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## Poly(ethylene glycol)-or silicone-modified hyaluronan for contact lens wetting agent applications

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**Abstract:** Hyaluronan (HA) is a hydrophilic biopolymer that has been explored as a wetting agent in contact lens applications. In this study, HA was modified with siloxy or polyethylene glycol moieties using click chemistry to make it more soluble in monomer solutions used to synthesize model contact lens materials; unmodified HA was not soluble in the same monomer solutions. The water contents of the silicone hydrogels were not increased by the presence of modified HA, nor was there a decrease in the surface contact angle. However, modified HA did lead to a reduction in lysozyme adsorption in some cases. The leaching rate of HA modified with polyethylene glycol from a 78:22 DMA:TRIS(OH) hydrogel was significantly slower than for unmodified HA. © 2014 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 103A: 2602–2610, 2015.

**Key Words:** HA, PEG, contact lens, protein fouling, hydrogel

### INTRODUCTION

Contact lenses are used by millions of patients who require vision correction. Despite their success, it has been estimated that only 5% of the total number of patients who require vision correction use contact lenses.<sup>1</sup> The two main issues that limit the widespread use contact lenses are dry eye, which may or may not be induced by the lenses,<sup>2</sup> and protein and/or microbe adsorption.<sup>3,4</sup> Although these issues are problematic for the uptake of contact lens use, they have also been reported to affect as much as 50% of the current contact lens wearing population. Problems of dry eye can vary: in some patients, it may only cause minor discomfort, but for other patients, it can be debilitating, preventing the use of contact lenses for vision correction.<sup>2</sup> In addition to patients for whom the inability to wear lenses is an inconvenience, there are cases where a lens is necessary. For example, patients who require bandage lenses post surgery are more susceptible to dry eye and other contact lens-related issues. Unfortunately, unlike patients who use contact lenses for vision correction, bandage lenses wearers do not have the option of removing the device in the event of dry eye.<sup>5</sup> In addition to being a cause of discomfort, including contributing to dry eye, adsorption of tear proteins can lead to more significant, sight

threatening effects including microbial keratitis and giant papillary conjunctivitis.<sup>6</sup>

Contact lens-associated dry eye, and protein and lipid adsorption issues with the current generation of silicone hydrogel materials can be traced back to the hydrophobicity of the silicone phase in these materials. Silicone-based contact lenses were introduced in the late 1990s and were touted as solving the issues of conventional lenses that were currently widely used at the time. Of particular note, the increased oxygen permeability of these materials, due to the connected silicone phases, has overcome issues of corneal hypoxia<sup>7</sup>—such as corneal swelling, blurred vision and increased risk of eye infection—and allowed the development of extended wear contact lenses.<sup>8,9</sup> The introduction of silicone addressed oxygen permeability issues because incorporation of silicone increased the hydrophobicity of these lenses compared with previous generation conventional hydrogel-based lenses.

In an effort to reduce the hydrophobicity of silicone containing contact lenses to help minimize dry eye and protein adsorption, wetting agents have been introduced into the lenses, either on the surface or as internal components of the lens itself. Wetting agents, as their name suggests, are additives included in the lens to make silicone-based lenses less hydrophobic, and, therefore, less likely to promote dry eye and protein adsorption issues. Although wetting agents have somewhat improved the comfort of silicone lenses, dry eye, and protein adsorption issues are still common among the contact lens wearing community.

The approaches used to incorporate wetting agents into contact lenses are relatively simple, but there is little published research looking at using specially designed wetting agents or novel incorporation methods. One wetting agent that has recently been used, shown significant promise, and could be developed further, is hyaluronan (HA). HA is a human biopolymer found in the vitreous humor, as well as other organs and tissue throughout the body, and has been investigated in ophthalmic applications.<sup>10–17</sup> An added advantage of HA over other wetting agents is that HA has therapeutic effects on the ocular surface; therefore, not only could HA address contact lens-associated dry eye and protein adsorption, it has the potential to increase the rate of healing.<sup>18</sup> Incorporation of HA into silicone-based contact lenses has shown to increase water uptake and decrease protein adsorption,<sup>14,19,20</sup> and it has also been shown to have the potential to control release kinetics for ophthalmic drugs from silicone-based contact lenses.<sup>21</sup> Unfortunately, increasing the amount of HA incorporated into these materials has been limited due to the poor solubility of HA in contact lens monomer solutions. In addition, the hydrophilic nature of HA and the hydrophobic silicone-based monomers tends to promote phase separation during polymerization, resulting in materials that have poor transparency. Therefore, the aim of this study was to chemically modify HA to make it more soluble in the monomer solutions used to synthesize contact lenses. A previous study<sup>22</sup> examined modification of HA to make it more soluble in nonaqueous solvents, but this study focused on temporary modification for synthetic applications. Yet, to the best of the authors' knowledge, no studies have examined modification of HA to improve its solubility for contact lens wetting agent applications. Therefore, the purpose of this study was to chemically modify HA to increase its solubility in monomer solutions used to make contact lens materials, and investigate how it acts as a wetting agent in contact lenses.

