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Hydrogel Nanocomposite as a Synthetic Intra-Ocular Lens Capable of Accommodation

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Summary: A hydrogel nanocomposite was designed, synthesized, and evaluated for use as an auto-focusing intra-ocular lens. The hydrogel scaffold was composed of a monomer-free, thiol that contained polyacrylamide (5%), which was allowed to gel in the presence of nanoparticles at pH 7.4, 25^o C. The nanoparticles consisted of a proteo-mimetic polyacrylamide nanogel (~42 nm), bovine serum albumin (BSA) (~6 nm), and hydrophilized silica (~3 nm). The extent of nanoparticle loading increased with decreasing particle size. The elastic modulus increased with increasing loading of the proteo-mimetic nanogels and BSA, and it decreased with hydrophilized silica. In this investigation, the hydrogel that contained silica was the most promising class of nanocomposite hydrogels with properties comparable to that of a young porcine lens. A nanocomposite that consisted of 10% hydrogel scaffold and 24% hydrophilized silica (elastic [E] modulus of ~1.0 kPa and refractive index [RI] of 1.42) was injected into a pre-evacuated porcine lens capsular bag. The composite lens was evaluated in a custom-designed four-arm radial stretcher, and its force-time spectrum was characterized by time constants of 60 ± 8.9 and 800 ± 32 ms. These results were comparable to a young porcine lens (E Modulus of 1.2 kPa; RI of 1.4105; time constants of 48.3 ± 0.58 and 668 ± 24.6 ms, respectively)

Keywords: accommodation; hydrogel; intra-ocular lens; nanocomposite; nanogels

Introduction

More than one million cataract surgeries are performed each year in the United States alone, and in almost all cases, the cataractous lens is replaced with synthetic intra-ocular lens (IOL). Most of the intraocular lenses are significantly thinner, have a higher modulus, are more transparent than the natural lens, and are typically located in the pre-evacuated lens capsular bag. They are all derivatives of silicones or acrylates, and their typical shapes and designs are shown in Figure 1. All of these intra-ocular lenses are

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prefabricated with varying refractive powers, with each IOL having a single focal length. Generally, because these lenses are incapable of changing focus, most patients are usually fitted with an IOL that enables them to see clearly at near distances, and are dispensed with bifocals.

Accommodation is the auto-focus mechanism of our visual system (Figure 2). This dynamic process is almost instantaneous and imperceptible when young, but its speed slows down and its amplitude decreases with increasing age. It is significantly reduced by the fourth decade and almost completely lost by the seventh decade of life, resulting in the inability to focus at arm's length[1-3] Although the causes of this condition, clinically referred to as *presbyopia,* are multi-factorial,[3-5] the progressive hardening of the lens seems to play an important role. Refilling the evacuated lens capsular bag with a transparent pliable material offers a potential surgical approach to restore the much needed accommodation. However, this approach has been met with only marginal success because of, among other things, the technical challenges in identifying a suitable material. [6-8] The ideal IOL material, in addition to having the optical, mechanical, and physical properties of the natural lens as listed in Table 1, should be easily injectable and dimensionally stable once formed within the lens capsular bag.

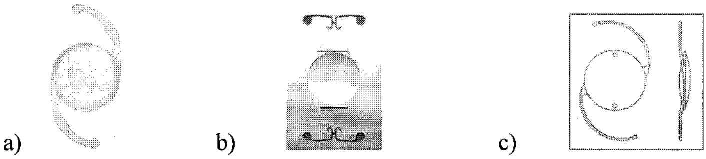


Figure 1. Examples of intra-ocular lenses. a) Alcon (soft flexible acrylate); b) Enovision (hinged silicone); c) Eagle Optics (rigid polymethylmethacrylate).

