Reprinted with permission from: Deng X, Korogiannaki M, Rastegari B, Zhang J, Chen M, Fu Q, Sheardown H, Filipe CDM, Hoare T. "Click" Chemistry-Tethered Hyaluronic Acid-Based Contact Lens Coatings Improve Lens Wettability and Lower Protein Adsorption. ACS Appl. Mater. Interfaces 2016, 8, 22064−22073 DOI: 10.1021/acsami.6b07433

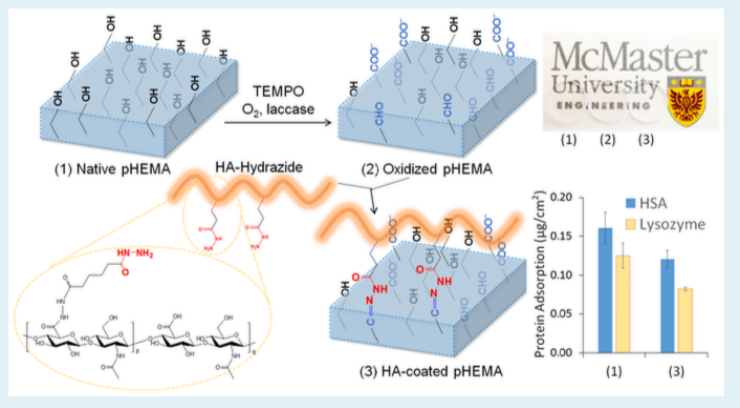
"Click" Chemistry-Tethered Hyaluronic Acid-Based Contact Lens Coatings Improve Lens Wettability and Lower Protein Adsorption

Xudong Deng,[†] Myrto Korogiannaki,[†] Banafsheh Rastegari,[†,‡] Jianfeng Zhang,[†] Mengsu Chen,[§] Qiang Fu,[†] Heather Sheardown,[†] Carlos D. M. Filipe,[*,†] and Todd Hoare[*,†]

[†]Department of Chemical Engineering, McMaster University, Hamilton, Ontario L8S 4L7, Canada [‡]Department of Biology, Faculty of Sciences, Shiraz University, Shiraz, Fars 71946-84636, Iran [§]School of Nursing, McMaster University, Hamilton, Ontario L8S 4L8, Canada

Improving the wettability of and reducing the protein adsorption to contact lenses may be beneficial for improving wearer comfort. Herein, we describe a simple "click" chemistry approach to surface functionalize poly(2-hydrox- yethyl methacrylate) (pHEMA)-based contact lenses with hyaluronic acid (HA), a carbohydrate naturally contributing to the wettability of the native tear film. A two-step preparation technique consisting of laccase/TEMPO-mediated oxidation followed by covalent grafting of hydrazide-functionalized HA via simple immersion resulted in a model lens surface that is significantly more wettable, more water retentive, and less protein binding than unmodified pHEMA while maintaining the favorable transparency, refractive, and mechanical proper- ties of a native lens. The dipping/coating method we developed to covalently tether the HA wetting agent is simple, readily scalable, and a highly efficient route for contact lens modification.

KEYWORDS: hyaluronic acid, poly(2-hydroxyethyl methacrylate), TEMPO oxidation, surface wettability, protein adsorption



1. INTRODUCTION

Soft contact lenses have been widely used for vision correction since their introduction by Wichterle in the 1960s.[1] The hydration and surface hydrophilicity of soft lenses significantly improve the oxygen permeability and wettability of the lens by the tear film, both of which are thought to enhance the overall comfort of the lens.[2,3] Hydroxyethyl methacrylate (HEMA) was the original hydrophilic monomer used for soft contact lens production and continues to be the most commonly used hydrophilic monomer in the production of both hydrogel-based and silicone hydrogel-based contact lenses.[4] However, poly- (hydroxyethyl methacrylate) (pHEMA)-based contact lenses tend to dehydrate over time when applied on the eye, particularly over longer wear times;[5] this drying leads to user discomfort in the form of eye dryness and scratchiness, both of which lead to lens dropout.[6] In addition, both hydrogel and silicone hydrogel contact lenses suffer from challenges related to the deposition of proteins from the tear fluid onto the lenses,[7–9] correlated to a variety of adverse effects including microbial cell attachment[10,11] and inflammatory complica- tions.[12]

In order to minimize the dehydration of and protein accumulation on soft contact lenses, a variety of chemical treatment methods have been employed to modify the lens surface and/or the lens as a whole before or after the polymerization process. Doping or tethering of highly hygroscopic polymeric wetting agents has been the most commonly pursued approach.[13,14] Synthetic polymers associ- ated with high water binding capacity and low protein adsorption, including poly(vinyl alcohol) (PVA), poly(ethylene glycol) (PEG),[15] and 2-methacryloyloxyethyl phosphoryl- choline (MPC),[16,17] have been either entrapped within the bulk of the lens (potentially enabling sustained release of the wetting agent over time)[18,19] or surface grafted to the lens (increasing the capacity for interfacial water retention).[20] However, these synthetic approaches have met with limited success in practice. For example, while PVA modification does increase lens wettability, PVA-containing lenses (FOCUS DAILIES with AquaComfort, nelfilcon A) have also performed worse than other daily disposable lenses such as 1-DAY ACUVUE (etafilcon A, Johnson & Johnson Vision Care, a copolymer of HEMA and methacrylic acid with a

polyvinyl- pyrrolidone embedded wetting agent) in terms of comfort, maximum wear time, corneal staining, and lens fit.[21]

Recent work has focused on the use of hyaluronic acid (HA), a natural polysaccharide present in the eye and used in several artificial tear formulations, as a preferred wetting agent. Coupled with its inherent wetting[22–24] and protein-resistance- promoting[25–27] properties, hyaluronic acid has also been associated with promoting corneal epithelial wound healing,[28] improving the integrity of superficial corneal cells,[29] reducing ultraviolet B (UVB) radiation-induced toxic effects,[30] protecting cells from oxidative damage,[31] and stabilizing the tear film.[32] Thus, it has the potential to provide multiple benefits in the context of contact lens coatings.

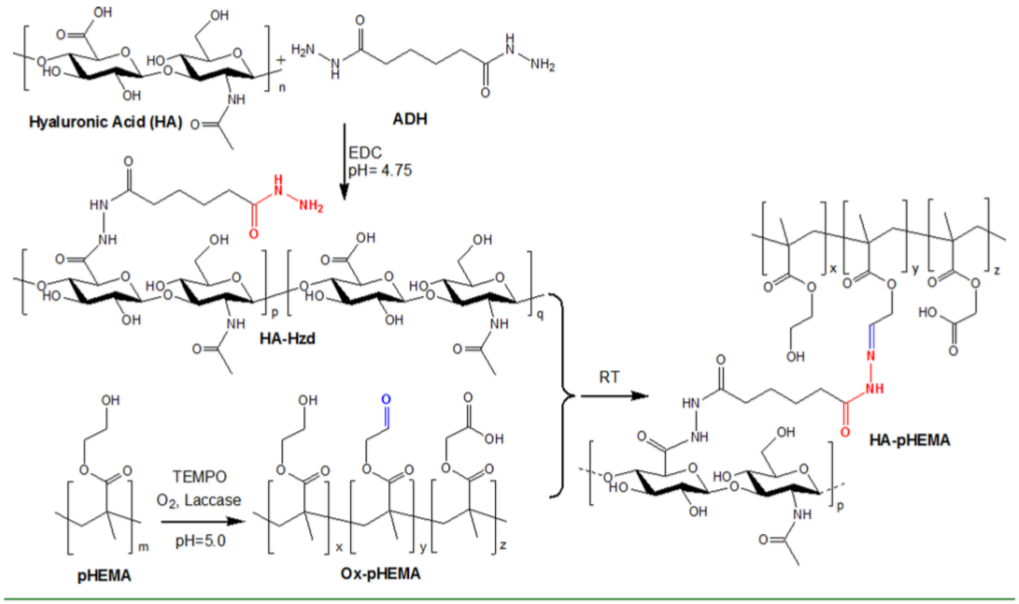
Various methods have been reported to incorporate HA into the bulk of the lens (facilitating wetting by either slow release of HA from the lens or self-assembly of HA at the lens–water interface) or immobilize HA on the lens surfaces (facilitating wetting directly at the interface). Cross-linking is the most commonly used method to introduce HA into the bulk hydrogel network, with HA incorporation having been demonstrated directly with the main lens material(s) (e.g., via photopolymerization of methacrylated photo-cross-linkable HA with HEMA[23]), within a secondary interpenetrating network (e.g., polyethylenimine cross-linked HA within an independ- ently cross-linked HEMA network[27]), or via physical entrap- ment of a higher molecular weight HA-based cluster or nanogel (e.g., conjugation of HA to polypropylenimine tetramine dendrimers that improve HA immobilization within the lens).[22,25,26,33] In comparison, given the relatively low reactivity of HEMA for chemical modification, surface modification methods generally require the generation of a reactive functional group or interfacial binding site for HA immobiliza- tion. Again, HA attachment to the surface may be accomplished either directly or via another mediating chemistry. As an example of the former, thermal cross-linking has been used to conjugate thiolated HA to acrylated pHEMA surfaces via Michael addition chemistry during autoclaving;[34] as an example of the latter, covalent linkage of an HA-binding peptide (HABpep, with or without a PEG spacer) via EDC/NHS chemistry to a PureVision (balafilcon A, Bausch and Lomb) contact lens surface mediates ligand–receptor binding of HA at the lens interface.[24] However, each surface modification method reported to date is practically hampered by the multistep nature of the required protocols, including chemical modification of HA, HA uptake and cross-linking, and/or preparation of special linkers like HABpep. As a result, the development of a simple and fast surface modification strategy requiring fewer steps and/or using simpler reactions would be preferred to facilitate a scalable and thus commercially relevant method for preparing surface-wetted contact lenses.