## MATERIALS AND METHODS

## Materials

HA (sodium salt, 7.5 kDa and 28.5 kDa) was purchased from LifeCore Biomedical (Chaska, MN) and used as received. Sodium ascorbate, N-(3-dimethylaminopropyl)-N<sup>0</sup> ethylcarbodiimide hydrochloride (EDC), N,N<sup>0</sup>-dimethacrylamide (DMA), ethyleneglycol dimethacrylate (EGDMA), sodium azide, 4-toluenesulfonyl chloride, copper (II) sulfate, ethylenediaminetetraacetic acid (EDTA), polyethylene glycol methyl ether (M<sub>w</sub> 5 2000 Da) and inhibitor remover were all purchased from Sigma Aldrich (Oakville, ON) and used as received. 1-(Bis(trimethylsiloxy)methylsilyl)propoxy-3-methacryloxy-2-propanol (TRIS(OH)) and 3-chloropropyltris(trimethylsiloxy)silane was purchased from Gelest (Morrisville, PA). Irgacure 184, the photoinitiator (PI), was provided by BASF Corp (Vandalia, IL). Plexiglas G-UVT for acrylic molds was kindly provided by Altuglas (Bristol, PA). All monomers were passed through a column of inhibitor remover (Sigma Aldrich, Oakville, ON) prior to polymerization. The synthesis of a-azido-polyethylene glycol methyl ether (3) was carried out according to the literature.<sup>23</sup>

## Synthesis of HA derivatives

Synthesis of HA-alkyne (1a and 1b). HA (7.5 kDa: 250 mg, 0.03 mmol; ca. 0.61 mmol CO<sub>2</sub>H) and propargyl amine (300  $\mu$ L, 4.68 mmol) was dissolved in DI H<sub>2</sub>O (50 mL). Once the pH was adjusted to 4.8 with 1M HCl, EDC (2.5 g, 13.0 mmol) was added and the solution was stirred for 12 h at room temperature; every 2 h the pH was measured and adjusted to 4.8 if needed. The resulting solution was dialyzed (MWCO 5 3500 Da) against water for three days then lyophilized to give a white solid (199 mg, ca. 80%). For the synthesis of 28.5 HA-alkyne, the same procedure and molar equivalents were used. The percentage alkyne incorporation was determined using <sup>1</sup>H NMR by comparing the integrals for the proton signals from the N-acetyl at  $\delta$  1.95 to the alkyne proton signal at  $\delta$  4.00 – 4.10.

Synthesis of 3-azidopropyltris(trimethylsiloxy)silane (2). Sodium azide (2.20 g, 36.9 mmol) was added to a solution of 3-chloropropyltris(trimethylsiloxy)silane (5.0 mL, 12.3 mmol) and sodium iodide (1.35 g, 12.3 mmol) in DMF (25 mL), and the resulting solution was stirred overnight at 90 °C. The reaction was diluted with water (200 mL) and extracted with dichloromethane (3  $\times$  100 mL). The organics were collected, washed with water (3  $\times$  150 mL) to remove any residual DMF, dried over MgSO<sub>4</sub>, and evaporated under reduced pressure to give a pale yellow oil (3.52 g, 75%).

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>) 3.19 (2H, d, J 6.95 Hz, CH<sub>2</sub>AN<sub>3</sub>), 1.62 (2H, m, CH<sub>2</sub>), 0.48 (2H, m, CH<sub>2</sub>ASi), 0.08 (27H, s, CH<sub>3</sub>). <sup>13</sup>C NMR (50.3 MHz, CDCl<sub>3</sub>) 53.93 (CH<sub>2</sub>AN<sub>3</sub>), 23.19 (CH<sub>2</sub>), 11.50 (CH<sub>2</sub>ASi), 1.74 (SiACH<sub>3</sub>). FT-IR (cm<sup>-1</sup>): 2958, 2096, 1251, 1052, 840. HR-MS (ES) m/z 364.1387 [M-CH<sub>3</sub>]<sup>+</sup> (C<sub>12</sub>H<sub>33</sub>O<sub>3</sub>Si<sub>4</sub>ACH<sub>3</sub> requires 364.1249)

Synthesis of HA-si (4a and 4b). The following method was used for 7.5 and 28.5 kDa HA-Si. A solution of 2 (35 mg, 0.09 mmol) in DMF (3 mL) was added to a solution of 1a (30 mg, ca., 0.03 mmol alkyne) in H<sub>2</sub>O (1 mL). To this was added CuSO<sub>4</sub> (50  $\mu$ L from 129 mg/mL aqueous stock solution) followed by sodium ascorbate (100  $\mu$ L from 30 mg/mL aqueous stock solution). The

solution was vigorously stirred for 2 days after which time the solution was dialyzed (MWCO 5 3500 Da) against methanol for 24 h, followed by saturated EDTA solution for 2 days then H<sub>2</sub>O for 3 days, and finally lyophilized to give a white solid (20.3 mg, ca. 67%). The degree of silicone functionalization was determined using <sup>1</sup>H NMR by comparing the integrals for the proton signals from the N-acetyl at d 1.95 to the triazole proton signal at d 5 7.70 – 8.00 and the trimethylsilane signals at d 0.10 – 0.10.

Synthesis of HA-PEG (5a and 5b). The following method was used for 7.5 and 28.5 kDa HA-PEG. Sodium ascorbate (50 IL from 30 mg/mL aqueous stock solution) was added to a solution of 1 (30 mg, ca. 0.03 mmol alkyne), 3 (189 mg, 0.09 mmol), and CuSO<sub>4</sub> (50 IL from 129 mg/mL aqueous stock solution) in DI H<sub>2</sub>O (XX mL). The solution was stirred for 48 h at room temperature, dialyzed (MWCO 5 3500 Da) against saturated EDTA solution for 2 days then H<sub>2</sub>O for 3 days, and finally lyophilized to give a white solid. The degree of PEG functionalization was determined using <sup>1</sup>H NMR by comparing the integrals for the proton signals from the N-acetyl at d 1.95 to the triazole proton signal at d 5 7.70 – 8.00.