Along this line of reasoning, several *in-situ*–forming hydrogels have been attempted.[6-10] For example, Murthy *et al*[9] used polyethylene-glycol acrylates to form an *in-situ* hydrogel. De Groot *et al* reported [6,7] the endocapsular photopolymerization of an acrylate-modified poly(N-vinylpyrrolidone/vinyl-alcohol)-copolymer. Aliyar *et al*[10] used a monomer-free, thiol-containing polyacrylamide solution that gelled spontaneously under physiological conditions. In all of these situations, if the modulus was in the correct range, then the refractive index (RI) was too low, or if the RI was correct, the modulus was too high when compared with the natural lens. In retrospect, this technical difficulty is well explained by network theories.[11,12] For a hydrogel formed from a homogeneous linear water-soluble polymer, the RI scales linearly with polymer concentration, whereas the modulus usually scales exponentially. Consequently, for most common hydrogels, an RI of 1.42 and a modulus of 1.2 kPa seem to be mutually exclusive.

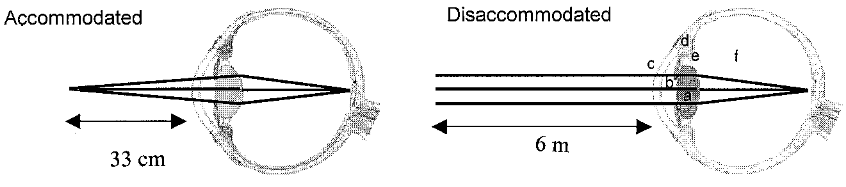


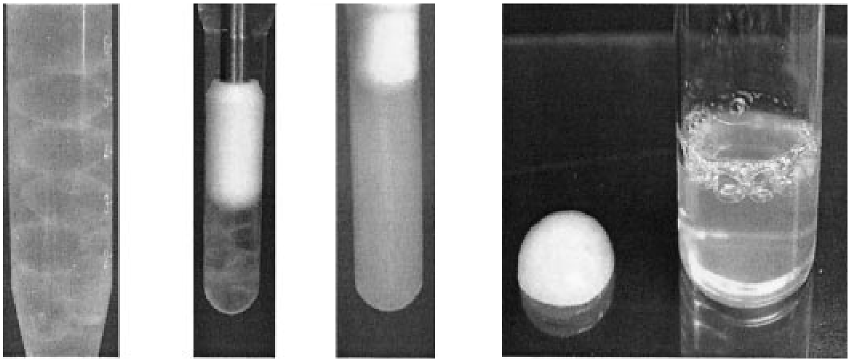
Figure 2. Accommodation of lens. a) Lens fibers; b) aqueous humor; c) cornea; d) ciliary muscles; e) zonules; f) vitreous. The lens is considered to be in the accommodated state when the object of regard is within 33 cm and in the disaccommodated state when it views object beyond 6 meters.

Table 1. Physical properties of natural porcine lens as determined in our laboratory.

Parameter	Values
Refractive index	1.405
Specific gravity	1.09
Transmission	0.95
Elastic modulus	1.2 ± 0.1 kPa
Relaxation time constants	50-500 ms

In an attempt to resolve this situation, we examined how nature solved this predicament within its limited arsenal of chemical compounds. To fully appreciate this, we briefly explain the salient feature of the lens. The lens is a biconvex aspherical structure that is composed of an outer capsular collagenous membrane. Within this membrane are hexagonal–shaped lens fibers that are long, numerous, closely packed, and well organized. These fibers typically arch from the anterior polar axis lens (or suture) to the posterior polar axis through the equator. The central lens fibers are devoid of any organelles to avoid scattering. Within each lens fiber the cytoplasm is filled with a protein-rich lens crystallin solution. The lens is composed of approximately 60% to 65% water. The cell-membrane lipids and proteins, the cytoskeletal elements, and the cytoplasmic lens crystallin proteins constitute the solids. The modulus of the lens substance is around 1.2 kPa and shows solid-like viscoelastic properties. On homogenization and centrifugation of the lens fibers, the components can be easily separated into a water-soluble, crystallin-rich fraction and a small soft pellet that contains lipids, membrane proteins, and cytoskeletal elements (Figure 3). The consistency of the crystallin protein solution, which represents the bulk of the solid, is almost similar to that of water, but with an RI of ~1.405. The pellet, if reconstituted to its original concentration in the lens, re-forms a gel and has a low RI. It therefore occurred to us that the way nature has circumvented the ideal polymer-network physics is by developing a two-component system: one component primarily provides the mechanical property and the other primarily provides the RI.