To this end, we propose a click chemistry process[35] to facilitate more direct and high yield HA functionalization of a contact lens surface. We have extensively explored the click reaction between aldehyde and hydrazide groups to facilitate the rapid and high-

yield formation of hydrazone bonds at physiological pH and have demonstrated the use of such chemistry to prepare a variety of bulk hydrogels,[36–39] electrospun hydrogel fibers,[40] and thin film hydrogels at the solid–liquid interface.[41] Hydrazide-functionalized HA (HA- Hzd) can be prepared at high yield via carbodiimide-mediated coupling of adipic acid hydrazide to the carboxylic acid on the glucuronic acid moieties of HA,[42,43] with such polymers already applied extensively in the preparation or modification of HA-based hydrogels for drug delivery and tissue engineer- ing.[39,44–47] Correspondingly, pHEMA contains primary hydroxyl groups that can be oxidized into aldehyde groups, but very limited work has been reported on the oxidation of pHEMA hydrogels,[48] and relatively few oxidation methods have been demonstrated to reliably convert alcohols to aldehydes without continuing to carboxylic acids. Catalytic oxidation using the stable nitroxyl radical 2,2′,6,6′-tetramethylpiperidinyl- 1-oxy (TEMPO) as an electron transfer mediator is one of the most promising procedures to oxidize primary alcohol groups and has been widely used in the oxidation of natural polysaccharides.[49] While traditional TEMPO oxidation proce- dures have used bleach (NaClO) as the oxidizer together with NaBr,[50] the undesirable residual halide and requirement of high pH in such procedures have spurred the more recent use of oxidative enzymes such as laccase in combination with oxygen at neutral pH to promote oxidation.[51,52] To the best of our knowledge, TEMPO-mediated oxidation of primary alcohol groups in synthetic polymers like pHEMA has not previously been demonstrated.

In this work, we demonstrate the use of a simple click chemistry procedure involving the reaction of hydrazide- functionalized HA with TEMPO-oxidized pHEMA to cova- lently tether HA to the surface of a model contact lens via a simple two-step process (Scheme 1). Furthermore, we demonstrate the potential of this modification to significantly improve the surface wettability of the lens while also substantially decreasing nonspecific protein adsorption, both of which are linked to improved comfort and the potential for longer term wear of soft contact lenses.

Scheme 1. Reaction Scheme Showing the Preparation of Hydrazide-Functionalized Hyaluronic Acid (HA-Hzd), Oxidation of pHEMA (Ox-pHEMA), and Coating HA-Hzd on Oxidized pHEMA (HA-pHEMA)



2. EXPERIMENTAL SECTION

2.1. Chemicals and Materials. Sodium hyaluronate (Lot # 010600, M = 336.8 kDa, Đ = 1.386 by GPC; see Supporting Information Figure S1) was obtained from Fidia Farmaceutici S.p.A. (Abano Terme, Italy). Adipic acid dihydrazyde (ADH, Sigma-Aldrich, 98%), N′-ethyl-N-(3-(dimethylamino)propyl)carbodiimide (EDC, Sigma-Aldrich, commercial grade), human serum albumin (HSA, Sigma-Aldrich, 97%), lysozyme from chicken egg white (Sigma- Aldrich, 41 800 units/mg solid), laccase from Trametes versicolor (EC 1.10.3.2, Sigma-Aldrich, ≥10 U/mg), TEMPO (Sigma-Aldrich, 99%), fluorescein isothiocyanate isomer I (FITC, Sigma-Aldrich, 97.5%), fluorescein-5-thiosemicarbazide (5-FTSC, Sigma-Aldrich, 80%), silver nitrate ($AgNO_3$, Sigma-Aldrich, ≥99.0%), and ammonium hydroxide (containing 28–30% NH_3, Caledon Laboratories) were all used as received. HEMA (2-hydroxyethyl methacrylate) and EGDMA (ethyl- ene glycol dimethacrylate) were purchased from Sigma-Aldrich, with the 4-methoxyphenol inhibitor in both monomers removed prior to photopolymerization using an inhibitor removal column (Sigma- Aldrich, product number 306312). Photoinitiator Irgacure 184 was purchased from BASF Canada Inc. Hydrochloric acid (1 M, 0.1 M) and sodium hydroxide (1 M, 0.1 M) were received from LabChem Inc. (Pittsburgh, PA). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin–streptomycin solution (10 000 units penicillin; 10 000 μg streptomycin) were obtained from Gibco (Gaithersburg, MD). MTT ([3-4,5-dimethylthiazol-2-yl]-2,5-diphenyl- tetrazolium bromide) was obtained from Sigma-Aldrich. For all experiments, Milli-Q grade distilled deionized water (DIW) was used. Phosphate buffered saline (PBS) was diluted from a 10× liquid concentrate (Bioshop Canada Inc.).

2.2. pHEMA Hydrogel Synthesis. To prepare a pHEMA hydrogel mimicking a contact lens, 3 g of HEMA, 90 mg of EDGMA, and 7 mg of Irgacure 184 were first mixed and shaken for 5 min until the initiator was completely dissolved. The mixture was then transferred to a custom-made poly(methyl methacrylate) mold (dimensions 70 mm × 70 mm × 1 mm) and placed in a chamber

equipped with a 400 W UV lamp (Cure Zone 2 CON-TROL-CURE, Chicago, IL) for 15 min. Before detaching from the mold, the cured HEMA plate was left at room temperature for 24 h to facilitate postcuring. After three cycles of extraction of this gel in Milli-Q water to remove unreacted monomer/cross-linker and initiator residues, the purified gel was immersed in Milli-Q water for 24 h to achieve an equilibrium swelling state prior to surface modification.

2.3. Laccase-Mediated TEMPO Oxidation of pHEMA Hydro- gel (Ox-pHEMA). A combination of TEMPO, laccase, and oxygen was used to oxidize the pHEMA hydrogel, based on a previously reported method for cellulose oxidation.[53] In a typical experiment, 50 mg of TEMPO was dissolved in 200 mL of sodium acetate aqueous buffer (50 mM, pH 5.0), and one piece of pHEMA hydrogel (10 cm × 10 cm × ~1 mm) was immersed in the solution for 30 min. Following, 50 mg of laccase was dissolved in the same sodium acetate buffer and added to the TEMPO/pHEMA mixture to create a 250 mL solution consisting of 250 mg/L TEMPO and 250 mg/L laccase. The oxidation solution was stirred under oxygen purging (1 bubble/s) for 24 h at room temperature. A polytetrafluoroethene (PTFE) grid was used to support the hydrogel and avoid direct contact between the magnetic stir bar and the hydrogel. After the oxidation procedure was complete, the oxidized pHEMA hydrogel was rinsed in the same sodium acetate buffer (3 × 5 min cycles) and then immersed in water for 8 h to remove the TEMPO and laccase.