### Hydrogel synthesis

Hydrogels were prepared according to the compositions in Table I. As an example, Hydrogel 5 is given. HA-PEG 5a (4.0 mg) was dissolved in a 1:1 H<sub>2</sub>O:DMF solvent system (8 IL; 2 vol % with respect to total monomer volume) with mild heating (37 °C). The solution containing 5a was diluted with DMA (157 IL, 1.80 mmol) and stirred, then diluted with EGDMA (21.7 IL, 0.12 mmol) and TRIS(OH) (212 mg, 0.50 mmol) and stirred until a homogeneous solution formed. The PI was added (2 IL from a 62.8 mg/mL Irgacure 184 in DMA stock solution) and the resulting prepolymer solution was injected into a mold consisting of two acrylic plates bolted together on either side of a 500 μm thick Teflon spacer. The mold was then placed in a 365 nm 400W UV chamber (Cure Zone 2 Control-cure, Chicago, IL) for 10 min. Upon removal from the mold, the hydrogel sheets were soaked in methanol for 3 h to remove unreacted monomers and then transferred to MilliQ water using serial dilutions to prevent fracturing the polymer during the solvent substitution. After soaking in for 24 h, the hydrogel sheet was punched into discs using a 5/16<sup>00</sup> or 1/4<sup>00</sup> cork borer. For samples containing 4a and 4b, the modified HA was dissolved in 30 vol % (with respect to total monomer volume) of a 2:1 DMF:H<sub>2</sub>O solvent system.

### Equilibrium water content (EWC)

Discs were weighed in a dry state after being placed in a 40 °C oven for 24 h and then transferred to room temperature for 24 h. Dry discs were then placed in 1 mL of PBS (pH 7.4) and incubated at room temperature for an additional 24 h. Hydrated discs were patted with lint-free wipes to remove any surface water droplets and then weighed. The EWC of each hydrogel composition was then determined using Eq. (1), where M<sub>H</sub> is the hydrated mass and M<sub>D</sub> is the dry mass of the hydrogel discs. All measurements were done in triplicate.

$$EWC\% = \frac{(M^H - M^D)}{M^D} \cdot 100 \quad (1)$$

## Surface wettability

The wettability of the hydrogel surface was analyzed by measuring the contact angle using the captive bubble technique. Hydrated discs were placed on top of a holder and then submerged in MilliQ water within a glass chamber. After waiting 5 min to ensure sample rehydration, a syringe was then used to place an air bubble (ca. 15  $\mu$ L) underneath the hydrogel materials and a goniometer (OCA 35, Dataphysics) was used to measure the captive bubble contact angle. All measurements were done in triplicate.

**HA-PEG release** Hydrogels used for 5a release studies were prepared as detailed in Section “Hydrogel synthesis.” A HA control was also prepared by substituting 5a with HA. After polymerization, the dry weight of the samples was measured and then the hydrogels were soaked in only enough PBS to cover the sample. After a soak time of 3 h, the hydrogels were weighed again, then punched into discs using a 5/16<sup>00</sup> cork borer, and wet weights of the discs were determined. Hydrogel discs were then placed into 2 mL Eppendorf tubes containing 1 mL of PBS and were incubated at 37 °C in an incubator shaker (90 rpm). At predetermined time points, the PBS solution was replaced to simulate infinite sink conditions. Release studies were carried out in triplicate. The concentration of 5a was determined using Enzyme-Linked Immunosorbent Assay (ELISA; Echelon, Salt Lake City, UT) using the procedure detailed in the kit. A 4 parameter logistic (4PL) nonlinear regression model was used for curve-fitting for standards analysis to extrapolate relative sample values, with the calibration curve covering a 50–1600 ng/mL range and had an average R<sup>2</sup> (nonlinear regression) of 0.98. An UV2vis spectrophotometer (Spectramax Plus 384, Molecular Devices, Corp, Sunnyvale, CA) was used for analysis. The amount of 5a or HA in the discs was calculated using Eq. (2), where M<sub>WD</sub> is the wet mass of the discs, M<sub>WH</sub> is the wet mass of the initial hydrogel sheet, and M<sub>5a/HA</sub> is the mass of 5a or HA incorporated into hydrogel sheet.

$$\text{Mass 5a or HA in disc} = (M_{WD}/M_{WH}) \times M_{5a/HA} \quad (2)$$

## Protein adsorption

Lysozyme was radiolabeled with Na<sup>125</sup>I using the iodine monochloride method.<sup>24</sup> Briefly, unbound <sup>125</sup>I was removed by passing the labeled samples through a 3 mL syringe packed with AG 1-X4 (Bio-Rad, Hercules, CA). Free iodide was measured by trichloroacetic acid precipitation of the protein. The free iodide of labeled lysozyme was 0.46% of total radioactivity. A 1.9 mg/mL lysozyme PBS solution containing 10% (w/w) labeled protein was used for the adsorption study.

Hydrogel discs 1/4<sup>00</sup> in diameter were placed vertically into a 96-well plate and the samples were soaked in PBS for 24 h. The discs were wiped dry using a Kimwipe, then lysozyme solution (250  $\mu$ L) was added to the wells and the samples were incubated for 2 h at room temperature. The samples were then rinsed with fresh PBS for 3 3 5 min to remove loosely adhered protein. The surfaces were subsequently counted for radioactivity for 10 min per sample using a Wizard 3 1480 Automatic Gamma Counter (PerkinElmer) and the adsorbed amounts were calculated using

background-corrected surface counts relative to the solution count for the individual protein solution. Samples were measured in triplicate and a PDMS blank was used for comparison.