In this context, we report our novel approach for reverse engineering the lens to preserve accommodation. We have now prepared and characterized a monomer-free, *in-situ*–forming hydrogel scaffold that provides the elastic modulus and within which is contained nanoparticles that provide the necessary RI. This composite system can now be easily formulated to match the RI and modulus of the natural lens.



a) b) c) d)

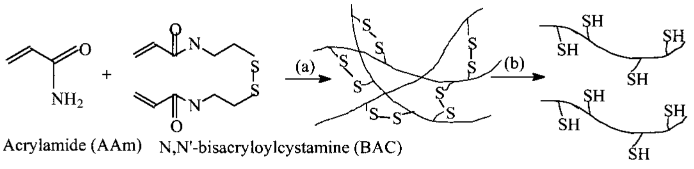
Figure 3. Lens fiber structures before and after homogenization. a), b) and c) Homogenization process. d) Separated components of lens fibers into soft pellets that contain lipids, membrane proteins, and cytoskeletal elements; and water-soluble, crystallin-rich solution.

Experimental Methods

Materials: All the chemicals were purchased from Sigma-Aldrich Chemical Co in St. Louis, Missouri and were used without further purification. All the other reagents used were of analytical grade.

Preparation and characterization of the monomer-free hydrogel scaffold: Synthesis and characterization of the hydrogel scaffold is explained in detail in our prior publication.[10] Briefly, copolymerization of acrylamide (AAm) and bisacryloylcystamine

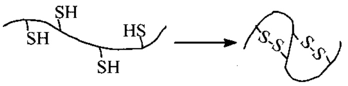
(BAC) was carried out at acrylic mole ratios of 96/4 at 5 % (w/w) monomer concentration in 25% ethanol (25:75 = ethanol:water v/v) (Scheme 1). The excipients in the hydrogel were removed through repeated washings, and the purified hydrogel was reduced with dithiothreitol (DTT), followed by precipitation in methanol to yield a water-soluble thiol-containing polymer (AB4SH). The polymer was characterized[10] by Raman spectroscopy, gel permeation chromatography (GPC) connected in series with an RI, viscosity, and laser-light scattering detectors, and the thiol content was determined with Ellman's analysis.[13]



Scheme 1. Schematic representation for preparation and liquefaction of copolymeric gel to yield AB4SH polymer. a) Copolymerization with ammonium persulfate; b) Reductive liquefaction of the gel followed by precipitation in methanol, filtration, and vacuum drying to get AB4SH polymer.

Preparation of nanoparticles: Three different types of nanoparticles were used in the preparation of the nanocomposite hydrogel. They consisted of non-degradable polyacrylamide proteo-mimetic nanogel (~42 nm), bovine serum albumin (BSA, ~6 nm), and hydrophilized silica (~3 nm). They are described as follows:

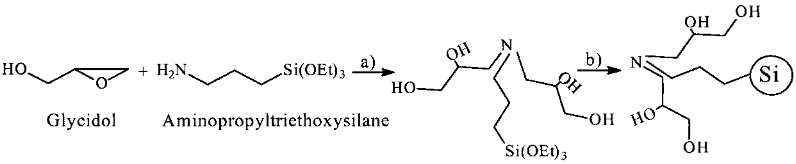
a) Nanogel from AB4SH thiol polymer: A large volume of 0.1% w/v of AB4SH thiol containing copolymer solution was dissolved in water at pH 4, and prior to oxidation, the pH was adjusted to 7.4 with a small amount of 1M NaOH and then bubbled with air for 3 days. Upon confirming the absence of the –SH by Ellman's analysis,[13] the solution was concentrated to 25% w/w. The nanoparticle (solution) was labeled as NP-AB4SS. A detailed information about the preparation and characterization of the proteo-mimetic nanogel was reported in our previous publications.[14-16]



Scheme 2. Thiol-containing polyacrylamide was cross-linked by air oxidation under dilute conditions to primarily favor intra-molecular disulfide bond formation.

b) Bovine serum albumin (BSA): A 30% w/w solution of BSA in buffer (20 mM Bis-Tris, pH 6.0, 0.1% sodium azide (NaN_3) was prepared. This solution was used in the hydrogel nanocomposite materials at different concentrations.

c) Silica: Water-soluble silica nanoparticles were prepared as reported by Mori *et al.*[17] The diameter of the nanoparticles was verified by TEM and GPC to be between 3-5 nm. A stock solution of 65% w/w was prepared and used in the nanocomposites at different concentrations. The reaction scheme is shown below (Scheme 3).



