Development of a Biomimetic Vitreous Substitute

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Statement of Purpose: The vitreous is a tenuous hydrogel composed principally of water and containing a small amount of solids consisting of a collagen network (rigid, helical and fibrillar) interspersed with hyaluronic acid (flexible, negatively charged, and randomly coiled). Silicone oils and perfluorocarbons are currently used as vitreous substitutes and are accompanied with many complications. We are developing a biomimetic vitreous substitute. For analogues of collagen and hyaluronic acid, we have chosen gellan, a rigid bacterial polysaccharide, and synthetic copolymethacrylate, an ionic flexible chain. These analogs, when endowed with thiol side groups and injected into the vitreous cavity, form a hydrogel with physical bonds by an ionic temperature transition followed by a gradual oxidation and formation of chemical bonds. The process results in a composite hydrogel with properties similar to the natural vitreous.

Methods: All chemicals were purchased from Sigma-Aldrich. The first component, Gellan was chemically-modified by amidation of the carboxyl group with cystamine [Du, Ravi, 2012]. The second component, a synthetic copolymer, was obtained by free-radical copolymerization of methacrylamide 78% (MAm), methacrylic acid 20% (MAa), and bis-methacryloylcystamine 2% (BMAC) cross-linker. Synthesis and characterization has been described elsewhere [Du, Ravi 2010]. The hydrogels were subsequently reduced and the copolymers were purified and characterized using GPC with light-scattering-viscometry and RI detectors, and capillary rheometry. Thermal scans for determining the transition temperature were performed on a Vilastic-3 Capillary Rheometer (Vilastic Scientific, Inc.) at a shear strain of 2%, and a frequency of 2Hz. Gellan-SH and reduced MAm:MAa co-polymer materials were prepared separately and warmed in a water bath at 60^{o}C for 15 minutes. Materials were mixed to give the desired final concentrations and immediately added to the preheated rheometer. Scans were carried out from 55^{o}C to 25^{o}C and were cooled at a rate of ~1^{o}C every 45 seconds, with one point being collected per degree. Frequency scans shown were performed at 5% shear strain at 37^{o}C. In vitro biocompatibility was performed by growing ARPE 19 retinal pigment epithelial cells on Millicell inserts as described by Wu et al. [2007].

Results: Figure 1 illustrates the change in transition temperature of gellan with and without –SH modification and the composite mixture of SH-gellan and MAm:MAa copolymer gels by thermal scan in the presence of PBS. Cystamine modification of gellan at 11% lowers the gelling temperature of the SH-gellan to below the range of the temperature scan, but when combined with the MAm:MAa copolymer, the composite material transitions to gel rapidly at 40-39^{o}C. The moduli of the gels are in the range of ~10 Pa, the RI is 1.335. In Figure 2, the in vitro biocompatibility shows no apparent toxic effects, indicating that there was no toxic material diffusing out of the hydrogels. The composite gel that was in the presence of the growing cells for three weeks was still well formed as demonstrated by a frequency scan of gels showing the storage modulus, indicating minimal swelling and negligible decomposition.

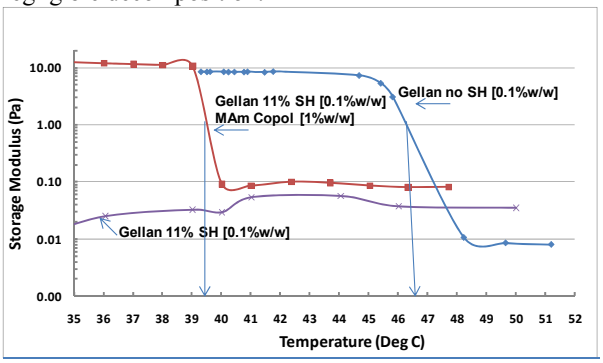

Figure 1. Gellan without –SH modification has a transition temperature of ~48^{o}C. Increased modification of gellan and incorporation of cystamine at 11% cross-linker lowers the transition temperature of the gellan-SH alone (below 35^{o}C) and the composite mixture to 40-39^{o}C , which is the target temperature that we are seeking. The storage modulus is close to the target range of ~10 Pa.

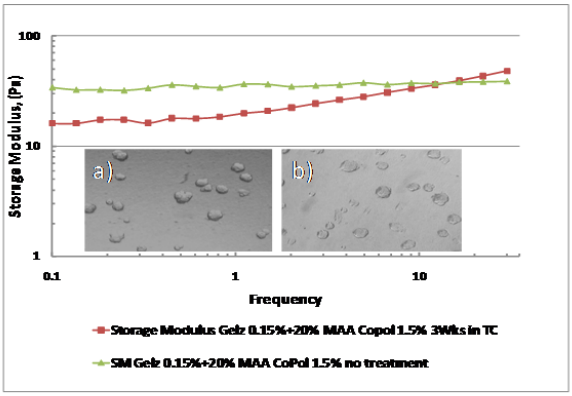

Figure 2. Hydrogels were formed in 24 well tissue culture trays and ARPE-19 cell were grown in the presence of the hydrogels on the apical side of microporous (0.4uM) membranes, Millicell-CM (teflon) inserts. 12 mm inserts were coated with collagen to cause the cause the cells to adhere. This setup allows for diffusion and for the gel and cells to be in close proximity. It was found that the cells formed colonies. Pictures were taken after 3 weeks of growth. a) Control media b) GellanS-S+20%MAA copolymer composite hydrogel. Frequency scan showing the storage modulus indicates that the composite gel was still well formed and did not degrade in the presence of the growing cells.

Conclusions: Our biomimetic hydrogel has its viscoelastic, optical, and physical properties similar to the natural vitreous. It also shows in-vitro biocompatibility. Rabbit studies are planned.

References:

Du H. Macromol Biosci. 2012;12(7):952-61.
Du H. Polym.Prepr. 2010; 55-2:408.
Wu H-J. Graefe's Arch. Clin. Exp. Ophthal. 2007; 245: 135-142.