The aldehyde content on the TEMPO-oxidized pHEMA hydrogel was assessed by both the silver mirror reaction and fluorescent labeling. For the silver mirror assay, Tollens' reaction was performed, exploiting the aldehyde-specific reduction of ammonia silver nitrate (a reaction that cannot be performed by either carboxylic acids or primary alcohols[54]). Tollens' reagent was first prepared from silver nitrate solution ($AgNO_3$, 0.02 M) and ammonia hydroxide (2.5 wt %),[55] after which one piece of unmodified pHEMA and one piece of TEMPO-oxidized pHEMA (1 cm × 2 cm × ~1 mm) were immersed in ~30 mL of the Tollens' reagent solution and warmed in a 50 °C water bath. After 3 min, the two samples were removed from the solution and rinsed three times with 50 mL of Milli-Q water. The relative aldehyde content was estimated based on visual observation of the silver mirror effect. For the fluorescent labeling technique, pHEMA hydrogels (1 cm × 2 cm × ~1 mm) were immersed in a 0.05 g/L solution of fluorescein-5-thiosemicarbazide (5-FTSC) in carbonate buffer (pH = 9.0, 0.106 g of Na_2CO_3 and 0.966 g of $NaHCO_3$ in 500 mL of water) for 4 h. The hydrogels were then rinsed with 2 mL of buffer (15 × 5 min cycles) to remove nonreacted fluorescein probe, leaving only FTSC covalently bound to aldehyde groups in the pHEMA gel. Aldehyde content was then visualized with the BioRad ChemiDoc imaging system, using an excitation wavelength of 488 nm.

2.4. Hydrazide-Functionalized Hyaluronic Acid (HA-Hzd) Synthesis and Characterization. HA-Hzd was prepared according to a protocol reported by Luo et al.[47] Briefly, 60 mg of sodium hyaluronate was dissolved at a concentration of 5 mg/mL in DIW. Solid ADH (1.1 g) was first dissolved in 12 mL of water and then added into the HA solution following syringe filtration (5 μm pore size), followed by magnetic stirring for 30 min. The pH of the reaction mixture was adjusted to 4.75 by adding HCl, after which 0.1 g of EDC was dissolved in 1 mL of water and added dropwise. The pH of the reaction mixture was maintained at 4.75–4.80 by adding 0.1 M HCl over the full 4 h reaction time, after which the reaction was stopped by adding NaOH to raise the pH to 7.0. The reaction mixture was poured into a prewashed dialysis membrane tube

(MWCO = 14 kDa) and dialyzed first against a large excess of 100 mM NaCl aqueous solution (3 cycles × 24 h) followed by a 1:3 (v/v) ethanol:water solution (1 cycles × 24 h) and then DIW (3 cycles × 24 h). The final solution was filtered through a 5 μm pore size syringe filter and then lyophilized to dryness. ^{1}H NMR was performed on a Bruker AVANCE 600 MHz spectrometer using D_2O (D, 99.96%, Cambridge Isotope Laboratories, Inc.) as the solvent. Results indicate that ~53% of carboxyl groups on HA were successfully converted to hydrazide groups (Supporting Information, Figure S2). The degree of hydrazide group conversion was also confirmed by base-into-acid conductometric and potentio- metric titration of both HA and HA-Hzd samples (Figure S3), with –COOH functionalization reduced from 2.23 ± 0.13 mM/g in HA to 1.07 ± 0.15 mM/g in HA-Hzd (52% substitution). Furthermore, FTIR spectroscopy using a KBr pellet technique (Nexus 6700 Fourier- transform infrared (FTIR) spectrometer, Thermo Fisher Scientific) indicated a slight shift of the peak around 1630 cm^{-1} (Figure S4), which could be attributed to the formation of a new amide bond between ADH and HA.

2.5. HA-Hzd Coating of Oxidized pHEMA Hydrogel (HA- pHEMA). HA-Hzd was dissolved overnight at 2 mg/mL in DIW under gentle shaking. The pHEMA hydrogel was cut or punched into the required shape, with most samples punched into disks with a diameter of 0.65 cm (1/4 in.) and thickness of ~1 mm unless otherwise specified. For coating, the gel disk was then immersed into 25 mL of HA-Hzd solution. The reaction between aldehyde groups in the hydrogel and hydrazide groups in HA-Hzd was allowed to proceed at room temperatures for 8 h, after which the hydrogel was rinsed with DIW (3 × 5 min cycles). The resulting HA-grafted hydrogel was compared to a noncoated pHEMA hydrogel before and after oxidation using infrared spectroscopy (Nexus 6700 Fourier-transform infrared (FTIR) spectrometer with an attenuated total reflection (ATR) attachment, Thermo Fisher Scientific) and X-ray photoelectron spectroscopy (PHI Quantera II XPS scanning microprobe, Physical Electronics (Phi), Chanhassen, MN, 1486.7 eV monochromatic Al Kα X-ray source, beam diameter 200 μm, 280 eV pass energy) to confirm surface functionalization.

2.6. Fluorescent Visualization of HA Coating on HA-pHEMA Hydrogel. To confirm the successful surface modification, the coating procedure outlined in section 2.5 was repeated by substituting HA- Hzd with FITC-labeled HA-Hzd. HA-Hzd was prepared by reacting a small fraction (1.1 mol %) of Hzd groups in HA-Hzd with fluorescein isothiocyanate (FITC). Typically, 50 mg of HA-Hzd was reacted with 0.25 mg of FITC (1.1 mol % equivalent of the Hzd groups) overnight (at least 12 h) under gentile mechanical agitation in carbonate buffer at pH 9 and room temperature. The resulting labeled polymers were dialyzed exhaustively against deionized water (MWCO = 14 kDa, 6 × 6 h cycles) to remove unbound FITC, lyophilized to dryness, dissolved in water to form a 2 mg/mL solution, and stored at 4 °C in the dark. All reactions and purification steps were performed in aluminum foil- covered reaction flasks or containers to prevent photobleaching during synthesis. Following surface modification of the pHEMA hydrogel with FITC-HA-Hzd as per the protocol in section 2.5, the dried sample was cut in cross section and exposed to blue (488 nm) light using a Zeiss 510 inverted confocal microscope to collect fluorescence images and define the localization of FITC-labeled HA-Hzd throughout the hydrogel.

2.7. Contact Angle. To assess changes in the wettability of the contact lens mimic throughout the HA tethering process, water contact angle measurements were conducted using a Model 100-00-

115 NRL contact angle goniometer (Rame-´Hart, Succasunna, NJ) equipped with a Sanyo VC8-3512T camera. Contact angles were measured by applying 25 μL droplets of DIW on the surface of the original and modified pHEMA hydrogel samples at 23 °C after gently wicking off the excess (i.e., nonbound/absorbed) water using a KimWipe. All the hydrogel samples were kept in OPTI-FREE Replenish contact lens solution for 14 days, changing the solution fresh every second day. Contact angles were tested following 1, 4, 7, and 14 days of soaking.

2.8. Dehydration Kinetics. Dried pHEMA samples were preweighed and then swollen in Milli-Q water for 24 h, a time point confirmed to reach the swelling equilibrium of the hydrogels in water. Following, the sample was removed from the water, wicked with a KimWipe to remove unbound water from the hydrogel surface, and weighed again to allow for calculation of the equilibrium water content. The samples were then incubated vertically on a holder in a constant temperature and humidity chamber to allow for evaporation from both sides (Platinous Sterling Series, ESL-2CA, ESPEC North America, 50% RH at 23 °C) and weighed again at time intervals of 0, 5, 10, 15, 20, 30, 60, 90, and 120 min to track the rate of evaporation as a function of pHEMA surface treatment.

2.9. Refractive Index and Light Transmittance Measure- ments. A digital hand-held pocket refractometer (Atago, Bellevue, WA) was used to measure the refractive index of the pHEMA hydrogel disks, with all measurements done in triplicate. The optical properties of the material were determined by measuring transmittance between 380 and 750 nm using a Beckman Coulter DU800 spectrophotometer (scan rate = 0.5 nm/s).

