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Biomimetic hydrogel with tunable mechanical properties for vitreous substitutes

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A B S T R A C T

The vitreous humor of the eye is a biological hydrogel principally composed of collagen fibers interspersed with hyaluronic acid. Certain pathological conditions necessitate its removal and replacement. Current substitutes, like silicone oils and perfluorocarbons, are not biomimetic and have known complications. In this study, we have developed an *in situ* forming two-component biomimetic hydrogel with tunable mechanical and osmotic properties. The components are gellan, an analogue of collagen, and poly(methacrylamide-co-methacrylate), an analogue of hyaluronic acid; both endowed with thiol side groups. We used response surface methodology to consider seventeen possible hydrogels to determine how each component affects the optical, mechanical, sol-gel transition temperature and swelling properties. The optical and physical properties of the hydrogels were similar to vitreous. The shear storage moduli ranged from 3 to 358 Pa at 1 Hz and sol-gel transition temperatures from 35.5 to 43 °C. The hydrogel had the ability to remain swollen without degradation for four weeks *in vitro*. Three hydrogels were tested for biocompatibility on primary porcine retinal pigment epithelial cells, human retinal pigment epithelial cells, and fibroblast (3T3/NIH) cells, by electric cell-substrate impedance sensing system. The two-component hydrogels allowed for the tuning and optimizing of mechanical, swelling, and transition temperature to obtain three biocompatible hydrogels with properties similar to the vitreous. Future studies include testing of the optimized hydrogels in animal models for use as a long-term substitute, whose preliminary results are mentioned.

Statement of Significance

Although hydrogels are researched as long-term vitreous substitute, none have advanced sufficiently to reach clinical application. Our work focuses on the development of a novel two component *in situ* forming hydrogel that bio-mimic the natural vitreous. Our thiol-containing copolymers can be injected as an aqueous solution into the vitreous cavity wherein, at physiological temperature, the rigid component will instantaneously form a physical gel imbedding the random coil copolymer. Upon subsequent oxidation, the two components will form disulfide cross-links and a stable reversible hydrogel capable of providing osmotic pressure to reattach the retina. It may be left in the eye permanently or easily removed by injection of a simple reducing agent to cleave the disulfide bonds, rather than surgery. This contribution is significant because it is expected to provide patients with a much better quality of life by improving surgical outcomes, creating much less post-operative burden, and reducing the need for secondary surgeries.

1. Introduction

Vitreous humor is a clear, jelly-like structure that occupies two-thirds of the posterior segment of the human eye (by volume) [1]. It is transparent to visual light, has a refractive index (RI) of

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1.3345–1.3348 and has a density of 1.0053–1.0089. It is a virtually acellular, highly hydrated, extracellular gel matrix primarily composed of bound and free water, with less than 1% (w) of collagen and hyaluronic acid (HA). Collagen (type II) is an abundant vitreous structural protein with a rigid-rod-like triple helix structure that forms scaffold-like network in the vitreous. HA is a disaccharide polymer with a randomly coiled structure in solution, which stabilizes the collagen network and exerts an osmotic pressure that holds the retina in its position [2]. Collagen and HA form an interpenetrating network that behaves as a visco-elastic solid [3] and acts as a shock absorber, dampening the intra-ocular motions and vibrations.

Ocular trauma and various ocular diseases require the removal of vitreous gel that is replaced with a substitute. Air, balanced salt solutions, perfluorocarbons (PFC), expansile gases, and silicone oils are used as replacements based on the clinical need [4]. Expansile PFC gases are used as short-term substitutes for post-operative endo-tamponade and PFC liquids as intra-surgery tool to temporarily flatten the retina, which are exchanged for long-term substitutes. Silicone oil is clinically accepted for short- or long-term tamponade to treat complex retinal detachments. However, there are various limitations with each substitute such as expansion of gases at high altitudes, the toxic nature of PFCs, elevated intraocular pressure, and lipophilic nature, emulsification and post-vitrectomic complications, particularly with the use of silicone oil, which requires a second surgery for its removal [5–7]. In addition, current vitreous substitutes that work by surface tension and pneumatic pressure to hold the retina in its position alter the refractive error of the eye, and cause significant patient burden. Hence, there is a need for long-term vitreous substitute. Our approach is to develop an *in situ* forming substitute that mimics the natural vitreous and in the process, enables us to gain more insight into the structure and physiology of the natural vitreous. Furthermore, a biomimetic gel will expand our knowledge of the role of the vitreous structure and its relation to its properties.