## Statistics

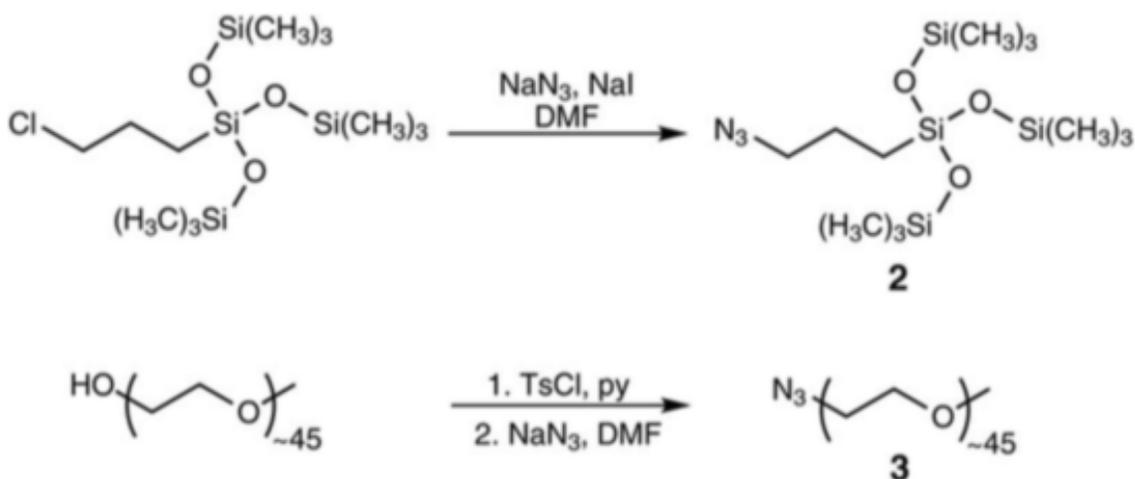
A one-factor analysis of variance was used to analyze hydrogel water content, water contact angle and protein adsorption using an  $\alpha = 0.05$ . Statistical analysis was performed using GenStat (VSN International Ltd, UK). All error bars represent standard deviation.

## RESULTS AND DISCUSSION

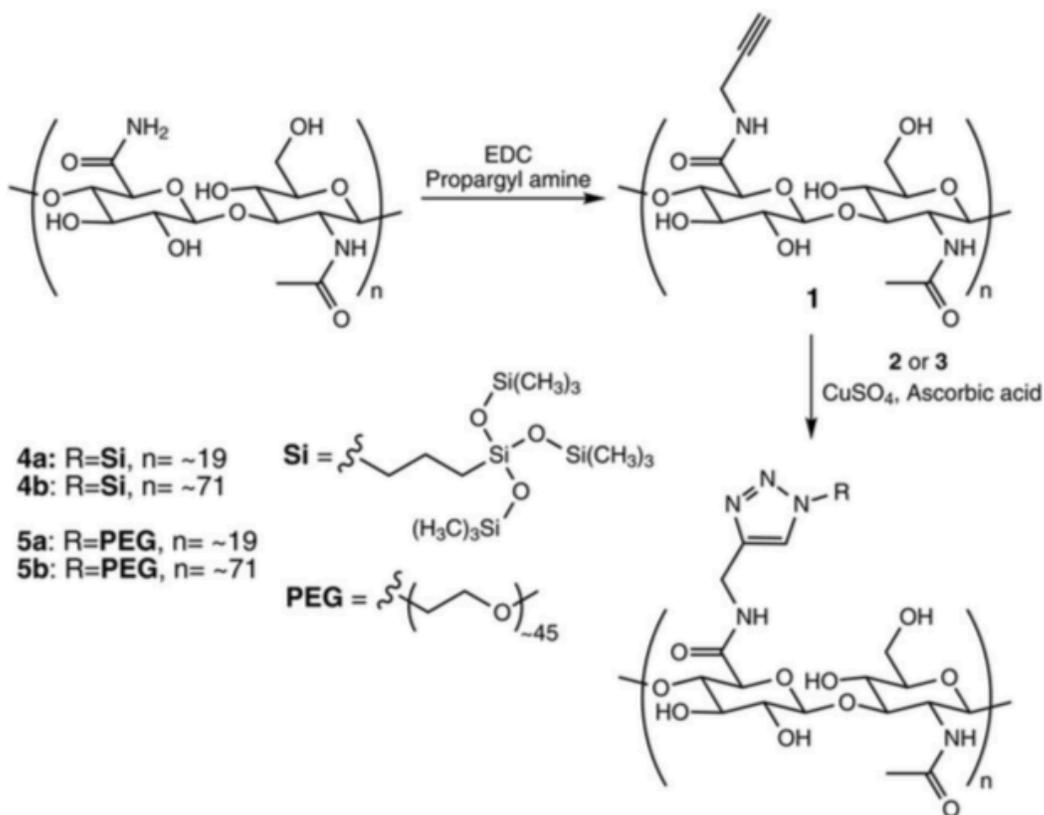
### Synthesis of HA derivatives

To improve the solubility of HA in polymerization solutions used to make contact lenses, it was thought that modification of HA with chemical groups that are similar to those present in the monomers would be needed. Modification of HA is not always easy because its hydrophilicity means that usually aqueous-based solvent systems can be used to solubilize HA. For modification with hydrophilic groups, this can usually be easily achieved using aqueous EDC coupling procedures, but for hydrophobic groups, this is not always the case. Fortunately, click chemistry can be used to overcome this issue. The robustness of click chemistry allows conjugations of very different chemical groups in a wide variety of solvent systems, and it has been used to synthesize HA-based materials.<sup>25-27</sup> However, there are few reports that use click chemistry to modify HA with different chemical moieties to control solubility in non-aqueous systems. To use click chemistry to modify HA, alkyne groups were first introduced using modified procedures to form a HA-alkyne adduct 1,<sup>28</sup> then reacted with azide-containing groups. As the molecular weight of HA would likely affect solubility in monomer solutions, both 7.5 and 28.5 kDa HA was used to make modified-HA.