2.10. Mechanical Properties. The hardness and mechanical strength of samples before and after surface modification were measured to assess the effects of oxidation and HA coating on the hydrogel mechanics. Prior to the test, freshly prepared pHEMA hydrogel samples were soaked in Milli-Q water for 24 h. Shore A (MFG. Co. Inc., U.S. Patent 2453042) and Shore OO (Rex Gauge Company, Inc. U.S. Patent 2421449) durometers were used to characterize the hardness of pHEMA before and after oxidation and HA modification. The elastic modulus and elongation-at-break were characterized using a Universal Test System (INSTRON 3366, 50 N load cell) on dumbbell pHEMA samples (ASTM 638-14/95 Type V) mounted to the tester using grips and subsequently stretched at a crosshead speed of 20 mm/min. The thickness and width of the pHEMA specimens under tension (measured at the center of the dumbbell at three random positions) were analyzed using an electronic digital micrometer (Mitutoyo, Japan; 0.001 mm sensitivity).

2.11. Cell Culture and Viability Test. Cell toxicity was assessed with NIH 3T3 mouse fibroblasts. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) heat-inactivated FBS and 1% (v/v) penicillin–streptomycin at 37 °C under a humidified atmosphere of 5% CO_2. For the cell toxicity assay, 5×10^4 cells/well were seeded in 24-well plates overnight, after which the cell medium was replaced by 500 μL of fresh culture medium. Disk samples of pHEMA, Ox-pHEMA, and HA-pHEMA were sterilized by immersing in ethanol solution (70% v/v) for 4 h and subsequently rinsed three times with sterile PBS prior to being inserted into the wells using tweezers. After 24 h of incubation, media was removed, 20 μL of the MTT solution (5.0 mg/mL in fresh culture medium) was added to each well, plates were wrapped with aluminum foil, and the plates were incubated for ~4 h at 37 °C.

Following, the culture medium was removed, and the resulting formazan crystals were dissolved in solubilization solution (40.0% (v/v) DMF, 16.0% (w/v) SDS, pH ~ 4.7). The absorbance of the solubilized formazan was measured at 570 nm using a Tecan infinite-200M Pro colorimeter (Tecan Co, Switzerland). Cell viability relative to a cell-only control and a well containing only cell media was calculated via the equation

$$\text{viability (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100$$

Six replicates were performed per sample, with the mean ± standard deviation reported for each.

2.12. Protein Adsorption. Proteins (human serum albumin and lysozyme from chicken egg white) were radiolabeled with $Na^{125}I$ using the iodine monochloride method (ICl).[56] Following, unbound ^{125}I was removed by passing the radiolabeled samples through two 3 mL syringes packed with AG 1-X4 resin (100–200 dry mesh in chloride form, Bio-Rad, Hercules, CA). Free iodide was measured by trichloroacetic acid precipitation of the protein. The free iodide in both radiolabeled HSA and lysozyme solutions was, in all cases, less than 1% of total radioactivity. To perform protein adsorption assays, pHEMA hydrogel disks were equilibrated in PBS for 24 h, blotted using a KimWipe to remove nonbound water, and subsequently placed in 96-well plates. HSA or lysozyme solution (1 mg/mL, contained 10% (w/w) radiolabeled protein, 250 mL) was added to the wells (n = 4), and the samples were incubated for 4 h at room temperature (23 °C). The samples were subsequently rinsed with fresh PBS buffer (3 × 5 min cycles) to remove any loosely bound protein. The surfaces were subsequently counted for radioactivity 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.

RESULTS AND DISCUSSION

3.1. Oxidation of pHEMA Hydrogel. The oxidation of primary alcohols with laccase/TEMPO is generally reported to result in the generation of both aldehyde groups and some fraction of carboxyl groups.[52] Therefore, to first confirm the targeted conversion of alcohol to aldehyde, three methods were applied to provide semiquantitative assessments of the aldehyde content. First, the silver mirror reaction is a classical method for identifying aldehyde groups,[57] tracking the reduction (precipitation) of $AgNO_3$ solution to silver nanoparticles by aldehydes to create a black or reflective film on the underlying substrate.[55] Figure 1A shows this characteristic dark black color on Ox-pHEMA, indicating the presence of aldehyde groups; in contrast, almost no color was observed on the native pHEMA hydrogel, indicating the presence of few if any aldehyde groups prior to oxidation. Second, the pHEMA hydrogels were reacted with 5-FTSC, an aldehyde-reactive fluorescent dye previously used to visualize the product of laccase/TEMPO oxidation of cellulose.[58] Figure 1B shows much stronger fluorescence on the Ox-pHEMA hydrogel compared to the native pHEMA hydrogel, again confirming the

generation of aldehyde groups via the laccase/TEMPO reaction. Note that the native pHEMA samples still have some mild background emission, which could be attributed to physically adsorbed fluorescent dye. Third, the content of the aldehydes in Ox-pHEMA was determined by hydroxylamine hydrochloride titration (see Supporting Information, Figure S5).[59] The titration result yields an aldehyde content of 26.7 ± 3.3 μM aldehydes/g of Ox-pHEMA sample, comparable to the aldehyde content in TEMPO-oxidized native cellulose fibers (mechanically fibrillated pulp) reported in previous research.[60] The ATR-FTIR spectra of pHEMA and Ox-pHEMA (Figure 2A,B) further confirm oxidation based on the significant increase in intensity observed for the C O carbonyl stretch at 1631 cm^{-1}, an observation consistent with previous TEMPO oxidation data.[61] While this peak may overlap with the ester signal, the appearance of additional peaks at 2750 cm^{-1} (Figure 2B,D) is consistent with the C–H stretch of aldehydes and offers further evidence of successful oxidation.[62,63] In addition, the change in the shape of the C–O–C peak at 1050–1200 cm^{-1} may suggest the formation of covalent bonds through hemiacetal and/or acetal linkages between aldehyde groups formed by the laccase/TEMPO oxidation and the hydroxyl groups in pHEMA;[60] while this reaction is not necessarily desirable in the context of contact lens modification, its occurrence would confirm the generation of aldehyde groups on Ox-pHEMA. Note that both spectra have a strong peak centered at 1705–1715 cm^{-1} primarily attributable to the ester group in HEMA;[64] the aldehyde group generated by oxidation is likely obscured by overlap with the ester signal. XPS (Figure 3), while unable to distinguish oxidation of C–OH to HC O in terms of atom percent, does indicate no presence of nitrogen in the oxidized pHEMA sample (Figure 3), suggesting that all free TEMPO and laccase have been successfully removed by the washing steps.

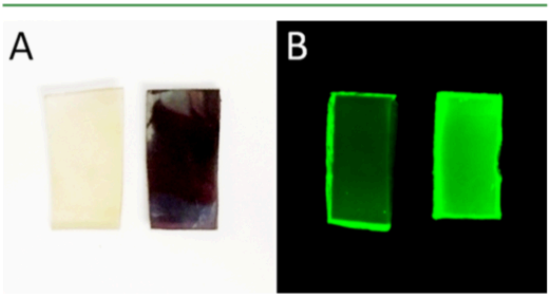


Figure 1. Visualizing the presence of aldehyde group on Ox-pHEMA by (A) silver mirror reaction on native pHEMA (left) and Ox-pHEMA (right); (B) fluorescein-5-thiosemicarbazide (5-FTSC) labeling on native pHEMA (left) and Ox-pHEMA (right).

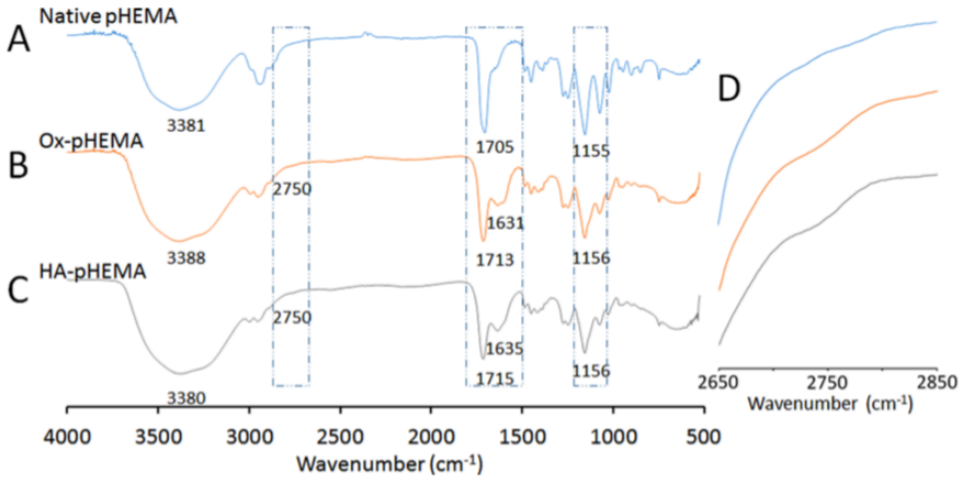


Figure 2. Attenuated total reflection (ATR)-FTIR spectra of (A) native pHEMA, (B) Ox-pHEMA, (C) HA-pHEMA, and (D) zoom of 2650−2850 cm^{-1} region showing evidence for aldehyde functionalization; key peaks discussed in the text are labeled by wavenumber.

