).

Results/Discussion: The 2% and 3% AB4.5N3.0 formulations regelled successfully *in vitro*. The AB3.0N4.5 formulation regelled at 3%, but remained a liquid at 2% concentration. In dialysis tubing, the AB4.5N3.0 solution gelled in 30 minutes at 20% oxygen and 1 hour at 5% oxygen. AB3.0N4.5 gelled in 2 hours at 20% oxygen and failed to gel at 5% oxygen. Both formulations failed to gel in the absence of oxygen.

Figures 1 and 2 compare the storage and loss moduli of the hydrogel formulations with that of the porcine vitreous humor. Higher storage modulus than loss modulus indicates viscoelastic solid, or gel-like, behavior. The porcine vitreous and three of the four gel formulations act as viscoelastic solids. Higher crosslinker concentration and polymer concentration produce stronger, more elastic gels. The concentration of crosslinker greatly impacts gelation kinetics.

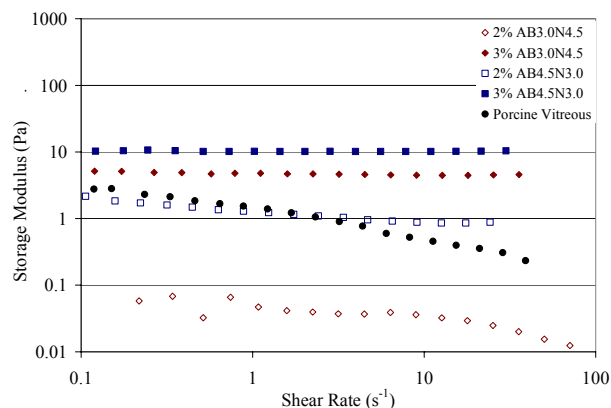


Figure 1. Storage Modulus at Frequency 2 Hz

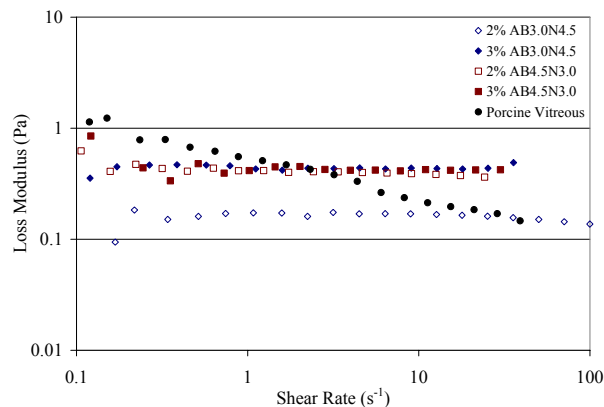


Figure 2. Loss Modulus at Frequency 2 Hz

Conclusions: The 2% AB4.5N3.0 and 3% AB3.0N4.5 formulations closely mimic the viscoelastic properties of the porcine vitreous. The AB4.5N3.0 formulation gelled at 5% oxygen concentration, indicating that it is a good candidate for *in situ* regelation. Future work will evaluate this formulation *in vivo*.

References:

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