Scheme 3. Schematic representation for preparation of silica nanoparticles through a) addition and b) acidic condensation reactions.

Preparation of the nanocomposites: Initially, a 15% w/w solution of AB4SH in nitrogen-saturated water was prepared at pH 4. This solution was mixed with various nanoparticles at different concentrations. For comparison among the three nanoparticles, the concentration of the polymer scaffold (AB4SH) was kept constant at 5% w/w. The concentration of the nanoparticles was varied from 0 to 36% w/w as shown in Table 2. An additional composition, 10% AB4SH with 25% hydrophilized silica, was used in the silica nanocomposite. This formulation was used to fill the lens capsular bag. To mimic the radial stretch of the ciliary body, we developed a custom multi-arm stretcher as described in the next paragraph. The relaxation time constants were determined with this multi-arm stretcher. The pH of the composite was adjusted to ~7 with 1 M NaOH just before the re-gelation. The nanocomposites were re-gelled with dithiodipropionic acid (DTDP) in equimolar amounts of the thiol content in the AB4SH polymer. The composition and concentration of polymer and nanoparticles are shown in the Table 2.

Determination of optical and mechanical properties of nanocomposites: The re-gelation of the composite materials was carried out in a cylindrical Teflon mold (10 mm diameter and 5 mm height). The mechanical properties of these cylindrical samples were determined by compression between parallel plates using a dynamic mechanical analyzer (DMA 7e, Perkin Elmer, Norwalk, CT, USA) from 0-25 mN at the rate of 1mN/min. The force-time spectra shown in Figure 4 were obtained by pushing the lens, using a custom-designed four-arm stretcher/pusher that mimics the ciliary muscles of the eye.[18] From previous experiments, the decay of force by pulling or pushing the lens at its equator, produced equivalent time constants. From the force-time spectra, the time constants were determined by fitting to exponentials using the following equation.

$$f_t = f_1 e^{-t/\tau_1} + f_2 e^{-t/\tau_2} + f_{Eq}$$

where, f_t = total force at any given time; τ_1, τ_2 = time constants corresponding to f_1, f_2 and f_{Eq} = force reached at equilibrium. The refractive indexes of the re-gelled composite materials were determined using an Abbe refractometer (ATAGO's Abbe refractometer 1T/4T, Kirkland, WA, USA).

Results and Discussion

The ocular tissue is exquisitely sensitive even to trace chemicals. Consequently, *in-vivo* free radical polymerization, which seldom occurs quantitatively, is not a desirable technique. In addition, such reactions are typically exothermic, and the monomers are usually toxic. Therefore, a monomer-free gelation technique was developed.[10] Briefly, copolymerization of AAm and BAC resulted in hydrogel (AB4SS). The key step in obtaining the desired water-soluble copolymers (AB4SH) from the crosslinked gels (AB4SS) involves the complete reduction of the disulfide bonds (-S-S-) in the gels into –SH groups as shown in the Scheme 1. More details on the preparation and characterization of the similar type of thiol polymer can be seen in our previous report.[10] Reduction of the gels with DTT resulted in almost complete reduction of the disulfide bonds, as evidenced by the –SH determination. Ellman's analysis showed –SH content to be 5.1 x 10^{-4} mol/g. The calculated value is 5.4 x 10^{-4} mol/g. The molecular weight distribution analysis of AB4SH showed a broad distribution with a polydispersity of 3.4 and a weight average molecular weight (Mw) of 3.8 x 10^{5} Da.

Preparation of nanoparticles: The objective for preparing water-dispersible nanoparticles was to mimic the role of the lens crystallins, which as a first approximation serves as an RI enhancer without significantly increasing the modulus of the composite.