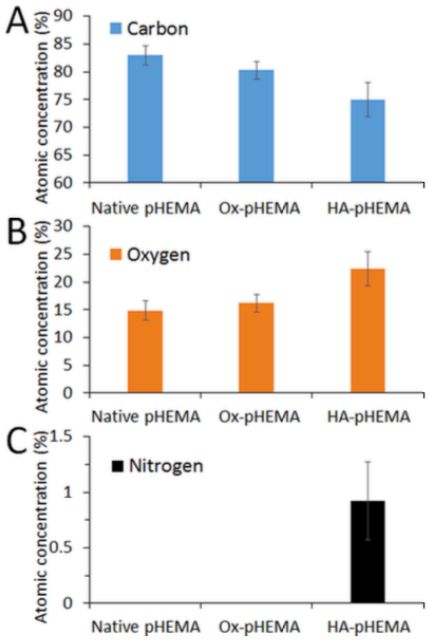


Figure 3. Elemental concentrations of native pHEMA, Ox-pHEMA, and HA-pHEMA from XPS analysis: (A) carbon; (B) oxygen; (C) nitrogen.

3.2. Surface Conjugation of HA-Hzd to Ox-pHEMA Hydrogel. HA-coated pHEMA was prepared by simple immersion of the Ox-pHEMA hydrogel into a 2 mg/mL HA- Hzd solution. An amide band I peak at 1635 cm^{-1} is present in the ATR-FTIR spectra of HA-modified pHEMA sample (Figure 2), consistent with the spectrum of HA-Hzd prior to conjugation (Figure S4); however the overlap of this peak with the C O stretching peak of the carboxyl group in Ox-pHEMA makes this result of limited utility for analysis. However, XPS shows an ~1 atom % nitrogen content only after HA conjugation (Figure 3), suggesting the HA conjugation on Ox-pHEMA gel; furthermore, when FITC-labeled

HA-Hzd is instead used for surface functionalization, confocal microscopy indicates that all bound HA-Hzd is localized at the surface of the model lens, with a continuous coating of FITC-HA-Hzd observed at both interfaces of the lens and no significant amount of HA detected in the bulk (Figure 4). We further confirmed that unfunctionalized HA could exhibits only weak physically adsorbance on either native pHEMA or Ox-pHEMA (Supporting Information, Figure S6), indicating that the chemical bond forming between aldehyde groups on Ox- pHEMA and hydrazide groups on HA-Hzd is primarily driving the tethering of HA on the surface. Thus, by simple dipping of the Ox-pHEMA hydrogel into a HA-Hzd solution, effective surface functionalization of the lens can be achieved. Note that the molecular weight of HA was >300 kDa, making it unlikely that HA will penetrate into the bulk hydrogel (consistent with this result).

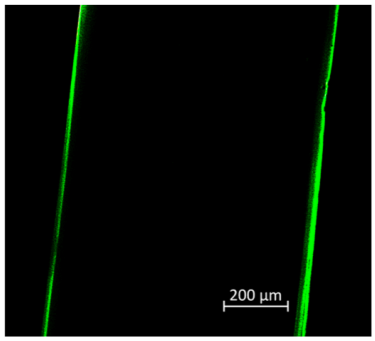


Figure 4. Cross section of pHEMA hydrogel coated with fluorescently labeled HA-Hzd as viewed by confocal laser scanning microscopy (CLSM).

3.3. Optical and Mechanical Properties of Modified pHEMA Hydrogels. Optical properties including refractive index and transparency are critical for materials to be used as contact lenses. The refractive indices of Ox-pHEMA and HA- pHEMA are not significantly different than that of native pHEMA ($p > 0.1$ in both pairwise comparisons), with all samples yielding refractive index values between 1.332 and 1.334 that closely approximate the refractive index of human tears[65] (Figure 5A). Furthermore, UV–vis spectrophotometry indicated that while a slight reduction in transparency was observed for Ox-pHEMA and HA-pHEMA hydrogels relative to native pHEMA, even the modified materials still transmitted more light (>88% transmittance over the full visible range) than the normal adult lens and appeared transparent to visual inspection (Figure 5B).[66] Both these results suggest the potential suitability of these materials for contact lens. From a chemical perspective, the lack of opacity or domain formation indicated by these results suggests that the modifications neither significantly change the bulk material morphology nor lead to the creation of phase-separated domains within the gels, supporting a surface grafting mechanism of modification.

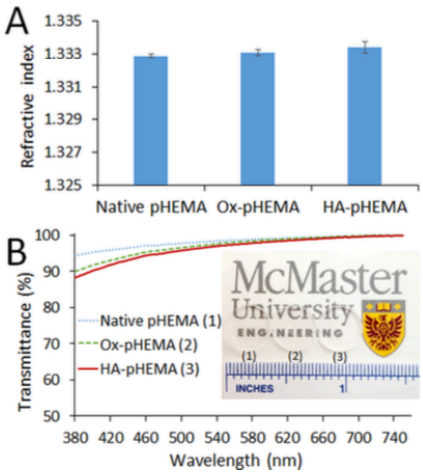


Figure 5. Optical properties of modified pHEMA hydrogels: (A) refractive index; (B) visible light transmittance and (B, inset) visual confirmation of the functional transparency (thickness = ~1 mm) of native pHEMA, Ox-pHEMA, and HA-pHEMA hydrogels.

The mechanics of the pHEMA hydrogel are also largely unaffected by the oxidation and HA tethering, with the compressive modulus, shear modulus, and tensile modulus all showing no significant increase after modification ($p > 0.05$ in all pairwise comparisons) and the softness (hardness) remaining in the same ranges (Table 1). However, the elasticity of the hydrogel (break elongation) did exhibit a significant decrease following laccase/TEMPO oxidation ($p = 0.004$ relative to native pHEMA). We attribute this result to a degree of depolymerization occurring during oxidation, caused by the in situ generation of active species such as hydroxyl radicals in side reactions of the hydroxylamine structure with oxygen during the oxidative treatment.[67] However, this degree of elongation is still similar to those of HEMA/MAA materials most commonly used in daily contact lens applications[68] and thus is not anticipated to be problematic in a practical application.

Table 1. Mechanical Properties of Native pHEMA, Ox-pHEMA, and HA-pHEMA[a]

	hardness		MicroSquisher		tensile test	
	shore A	shore OO	compression modulus (MPa)	shear modulus (MPa)	tensile modulus (MPa)	break elongation (%)
native pHEMA	55–60	85–90	1.76 ± 0.17	0.59 ± 0.06	11.2 ± 2.8	33.6 ± 5.3
Ox- pHEMA	55–60	85–90	2.15 ± 0.82	0.72 ± 0.27	12.2 ± 4.0	15.3 ± 4.5[a]
HA-pHEMA	45–50	85–90	2.65 ± 0.80	0.88 ± 0.26	14.2 ± 4.4	15.7 ± 4.6[a]

[a] $p \ll 0.01$, compared to native pHEMA gel.