Two different azido groups were prepared, siloxy-based (2) or PEG-based (3, Scheme 1). It was thought that the siloxy groups on the backbone of HA would reduce the hydrophilicity of HA, in addition to allowing favorable interactions with the siloxy monomers to improve solubility, and that the amphiphilic nature of PEG would interact favorably with all monomer components. Groups 2 or 3 were conjugated to 1a and 1b using copper catalyzed click reaction conditions, but a 3:1 DMF:H<sub>2</sub>O solvent system was required for conjugation of 2 to aid solubilization. Purification of the conjugates was carried out using dialysis against methanol to remove any hydrophobic component, and aqueous EDTA solution to remove residual copper, followed by lyophilization. The degree of functionalization was 25% for 4a, 4b, 5a, and 5b, as determined using <sup>1</sup>H NMR (Scheme 2). In an attempt to form a siloxy-PEG-modified HA, the click reaction was performed in the presence of both 2 and 3 using a 3:1 DMF:H<sub>2</sub>O solvent system. Unfortunately, the only products to form were 5a and 5b, most likely due to miscibility issues with 2 and the aqueous-based solvent system. Nonetheless, the chemical properties of 2 and 3 are quite different, yet both were easily conjugated to 1a and 1b using click chemistry, further demonstrating the robustness of this method for modification of HA.



**SCHEME 1.** Synthesis of azido-containing siloxy (**2**) or PEG (**3**) groups.



**SCHEME 2.** Synthesis of siloxy-(**4a** and **4b**) or PEG-functionalized (**5a** and **5b**) HA.

Incorporation of modified-HA into model contact lens materials. One of the key prerequisites for contact lenses is that they are optically transparent. Therefore, it is imperative that the

prepolymer solution and the resulting polymer remain homogeneous. In cases where a homogeneous solution phase separates during polymerization, it is sometimes possible to optimize the monomer formulation to produce homogeneous, transparent materials. However, it is much more difficult to produce a homogeneous hydrogel with a heterogeneous prepolymer solution. Thus, for the purpose of this study, only samples that had homogeneous prepolymer were investigated. It should also be noted that unmodified HA was not soluble in any of the monomer formulations tested in this study, instead it was only dispersed in the monomer solution and subsequently formed a hydrogel with heterogeneous HA distribution. This distribution made it difficult to characterize these materials. As a result, samples that contained unmodified HA were not examined for their EWC nor surface wettability.

Incorporation of HA-Si. To test how modification of HA altered its solubility in polymerization solutions used to make contact lenses, modified-HA was incorporated into a [78]:[22] DMA:TRIS(OH) monomer solution. Although commercial silicone contact lenses have additional components, this monomer solution was used as a model contact lens monomer solution to test modified-HA solubility. For reference, unmodified HA was also incorporated into the same model monomer solution. Modified-HA was incorporated at 0.25 wt % as this is the concentration that unmodified HA becomes insoluble in contact lens monomer solutions (it should be noted that HA is very rarely soluble in monomer solutions, it merely is dispersed and below 0.25 wt % the dispersion is too fine to be noticed). When 4a was incorporated into DMA:TRIS(OH) monomer solutions (Hydrogel 4, Table I), it was not soluble in the monomer mixture even when a 2:1 DMF:H<sub>2</sub>O solvent system was introduced. Interestingly, when 4b (Hydrogel 5, Table I) was incorporated into DMA:TRIS(OH) monomer solutions, the use of a 30 vol % (with respect to total monomer volume) of a 2:1 DMF:H<sub>2</sub>O solvent system enabled complete dissolution of the modified HA. It should be noted that without the addition of the solvent system, 4b could only be dispersed in the polymerization solution. Despite the improved solubility of modified HA in the monomer solution, phase separation occurred during polymerization of the hydrogels that contained 4b (Hydrogel 5) as well as the associated control prepared with 2:1 DMF:H<sub>2</sub>O (Hydrogel 2, Table I), resulting in nontransparent materials. As the 2:1 DMF:H<sub>2</sub>O control hydrogel was also nontransparent, it indicates that the DMF:H<sub>2</sub>O solvent system and ratios most likely needed changing to produce a transparent material. Unfortunately, any adjustments to the solvent to produce transparent materials resulted in 4b being insoluble in the monomer solution.

**TABLE I. Composition of [78]:[22] DMA:TRIS(OH) Hydrogels used to Study the Effect of HA-Modification on its Solubility of Monomer Solutions and the Materials Properties**

Hydrogel <sup>a</sup>	Modified-HA <sup>b,c</sup>	EGDMA (mol %) <sup>d</sup>	DMF/H <sub>2</sub> O	Water Content <sup>e</sup>	Contact Angle <sup>e</sup>
1	–	5	–	19.3 ± 0.85	41.1 ± 0.97 <sup>ii</sup>
2	–	5	2:1 <sup>f</sup>	21.0 ± 1.75 <sup>i</sup>	34.2 ± 4.48
3	–	5	1:1 <sup>g</sup>	17.1 ± 1.47 <sup>^</sup>	36.7 ± 1.48 <sup>Å<sup>ii</sup></sup>
4	HA-Si (7.5), <b>4a</b>	5	2:1 <sup>f</sup>	–	–
5	HA-Si (28.5), <b>4b</b>	5	2:1 <sup>f</sup>	18.0 ± 1.03 <sup>i</sup>	34.0 ± 0.25
6	HA-PEG (7.5), <b>5a</b>	5	1:1 <sup>g</sup>	15.7 ± 0.55	46.1 ± 0.85 <sup>Å</sup>
7	HA-PEG (28.5), <b>5b</b>	5	1:1 <sup>g</sup>	13.6 ± 1.19 <sup>^</sup>	41.4 ± 1.91
8	–	1	–	32.0 ± 0.41	31.9 ± 1.17
9	–	1	1:1 <sup>g</sup>	32.2 ± 0.97 <sup>^</sup>	31.4 ± 0.92
10	HA-PEG (7.5), <b>5a</b>	1	1:1 <sup>g</sup>	33.6 ± 0.20	32.2 ± 0.95
11	HA-PEG (28.5), <b>5b</b>	1	1:1 <sup>g</sup>	31.2 ± 0.14	34.2 ± 2.99

<sup>a</sup>All polymerizations contained [78]:[22] DMA:TRIS(OH) and were initiated using 0.01 mol % PI.