We used three nanoparticles: (1) Polymer-containing pendent –SH groups (AB4SH) was used to prepare the nanoparticles through primarily intramolecular crosslinking between –SH groups at very dilute concentrations utilizing "green-chemistry". While very dilute concentrations were expected to favor the nanoparticle formation by intramolecular crosslinking (Scheme 2), the concentration investigated (0.1% w/v) in this work did show some intermolecularly formed nanoparticles. The concentrated nanoparticle solution (25% w/v) was used in the preparation of nanocomposites at different concentrations. Higher concentrations were difficult to obtain. (2) BSA (30% w/w), was used as a nanoparticle solution in the nanocomposites, higher concentration resulted in an opaque composite. (3) The silica nanoparticles were prepared by the addition reaction between glycidol and aminopropyltriethoxysilane followed by the acidic condensation of the addition product through sol-gel technique as reported by Mori *et al.*[17] The particle size was reported as ~ 3 nm diameter. Because of higher hydroxyl group functionality of each silicon atom and very small size, these particles are well dispersed and behave like a dissolved molecule in water. A very high concentrated (65% w/w) solution was prepared and used in the nanocomposite hydrogel composition at different concentrations.

Optical and mechanical properties: Porcine eyes were utilized in the studies as they are obtained from pigs sacrificed at approximately 180 to 200 lbs and thus the lenses are very uniform in size, weight, specific gravity and modulus. Although the porcine lens does not accommodate, the morphology, fiber architecture and composition is evolutionarily well preserved, and similar to the human eye. The refractive index (RI) and moduli values are presented in Table 2 for all the nanocomposites. As expected, as the nanoparticle concentration increases the RI also increases in all the composites. In the composite containing NP-AB4SS, the moduli values also increased. This may be due to the interaction of the polyacrylamide backbones along the polymer chain with those of the nanogels. Thus, the nanogels may be behaving as interacting filler. It is also possible that if the pH becomes alkaline, then sulfide exchange may occur at these high concentrations, forming non-ideal networks. Refractive index showed a similar trend with the BSA hydrogel composite, that is, the RI increased with increasing concentration of BSA loading. More BSA could be loaded than the proteo-mimetic nanogels and the modulus did not increase as sharply as it did with the nanogels. At the maximum loading of 24% of BSA in a 5% hydrogel scaffold, the RI was 1.386 and the modulus was 0.46 kPa.

Interestingly, in the case of the silica nanocomposite, the modulus decreased with increasing silica. Here, the silica dimensions are about 3 nm, at the network concentration of 5% it may be smaller than the average network pore size. Both the polymer and the silica nanogels have amine groups which may carry a slight positive charge at pH 7.4 thus minimizing any attraction between the two. Thus the silica may act as a lubricant or plasticizer. It was only in the hydrogel containing silica nanocomposite that the correct combination of the RI index and modulus was obtained. In fact, the modulus was lower than 1.2 kPa. To increase the modulus, the hydrogel composition of 10% AB4SH + 25% silica was subsequently tested in a porcine lens capsular bag in a four-arm mechanical analogue of the ciliary body. The lens was allowed to accommodate by radially pushing in on the lens and the force was monitored with time. Figure 4 exhibits typical force-time spectra of the natural and the nanocomposite lens. The spectra were fitted to a double exponential decay, and correlated well with time constants of 60 ± 8.9 and 800 ± 32 ms for the nanocomposite lens. In comparison, the time constants for the natural lens were 48.3 ± 0.58 and 668 ± 24.6 ms. Figure 5 shows the photographs of the lens capsule filled with the silica nanocomposite. A natural lens is also depicted for comparison. Although preliminary work seems promising in terms of RI, modulus, relaxation times,