3.4. Surface Hydrophilicity and Water Retention. HA modification of pHEMA significantly improved both the surface hydrophilicity and the water retention of the model lens (Figure 6). Relative to unmodified pHEMA (water contact angle 69.7 ± 1.5°), oxidation reduced the contact angle to 41.5 ± 0.9° while subsequent HA grafting further reduced the contact angle to 35.0 ± 1.0° (Figure 6A). Of note, while the contact angle of unmodified pHEMA decreased from 69.7 ± 1.5° to 52.5 ± 0.3° over 14 days of storage in a contact lens care solution (likely attributable to the physical absorption of organic acids

in the care solutions to surface hydroxyl groups), there was no significant change in the contact angles of Ox- pHEMA over this two week period in the same care solution ($p > 0.05$), while for HA-pHEMA, the contact angles show a small but significant decrease after 7 days of storage in the care solution to 31.3 ± 1.2° on day 7 and 31.6 ± 1.9° on day 14 (both $p < 0.05$ relative to the initial gel). Fundamentally, this result suggests the oxidized and HA-grafted interfaces have lower nonspecific adsorption and can maintain a higher degree of hydration at the surface. Practically, the more stable contact angles observed following soaking of the Ox-pHEMA and HA- pHEMA model lenses indicate that the products of the laccase/ TEMPO oxidation and the HA coating step both remain intact and functional following 2 weeks of soaking in the care solution, suggesting the potential suitability of these materials as biweekly deposable lenses and/or for extended storage. This higher interfacial hydrophilicity in HA-pHEMA also acts to retard the evaporation of water from pHEMA hydrogels (50% RH in air, 23 °C), with HA-pHEMA in particular exhibiting a significantly lower rate of water evaporation relative to unmodified and base pHEMA hydrogels (Figure 6B). These observations, both strongly contributing to the comfort of the lens for the wearer, are all consistent with previous surface modifications of contact lenses with HA.[24]

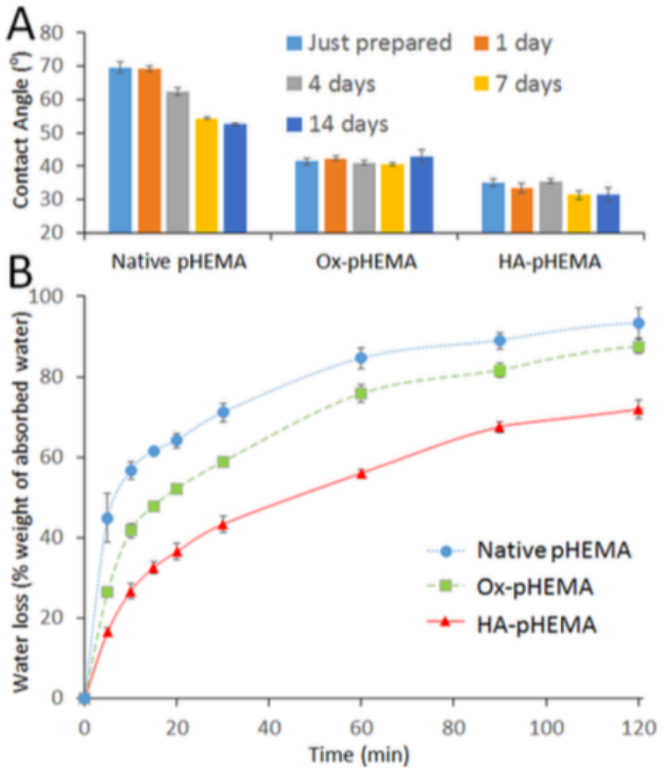


Figure 6. Hydrophilicity of pHEMA as a function of surface treatment: (A) water contact angle of native pHEMA, Ox-pHEMA, and HA-pHEMA; (B) water loss from native pHEMA relative to oxidized and HA-grafted pHEMA in a controlled temperature/humidity chamber (50% RH, 23 °C).

3.5. Cell Compatibility and Protein Adsorption. No significant cytotoxicity was observed for 3T3 fibroblasts (used as corneal feeder cells when cultured on contact lenses[69]) after 24 h of culturing in the presence of Ox-pHEMA and HA- pHEMA hydrogels (Figure 7). This result confirms the effective removal of free TEMPO and laccase via the rinsing process used and, in the case of HA-pHEMA, is consistent with the noted compatibility of HA in culturing ocular cells.[30] Furthermore, protein adsorption to HA-pHEMA lenses (key to reducing fouling and optimizing wear times) was reduced by ~25% for HSA and ~35% for lysozyme compared to the native pHEMA hydrogel following 4 h of adsorption time (Figure 8). We have further tested HSA adsorption over both 12 and 24 h periods using fluorescently labeled protein to assess the potential longer term impact of any residual aldehydes in the Ox-pHEMA gel on protein adsorption (Supporting Information, Figure S7). While protein adsorption increases with time for both native pHEMA and oxidized pHEMA (the latter of which presents aldehyde groups directly at the interface), HA- pHEMA shows no significant change in protein adsorption ($p = 0.08$) as a function of time, maintaining its low HSA adsorption over even the longer test periods. This result suggests that HA effectively screens any residual aldehyde groups on Ox-pHEMA to avoid any potential issues with imine-promoted protein deposition over time and is consistent with previous research indicating that HA modification reduces protein adsorption on various types of biomaterials.[22,23,25–27] Thus, HA modification of the model lens surface through our click chemistry technique significantly reduces protein adsorption while increasing wetting without inducing any significant cytotoxicity, suggesting the potential translatability of this coating to practical applications.

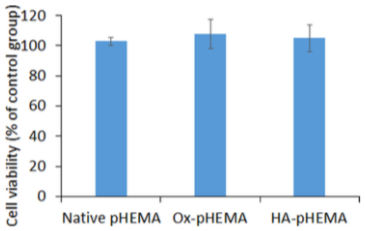


Figure 7. Cell viability (relative to cell-only control) of 3T3 fibroblasts in the presence of native pHEMA, Ox-pHEMA, and HA-pHEMA hydrogel disks via the MTT assay.

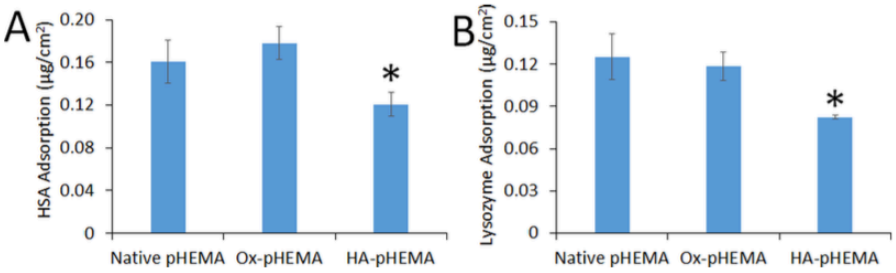


Figure 8. Protein adsorption on the surface of native pHEMA, Ox-pHEMA, and HA-pHEMA in 4 h at 23 °C: (A) human serum albumin (HSA, 1.0 mg/mL); (B) lysozyme (1.0 mg/mL). *$p < 0.01$, compared to the native pHEMA group.

4. CONCLUSIONS

In this study, we demonstrate a facile click chemistry-based strategy for covalently tethering an HA layer on the surface of pHEMA hydrogel that mimics a daily wear contact lens. Only twostepsarerequiredinourcoatingmethod:laccase/TEMPO oxidation and subsequent dipping in a hydrazide-functionalized HA solution. The resulting modified surfaces exhibit signifi- cantly improved wettability and water retention and reduced protein adsorption while largely maintaining the favorable transparency, refractive, mechanical, and storage stability properties of pHEMA. Given the capacity for efficient surface modification provided by this simple dipping or coating process, we anticipate that our method has potential for translation to industrial contact lens manufacturing.

Supporting Information The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.6b07433.

GPC curve of HA, ^{1}H NMR and FTIR spectra of HA and HA-Hzd, titration curves of HA and HA-Hzd, hydroxylamine hydrochloride titration of native pHEMA and Ox-pHEMA, adsorption of fluorescently labeled HA/HA-Hzd on native pHEMA and Ox-pHEMA, and adsorption of fluorescently labeled HSA on native pHEMA, Ox-pHEMA, and HA-pHEMA (PDF)

AUTHOR INFORMATION Corresponding Authors

*E-mail filipec@mcmaster.ca (C.D.M.F.). *E-mail hoaretr@mcmaster.ca (T.H.).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

The authors thank Ms. Fei Xu and Ms. Maryam Badv for their generous help with the mechanical and NMR tests. The authors thank the Natural Sciences and Engineering Research Council of Canada (CREATE Biointerfaces Training Program- 10532261, and Discovery Grants RGPIN-356609-12 and RGPIN-261686-13), the Sentinel Bioactive Paper Network (NSERC NETGP 398408), the Canada Foundation for Innovation (Leaders Opportunity Fund Grant # 29131), and the Ontario Innovation Trust for financial support.

REFERENCES

(1) Wichterle, O.; Lim, D. Hydrophilic Gels for Biological Use. Nature 1960, 185, 117–118.