<sup>b</sup>Values in parentheses indicates molecular weight (kDa) of HA used in modified-HA.

<sup>c</sup>Added as 0.25 wt % with respect to total monomer [i.e., DMA:TRIS(OH)] weight.

<sup>d</sup>With respect to total moles of monomer.

<sup>e</sup>*n* = 3, +/-SD. Symbols indicate values that are statistically different from one another (*p* < 0.05).

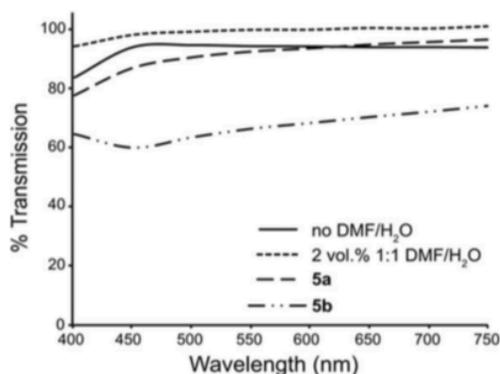
<sup>f</sup>Total volume of solvent was 30 vol % with respect to total monomer volume.

<sup>g</sup>Total volume of solvent was 2 vol % with respect to total monomer volume.

The water content of hydrogels that contained 4b (18.0 ± 1.03%) decreased compared with the controls (21.0 ± 1.75%; Hydrogel 2, Table I), with the results were statistically different. The addition of additional siloxy groups from 4b most likely increases the hydrophobicity of the material, despite the fact hydrophilic HA is being added to the material. In addition, phase separation during polymerization may also rearrange the distribution of 4b, the hydrophilic and hydrophobic domains in the hydrogel, meaning 4b may adopt a conformation that prevents the HA backbone from adsorbing water and acting as a wetting agent, although further studies would be required to support this hypothesis. As the hydrogels containing 4a and 4b contained dispersed HA-Si and/or were phase separated, we did not carry out transparency and release studies on these materials because (i) the results obtained from these studies would be highly variable and hard accurately draw any conclusions from, and (ii) these materials, in their current form, would have little potential as materials for contact lens applications.

Incorporation of HA-PEG. When 5a or 5b (Hydrogel 6 and 7, Table I) was incorporated into the monomer solution, only 2 vol % of a 1:1 DMF:H<sub>2</sub>O solvent system was needed for complete dissolution. The reduction in solvent needed for dissolution meant that the polymerization solution remained homogenous throughout polymerization, leading to materials that were mostly optically transparent (Fig. 1). Although transparent, samples prepared with 5b (Hydrogel 7, Table I) were transparent up to a maximum of 80% (Fig. 1). However, samples prepared with 5a (Hydrogel 6, Table I) had transparencies mostly >95%; for contact lens applications, materials should have transparencies [at]80%. Like 4a and 4b, 5a and 5b were only dispersed in the monomer solution in the absence of a solvent system and the resulting hydrogels had heterogeneous HA-PEG distributions. Likewise, unmodified HA was not soluble in the polymerization solution. The EWC of hydrogels that contained 5a (15.7±0.55%) or 5b (13.6±1.19%) were lower than control hydrogels that did not contain 5a or 5b (19.3% / 20.43, Hydrogel 1; 17.1% / 20.73, Hydrogel 3), although only samples with 5b were statistically different to the control (Hydrogel 3, Table I). The low EWC values of these hydrogels are most likely a consequence of the relatively high concentration of crosslinking (5 mol %). At such high crosslinking concentrations, the polymer chains have limited mobility, meaning they have

limited capacity for hydration. If the polymer chains are unable to accommodate any additional hydration, then the presence of wetting agents 5a or 5b will likely have no effect on the EWC for these materials.



**FIGURE 1.** Transparency of [78]:[22] DMA:TRIS(OH) hydrogels cross-linked at 5 mol % without any 1:1 DMF:H<sub>2</sub>O solvent (hydrogel 1), with 2 vol % of 1:1 DMF:H<sub>2</sub>O solvent (hydrogel 3), and 5a (hydrogel 6) or 5b (hydrogel 7).