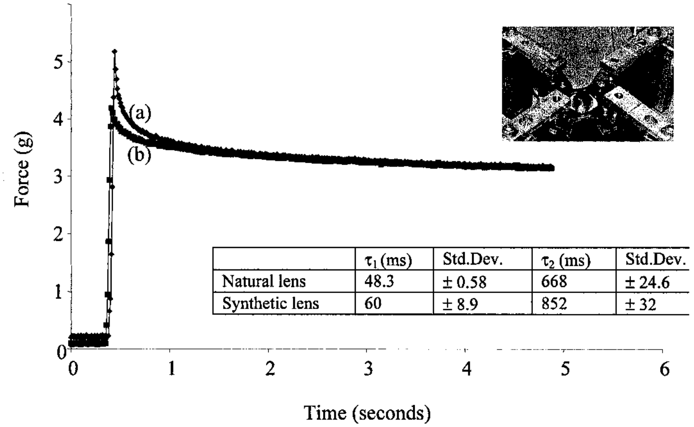
Table 2. Refractive index and moduli of nanocomposites.

Formulation type	Total solid content (% w/w)	Refractive index	Moduli (kPa)
1 (with NP-AB4SS) (~42 nm)	5	1.344	0.23 ±0.05
	11	1.348	0.47
	17	1.353	1.93 ±0.12
	23	1.358	2.69± 0.51
2 (with BSA) (~6 nm)	5	1.343	0.19 -
	11	1.352	0.29 -
	17	1.362	0.46 -
	23	1.375	0.47 -
	29	1.386	0.46 -
3 (with silica) (~3 nm)	5	1.346	0.49 -
	17	1.379	0.47 ±0.07
	29	1.402	0.42 -
	41	1.422	0.41 ±0.02
	*35	1.410	-

Due to insufficient quantity of AB4SH only a few samples were performed in triplicate. The % std. dev. of modulus ranged from 2 to 19 %, and that of RI was generally less than 1.0%.

* this has 10% AB4SH with 25% hydrophilized silica, the modulus of this composition was not determined, however appeared to be lower than the regelled hydrogel from 10% of AB4SH alone, which showed the modulus of 1.1 kPa.

biocompatibility,[19,20] and has for the first time provided significant insight into the molecular architecture of the lens, many more questions need to be answered. For example, the swelling pressure inside the lens capsule needs to be determined and maintained approximately between 290-310 milli-osmoles. The lens capsule has a modulus that is three orders of magnitude greater than the lens composite and is permeable to molecules of 5-10 K Daltons. Therefore, future work will be focused on developing dimensionally stable neutral gels that contain nanoparticles in the 30-60 K Dalton range, embedded in a hydrogel scaffold. Also, various methods of regelling, particularly photochemical oxidation of thiols, as alternatives to DTDP are being investigated, and will be published shortly.



	τ_1 (ms)	Std.Dev.	τ_2 (ms)	Std.Dev.
Natural lens	48.3	± 0.58	668	± 24.6
Synthetic lens	60	± 8.9	852	± 32

Figure 4. Force-time spectra of the natural lens (a) and the nanocomposite hydrogel (b). The time constants were obtained by fitting the force to a double exponential (n=3). The in-set shows the lens in the four-armed stretcher.

a) b)

Figure 5. a) Natural lens capsules; b) Lens capsule filled with silica nanocomposite (total solid content 35% w/w).

Conclusion

Various hydrogel compositions containing nanoparticles were synthesized to mimic the optical and mechanical properties of the porcine lens. Hydrogel was prepared by the copolymerization of acrylamide and disulfide containing cross-linker. After exhaustive washing of the hydrogel, it was liquefied, precipitated in methanol and vacuum dried to yield a monomer-free copolymer containing pendant thiols. The hydrogel scaffold was prepared by utilizing the above polyacrylamide copolymer. The nanocomposite hydrogels were formed by adding proteo-mimetic nanoparticles (~42 nm), BSA (~5 nm), and hydrophilized silica (~3 nm). The extent of loading of nanoparticle increased with decreasing particle size. The modulus increased with increasing amounts of nanogels and BSA, but decreased with addition of hydrophilized silica. In this current investigation, the silica containing hydrogels exhibited properties similar to those of the porcine natural lens. The hydrogel containing 10% polyacrylamide scaffold and 25% silica exhibited a RI of 1.410 and relaxation time constants of 60 ± 8.9 and 800 ± 32 ms.

Acknowledgements

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