(2) Lai, Y. C.; Friends, G. D. Surface Wettability Enhancement of Silicone Hydrogel Lenses by

Processing with Polar Plastic Molds. J. Biomed. Mater. Res. 1997, 35, 349–356.

(3)Loṕez-Alemany,A.;Compañ,V.;Refojo,M.F.PorousStructure of Purevision Versus Focus® Night&Day and Conventional Hydrogel Contact Lenses. J. Biomed. Mater. Res. 2002, 63, 319–325.

(4) Nicolson, P. C.; Vogt, J. Soft Contact Lens Polymers: An Evolution. Biomaterials 2001, 22, 3273–3283.

(5) Pritchard, N.; Fonn, D. Dehydration, Lens Movement and Dryness Ratings of Hydrogel Contact Lenses. Ophthal. Physiol. Opt. 1995, 15, 281–286.

(6) Brennan, N. A.; Efron, N. Symptomatology of Hema Contact Lens Wear. Optom. Vision Sci. 1989, 66, 834–838.

(7) Luensmann, D.; Jones, L. Albumin Adsorption to Contact Lens Materials: A Review. Cont. Lens. Anterior. Eye 2008, 31, 179–187.

(8) Luensmann, D.; Jones, L. Protein Deposition on Contact Lenses: The Past, the Present, and the Future. Cont. Lens. Anterior. Eye 2012, 35, 53–64.

(9) Lord, M. S.; Stenzel, M. H.; Simmons, A.; Milthorpe, B. K. The Effect of Charged Groups on Protein Interactions with Poly (Hema) Hydrogels. Biomaterials 2006, 27, 567–575.

(10) Taylor, R. L.; Willcox, M. D.; Williams, T. J.; Verran, J. Modulation of Bacterial Adhesion to Hydrogel Contact Lenses by Albumin. Optom. Vis. Sci. 1998, 75, 23–29.

(11) Miller, M. J.; Wilson, L. A.; Ahearn, D. G. Effects of Protein, Mucin, and Human Tears on Adherence of Pseudomonas Aeruginosa to Hydrophilic Contact Lenses. J. Clin. Microbiol. 1988, 26, 513–517.

(12) Tan, M. E.; Demirci, G.; Pearce, D.; Jalbert, I.; Sankaridurg, P.; Willcox, M. D. Contact Lens-Induced Papillary Conjunctivitis Is Associated with Increased Albumin Deposits on Extended Wear Hydrogel Lenses. In Lacrimal Gland, Tear Film, and Dry Eye Syndromes 3; Springer: 2002; pp 951–955.

(13) Fonn, D. Targeting Contact Lens Induced Dryness and Discomfort: What Properties Will Make Lenses More Comfortable. Optom. Vis. Sci. 2007, 84, 279–285.

(14) Papas, E. B.; Ciolino, J. B.; Jacobs, D.; Miller, W. L.; Pult, H.; Sahin, A.; Srinivasan, S.; Tauber, J.; Wolffsohn, J. S.; Nelson, J. D. The Tfos International Workshop on Contact Lens Discomfort: Report of

the Management and Therapy Subcommitteemanagement and Treatment of Cld. Invest. Ophthalmol. Visual Sci. 2013, 54, TFOS183–TFOS203.

(15) Thissen, H.; Gengenbach, T.; du Toit, R.; Sweeney, D. F.; Kingshott, P.; Griesser, H. J.; Meagher, L. Clinical Observations of Biofouling on Peo Coated Silicone Hydrogel Contact

Lenses. Biomaterials 2010, 31, 5510–5519.

(16) Goda, T.; Matsuno, R.; Konno, T.; Takai, M.; Ishihara, K. Protein Adsorption Resistance and Oxygen Permeability of Chemically Crosslinked Phospholipid Polymer Hydrogel for Ophthalmologic Biomaterials. J. Biomed. Mater. Res., Part B 2009, 89, 184–190.

(17) Shimizu, T.; Goda, T.; Minoura, N.; Takai, M.; Ishihara, K. Super-Hydrophilic Silicone Hydrogels with Interpenetrating Poly (2- Methacryloyloxyethyl Phosphorylcholine) Networks. Biomaterials 2010, 31, 3274–3280.

(18) Winterton, L. C.; Lally, J. M.; Sentell, K. B.; Chapoy, L. L. The Elution of Poly (Vinyl Alcohol) from a Contact Lens: The Realization of a Time Release Moisturizing Agent/Artificial Tear. J. Biomed. Mater. Res., Part B 2007, 80, 424–432.

(19) Peterson, R. C.; Wolffsohn, J. S.; Nick, J.; Winterton, L.; Lally, J. Clinical Performance of Daily Disposable Soft Contact Lenses Using Sustained Release Technology. Cont. Lens. Anterior. Eye 2006, 29, 127–134.

(20) Xu, L.; Ma, P.; Yuan, B.; Chen, Q.; Lin, S.; Chen, X.; Hua, Z.; Shen, J. Anti-Biofouling Contact Lenses Bearing Surface-Immobilized Layers of Zwitterionic Polymer by One-Step Modification. RSC Adv. 2014, 4, 15030–15035.

(21) Walker, J.; Young, G.; Hunt, C.; Henderson, T. Multi-Centre Evaluation of Two Daily Disposable Contact Lenses. Cont. Lens. Anterior. Eye 2007, 30, 125–133.

(22) Weeks, A.; Luensmann, D.; Boone, A.; Jones, L.; Sheardown, H. Hyaluronic Acid as an Internal Wetting Agent in Model Dmaa/Tris Contact Lenses. J. Biomater. Appl. 2012, 27, 423–432.

(23) Weeks, A.; Morrison, D.; Alauzun, J. G.; Brook, M. A.; Jones, L.; Sheardown, H. Photocrosslinkable Hyaluronic Acid as an Internal Wetting Agent in Model Conventional and Silicone Hydrogel Contact Lenses. J. Biomed. Mater. Res., Part A 2012, 100, 1972–1982.

(24) Singh, A.; Li, P.; Beachley, V.; McDonnell, P.; Elisseeff, J. H. A Hyaluronic Acid-Binding Contact Lens with Enhanced Water Retention. Cont. Lens. Anterior. Eye 2015, 38, 79–84.

(25) Van Beek, M.; Jones, L.; Sheardown, H. Hyaluronic Acid Containing Hydrogels for the Reduction of Protein Adsorption. Biomaterials 2008, 29, 780–789.

(26) Van Beek, M.; Weeks, A.; Jones, L.; Sheardown, H. Immobilized Hyaluronic Acid Containing Model Silicone Hydrogels Reduce Protein Adsorption. J. Biomater. Sci., Polym. Ed. 2008, 19, 1425–1436.

(27) Kim, H.-J.; Ryu, G.-C.; Jeong, K.-S.; Jun, J. Hydrogel Lenses Functionalized with Polysaccharide for Reduction of Protein Adsorption. Macromol. Res. 2015, 23, 74–78.

(28) Nishida, T.; Nakamura, M.; Mishima, H.; Otori, T. Hyaluronan Stimulates Corneal Epithelial Migration. Exp. Eye Res. 1991, 53, 753– 758.

(29) Yokoi, N.; Komuro, A.; Nishida, K.; Kinoshita, S. Effectiveness of Hyaluronan on Corneal Epithelial Barrier Function in Dry Eye. Br. J. Ophthalmol. 1997, 81, 533–536.

(30) Pauloin, T.; Dutot, M.; Joly, F.; Warnet, J.-M.; Rat, P. High Molecular Weight Hyaluronan Decreases Uvb-Induced Apoptosis and Inflammation in Human Epithelial Corneal Cells. Mol. Vis. 2009, 15, 577–583.

(31) Presti, D.; Scott, J. E. Hyaluronan-Mediated Protective Effect against Cell Damage Caused by Enzymatically Produced Hydroxyl (Oh·) Radicals Is Dependent on Hyaluronan Molecular Mass. Cell Biochem. Funct. 1994, 12, 281–288.

(32) Aragona, P.; Papa, V.; Micali, A.; Santocono, M.; Milazzo, G. Long Term Treatment with Sodium Hyaluronate-Containing Artificial Tears Reduces Ocular Surface Damage in Patients with Dry Eye. Br. J. Ophthalmol. 2002, 86, 181–184.