Several *in situ* forming hydrogels have been proposed as vitreous substitutes, including HA [8,9], polyvinylalcohol [10], polyethylene glycol [11–13], and polyacrylamide (PAM) [14,15]. An *in situ* forming hydrogel has several substantial benefits other than the physical and optical properties similar to native vitreous; it is easily injected into the vitreous cavity without any modification of the current vitrectomy procedure, and it does not shear degrade during the injection, a process which causes fragmentation of the pre-formed gel and introduces free radicals into the cavity. Our laboratory has achieved a reversible *in situ* forming PAM hydrogel using thiol cross-linker, which holds potential as a long-term vitreous substitute [15]. The thiol groups in polymer chains, upon oxidation, establish disulfide bonds that can be reduced back to thiols by dithiotheritol (DTT) *ex vivo* or with glutathione *in vivo*. The reversibility potentially eliminates the need for a second surgery in case the hydrogel needs to be removed.

The current work significantly extends our previous work on the use of co-polyacrylamide [15]. Here, we reverse-engineered the critical elements of the natural vitreous (collagen and HA) and consequently have focused on the development of a two-component (rigid and random coil) *in situ* forming hydrogel that mimics the natural vitreous with tunable osmotic/swelling pressure and other mechanical properties. The rigid component of our hydrogel, Gellan, is a bio-polysaccharide, which undergoes sol-gel transition at certain temperature and forms a physical gel almost instantaneously. This transition also occurs in the presence of mono/divalent cations. It is a stiff-biopolymer that has helical conformation at low temperature and swells minimally, analogues to collagen. In addition, it is used in ophthalmic applications [8,16]. On the other hand, the poly(methacrylamide-co-methacrylate-co-*N′*,*N′*-bis(methylacryloyl-cystamine)) (poly(MAM-co-MAA-co-BMAC) is a random coil, ionic polyelectrolyte, that allows for variation in the swelling (osmotic pressure) and gelling properties of the hydrogels [17], analogues to HA at a mesoscopic level. Nakagawa [18] observed that a simple combination of collagen and HA does not last long as a vitreous substitute as they diffuse out of the vitreous cavity. To overcome this obstacle, we modified the components of our hydrogel by introducing thiol (-SH) side groups that can form chemical cross-links (S-S) upon physiological oxidation. Our polymer solution can be safely and easily injected into the vitreous cavity at 42 °C that will rapidly form a physically-crosslinked hydrogel instantaneously as it cools to body temperature. The thiol groups will oxidize over time to form a reversible chemically crosslinked hydrogel capable of generating osmotic pressure to keep the retina in its position (Fig. 1). In addition, the properties of the hydrogel are tunable with change in concentration of each component. To ascertain the composition of this two-component hydrogel that can mimic the properties of natural vitreous, we evaluated the mechanical, optical, physical, transition

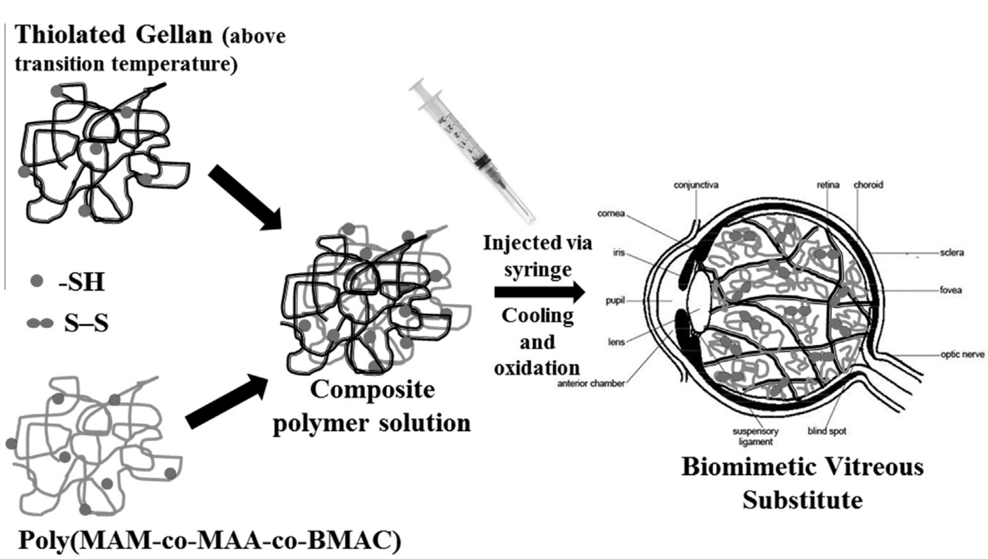

Fig. 1. Schematic representation of our two-component polymer solution injected via syringe into the vitreous cavity, which forms an instantaneous physically crosslinked hydrogel upon cooling and a chemically crosslinked network with established disulfide bonds on oxidation.