To try and increase the water content of the hydrogels prepared with 5a or 5b, and, thus, for 5a or 5b to act as a better wetting agent, the crosslinking concentration was decreased from 5 to 1 mol % to increase chain mobility and allow the polymer chains to adsorb more water. As expected, a decrease in the crosslinking concentration increased the EWC of the hydrogels from 17.1 ± 1.47% (Hydrogel 3, Table I) to 32.2 ± 6.097% (Hydrogel 9, Table I). Incorporation of 5a did slightly increase the EWC of the hydrogels to 33.6 ± 6.020% (Hydrogel 10, Table I), but this increase was not statistically significant; incorporation of 5b slightly decreased the EWC (31.2 ± 6.014%, Hydrogel 11, Table I) and again this decrease was not statistically significant. Although the EWC of these hydrogels was higher compared with samples crosslinked at 5 mol %, the transparency of these materials was poorer (Fig. 2). The lack of transparency is likely a consequence of the higher water content and lower crosslinking concentration. As the crosslinking density is lower, chain mobility should increase and the hydrophilic polymer chains should be able to adsorb more water. If there is sufficient chain mobility and hydration, the polymer chains may phase separate on the nanoscale to give hydrophilic and hydrophobic domains. This hypothesis is supported by the fact that these materials were optically transparent when soaked in methanol (a good solvent for both the hydrophilic and hydrophobic regions phases of the hydrogels), but became opaque when soaked in water (only a good solvent for the hydrophilic phases). It should be noted, however, that samples prepared with 5a and 5b at 1 mol % crosslinking had poor transparencies, while control hydrogels (Hydrogel 8 and 9, Table I) exhibited transparency mostly [mt]90%. This result suggests that it is the addition of the hydrophilic wetting agents that promotes phase separation in samples with lower crosslinking concentrations, and that the presence of wetting agents has minimal effects on water content as the biphasic nature of these materials has a more dominant role in controlling EWC. Nonetheless, as with the hydrogels prepared with 4a and 4b, it is likely that formulation optimization will produce a material that contains 5a or 5b that has optimal EWC and optical transparency.

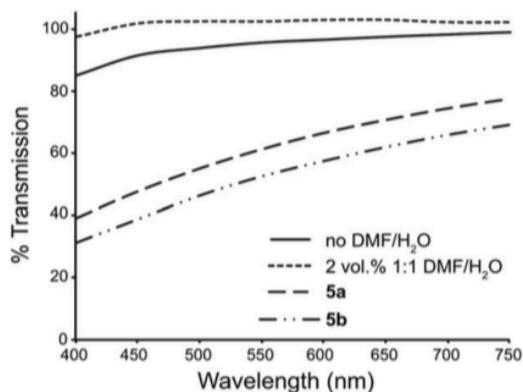


FIGURE 2. Transparency of [78]:[22] DMA:TRIS(OH) hydrogels cross-linked at 1 mol % without any 1:1 DMF:H<sub>2</sub>O solvent (hydrogel 8), with 2 vol % of 1:1 DMF:H<sub>2</sub>O solvent (hydrogel 9), and 5a (hydrogel 10) or 5b (hydrogel 11).

## Surface wettability

Contact angle can usually be related to the water content of hydrogels. However, migration of polymer chains and/or wetting agents can alter the surface chemistry of a hydrogel, which will alter the surface wettability. For contact lens applications, having a hydrophilic surface is advantageous as it helps to minimize occurrence of conditions such as dry eye and excessive protein adsorption. Therefore, a lens that has a low EWC but a high surface wettability may be more useful than a lens with opposite characteristics. For samples crosslinked at 5 mol %, the inclusion of 30 vol % of a 2:1 DMF:H<sub>2</sub>O solvent system or 2 vol % of a 1:1 DMF:H<sub>2</sub>O solvent system decreased the contact angle from 41.160.97° (Hydrogel 1, Table I) to 34.264.48° (Hydrogel 2, Table I) and 36.761.48° (Hydrogel 3, Table I;  $p < 0.05$ ), respectively. This decrease is not surprising considering the network density is lower when solvents are included during polymerization, leading to the polymer chains at the surface being more mobile and able to adsorb more water. Inclusion of 4b had negligible effect on the contact angle (34.060.25°), and inclusion of 5a (Hydrogel 6) or 5b (Hydrogel 7) increased the contact angle to 46.160.85° ( $p < 0.05$ ) and 41.461.91°, respectively. An increase in the contact angle indicates the surface is more hydrophobic, which is the opposite of what is expected when an internal wetting agent is included in hydrogels. As discussed in Section “Synthesis of HA derivatives,” the presence of 5a or 5b may lead to a rearrangement of the polymer chains so more hydrophilic domains are located around the modified-HA. If 5a or 5b are located more toward the center of the material, then the surface will have a greater proportion of hydrophobic domains. The contact angles for hydrogels crosslinked at 1 mol % were lower (ca. 31°) compared with materials crosslinked at 5 mol %. Again, this result is not surprising due to the increased chain mobility at the lower crosslinking concentration. However, there was no statistical difference in the contact angle for hydrogels that included 5a or 5b and the corresponding control. This result, along with EWC results, indicate that there either is not enough modified-HA to significantly affect EWC and contact angle, and/or the polymer formulation needs optimization.

## Protein adsorption

Adsorption of proteins onto contact lenses can have deleterious effects to their wearers, and can cause sight threatening effects including microbial keratitis and giant papillary conjunctivitis.<sup>6</sup> Increasing the water content and decreasing the surface contact angle of silicone hydrogel lenses through the use of wetting agents can minimize nonspecific protein adsorption. In this study, the ability for modified HA to minimize protein adsorption was examined using radiolabeled lysozyme. Lysozyme is the most abundant protein in the tear film, comprising of on average 36% of the total protein content,<sup>29</sup> and can be used to model in vivo protein interactions with contact lens hydrogels. A lysozyme concentration of 1.9 mg/mL was also used in the study as it closely matches average in vivo lysozyme tear film concentrations.<sup>30</sup> Hydrogel discs were incubated in radio labeled lysozyme solution and then analyzed. Incorporation of 4b (Hydrogel 5) did not significantly decrease the amount of adsorbed lysozyme (Fig. 3) compared with its control (Hydrogel 2). The presence of 5a (Hydrogel 6) or 5b (Hydrogel 7) in hydrogels crosslinked at 5 mol % increased the amount of adsorbed lysozyme compared to the control (Hydrogel 3). However, as the EWC of the hydrogels that contained 5a or 5b decreased compared with the control, this result is not surprising as protein adsorption typically increases with increasing material hydrophobicity. Interestingly, a decrease in the crosslinking concentration from 5 mol % (Hydrogel 3) to 1 mol % (Hydrogel 9) caused an significant increase in the adsorption of lysozyme. This result was unexpected as the water content of hydrogels crosslinked at 1 mol % had EWC values roughly double for hydrogels crosslinked at 5 mol %. Despite the increase in lysozyme adsorption for samples crosslinked at 1 mol % EGDMA, the presence of 5a (Hydrogel 10) decreased the amount of adsorbed lysozyme compared with its control (Hydrogel 9), despite the little change in the EWC and contact angle for Hydrogel 9 and 10. Although not conclusive, this result does highlight the potential for modified HA to act as an internal wetting agent to minimize protein adsorption and to increase EWC.

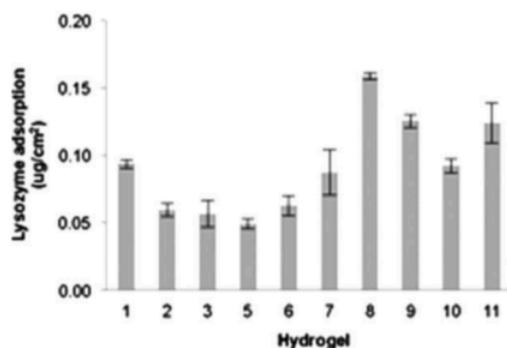
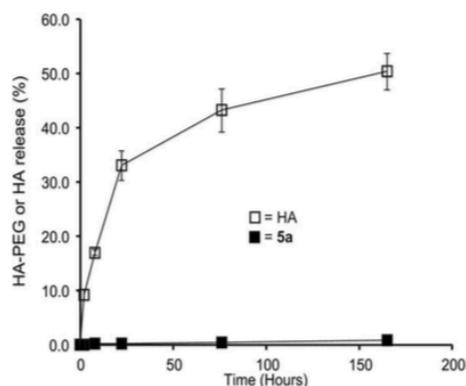


FIGURE 3. Lysozyme adsorption onto [78]:[22] DMA:TRIS(OH) discs prepared with and without 4b, 5a, or 5b at 1 or 5 mol % EGDMA (hydrogel list found in Table I).

## ELISA release study

Research has shown that HA can be used to control the release kinetics of ophthalmic drugs from silicone-based contact lenses. Because diffusion of HA through the hydrogel is significantly slower than the drug itself, interactions between the drug and HA means the drugs diffusion

constant is lowered to that of approximately HA.<sup>21</sup> Being able to control the release kinetics means that ophthalmic drugs can be delivered with greater efficacy over a longer period of time. HA in its unmodified form is a linear polysaccharide. Modification with PEG gives rise to HA with comb architectures. Polymers with comb architectures should interact more with the matrix (i.e., hydrogel) compared to linear polymer, and, thus, should diffuse more slowly. Therefore, it would be reasonable to assume that modified-HA should diffuse more slowly although a siliconebased hydrogel compared to unmodified HA. To test this theory, the release of 5a from Hydrogel 6 was monitored using an ELISA; to compare how modification of HA alters release kinetics, release of unmodified HA was measured as a control. ELISA was chosen due to its sensitivity and because 5a and HA do not have any chromophores that are easily detected. Infinite sink conditions were chosen to simulate the continual renewal of tear film in vivo. As seen in Figure 4, the rate of release of 5a was orders of magnitude lower than the rate of release for HA for the same hydrogel formulation. After 165 h of incubation only 0.8% of incorporated 5a was released, whereas 50% of HA was released over the same time period. Although this result suggests that the architecture of the wetting agent strongly affects its release kinetics, 5a was homogeneously distributed throughout the hydrogel whereas unmodified HA was only dispersed heterogeneously throughout the hydrogel. This difference would likely alter release kinetics. However, it would also not be unreasonable to assume that the heterogeneous distribution of unmodified HA should have slower release kinetics compared with the homogeneously distributed 5a since the release kinetics of unmodified HA would be determined by many more factors (degree of hydration, solubility, etc.); therefore, this result still suggests that the differences in polymer architecture strongly affects the rate of release. For some applications, it is desirable for the wetting agent to slowly leach out of the contact lens. In these circumstances, it would be advantageous to be able to control the release of wetting agent by simply changing the polymer architecture of the wetting agent. Reducing the number of PEG groups on modified-HA should lead to a polymer architectures with lower brush densities, and this, in theory, should increase the rate of release.



**FIGURE 4.** Percentage release of 5a (closed boxes) or HA (open boxes) from a [78]:[22] DMA:TRIS(OH) hydrogel crosslinked at 5 mol % (polymer formulation hydrogel 6). the error bars represent  $\pm$  SD. Note: some error bars are less than the size of the data point.

## CONCLUSION

HA was modified with siloxy or PEG moieties using click chemistry. Compared with HA,

modified HA was significantly more soluble in monomer solutions used to synthesize model contact lens materials. Incorporation of modified HA did not increase the water content nor decrease the surface contact angle of the silicone hydrogels, but in some samples it did help to reduce lysozyme adsorption. The release rates of HA-PEG (5a) from [78]:[22] DMA:TRIS(OH) hydrogels was significantly slower compared to unmodified HA. This result was attributed to the differences in polymer architectures. While this study did not optimize the polymer formulation to help increase the amount of modified HA that could be incorporated into the silicone hydrogels, this study did highlight the potential of modification of HA to broaden the range of available wetting agents that could be used for specific roles—drug delivery, for example—in contact lens applications.

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