(33) Weeks, A.; Boone, A.; Luensmann, D.; Jones, L.; Sheardown, H. The Effects of Hyaluronic Acid Incorporated as a Wetting Agent on Lysozyme Denaturation in Model Contact Lens Materials. J. Biomater. Appl. 2013, 28, 323–333.

(34) Korogiannaki, M.; Sheardown, H. Surface Modified Contact Lenses with a Wetting Agent for Improved Comfort. Invest. Ophthalmol. Visual Sci. 2014, 55, 4658–4658.

(35) Kolb, H. C.; Finn, M.; Sharpless, K. B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. Angew. Chem., Int. Ed. 2001, 40, 2004–2021.

(36) Smeets, N. M.; Bakaic, E.; Patenaude, M.; Hoare, T. Injectable and Tunable Poly (Ethylene Glycol) Analogue Hydrogels Based on Poly (Oligoethylene Glycol Methacrylate). Chem. Commun. 2014, 50, 3306–3309.

(37) Smeets, N. M.; Bakaic, E.; Patenaude, M.; Hoare, T. Injectable Poly (Oligoethylene Glycol Methacrylate)-Based Hydrogels with Tunable Phase Transition Behaviours: Physicochemical and Biological Responses. Acta Biomater. 2014, 10, 4143–4155.

(38) Patenaude, M.; Hoare, T. Injectable, Degradable Thermores- ponsive Poly (N-Isopropylacrylamide) Hydrogels. ACS Macro Lett. 2012, 1, 409–413.

(39) Patenaude, M.; Hoare, T. Injectable, Mixed Natural-Synthetic Polymer Hydrogels with Modular Properties. Biomacromolecules 2012, 13, 369–378.

(40) Xu, F.; Sheardown, H.; Hoare, T. Reactive Electrospinning of Degradable Poly (Oligoethylene Glycol Methacrylate)-Based Nano- fibrous Hydrogel Networks. Chem. Commun. 2016, 52, 1451–1454.

(41) Deng, X.; Smeets, N. M.; Sicard, C.; Wang, J.; Brennan, J. D.; Filipe, C. D.; Hoare, T. Poly (Oligoethylene Glycol Methacrylate) Dip-Coating: Turning Cellulose Paper into a Protein-Repellent Platform for Biosensors. J. Am. Chem. Soc. 2014, 136, 12852–12855.

(42) Pouyani, T.; Prestwich, G. D. Functionalized Derivatives of Hyaluronic Acid

Oligosaccharides: Drug Carriers and Novel Biomaterials. Bioconjugate Chem. 1994, 5, 339–347.

(43) Pouyani, T.; Harbison, G. S.; Prestwich, G. D. Novel Hydrogels of Hyaluronic Acid: Synthesis, Surface Morphology, and Solid-State Nmr. J. Am. Chem. Soc. 1994, 116, 7515–7522.

(44) Bajaj, G.; Kim, M. R.; Mohammed, S. I.; Yeo, Y. Hyaluronic Acid-Based Hydrogel for Regional Delivery of Paclitaxel to Intra- peritoneal Tumors. J. Controlled Release 2012, 158, 386–392.

(45) Oh, E. J.; Kang, S. W.; Kim, B. S.; Jiang, G.; Cho, I. H.; Hahn, S. K. Control of the Molecular Degradation of Hyaluronic Acid Hydrogels for Tissue Augmentation. J. Biomed. Mater. Res., Part A 2008, 86, 685–693.

(46) Prestwich, G. D.; Marecak, D. M.; Marecek, J. F.; Vercruysse, K. P.; Ziebell, M. R. Controlled Chemical Modification of Hyaluronic Acid: Synthesis, Applications, and Biodegradation of Hydrazide Derivatives. J. Controlled Release 1998, 53, 93–103.

(47) Luo, Y.; Kirker, K. R.; Prestwich, G. D. Cross-Linked Hyaluronic Acid Hydrogel Films: New Biomaterials for Drug Delivery. J. Controlled Release 2000, 69, 169–184.

(48) Noolandi, J.; Ta, C.; Huie, Jr., P.; Smith, A. J.; Waymouth, R.; Blumenkranz, M. Artificial Cornea. U.S. Patent No. 6976997B2, 2005. (49) Bragd, P.; Van Bekkum, H.; Besemer, A. Tempo-Mediated Oxidation of Polysaccharides: Survey of Methods and Applications.

Top. Catal. 2004, 27, 49–66. (50) Kitaoka, T.; Isogai, A.; Onabe, F. Chemical Modification of Pulp

Fibers by Tempo-Mediated Oxidation. Nord. Pulp Pap. Res. J. 1999, 14, 279–284.

(51) Fabbrini, M.; Galli, C.; Gentili, P.; Macchitella, D. An Oxidation of Alcohols by Oxygen with the Enzyme Laccase and Mediation by Tempo. Tetrahedron Lett. 2001, 42, 7551–7553.

(52) Aracri, E.; Valls, C.; Vidal, T. Paper Strength Improvement by Oxidative Modification of Sisal Cellulose Fibers with Laccase–Tempo System: Influence of the Process Variables. Carbohydr. Polym. 2012, 88, 830–837.

(53) Shi, S.; Pelton, R.; Fu, Q.; Yang, S. Comparing Polymer- Supported Tempo Mediators for Cellulose Oxidation and Subsequent Polyvinylamine Grafting. Ind. Eng. Chem. Res. 2014, 53, 4748–4754.

(54) Benet, W. E.; Lewis, G. S.; Yang, L. Z.; Hughes, P. D. The Mechanism of the Reaction of the Tollens Reagent. J. Chem. Res. 2011, 35, 675–677.

(55) Shi, Y.; Li, Y.; Zhang, J.; Yu, Z.; Yang, D. Electrospun Polyacrylonitrile Nanofibers Loaded with Silver Nanoparticles by Silver Mirror Reaction. Mater. Sci. Eng., C 2015, 51, 346–355.

(56) Ceska, M.; Sjödin, A.; Grossmüller, F. Some Quantitative Aspects of the Labelling of

Proteins with 125i by the Iodine Monochloride Method. Biochem. J. 1971, 121, 139–143.

(57) Siggia, S.; Segal, E. Determination of Aldehydes in Presence of Acids, Ketones, Acetals, and Vinyl Ethers. Anal. Chem. 1953, 25, 640– 642.

(58) Liu, J.; Pelton, R.; Obermeyer, J. M.; Esser, A. Laccase Complex with Polyvinylamine Bearing Grafted Tempo Is a Cellulose Adhesion Primer. Biomacromolecules 2013, 14, 2953–2960.

(59) Zhao, H.; Heindel, N. D. Determination of Degree of Substitution of Formyl Groups in Polyaldehyde Dextran by the Hydroxylamine Hydrochloride Method. Pharm. Res. 1991, 8, 400–402.

(60) Saito, T.; Isogai, A. Introduction of Aldehyde Groups on Surfaces of Native Cellulose Fibers by Tempo-Mediated Oxidation. Colloids Surf., A 2006, 289, 219–225.

(61) Gomez-Bujedo, S.; Fleury, E.; Vignon, M. R. Preparation of Cellouronic Acids and Partially Acetylated Cellouronic Acids by Tempo/Naclo Oxidation of Water-Soluble Cellulose Acetate. Biomacromolecules 2004, 5, 565–571.

(62)Mansur,H.S.;Oref´ice,R.L.;Mansur,A.A.Characterizationof Poly (Vinyl Alcohol)/Poly (Ethylene Glycol) Hydrogels and Pva- Derived Hybrids by Small-Angle X-Ray Scattering and Ftir Spectros- copy. Polymer 2004, 45, 7193–7202.

(63) Mansur, H. S.; Sadahira, C. M.; Souza, A. N.; Mansur, A. A. Ftir Spectroscopy Characterization of Poly (Vinyl Alcohol) Hydrogel with Different Hydrolysis Degree and Chemically Crosslinked with Glutaraldehyde. Mater. Sci. Eng., C 2008, 28, 539–548.

(64) Castillo, E.; Koenig, J.; Anderson, J.; Kliment, C.; Lo, J. Surface Analysis of Biomedical Polymers by Attenuated Total Reflectance- Fourier Transform Infra-Red. Biomaterials 1984, 5, 186–193.

(65) Craig, J.; Simmons, P.; Patel, S.; Tomlinson, A. Refractive Index and Osmolality of Human Tears. Optom. Vis. Sci. 1995, 72, 718–724.

(66) van den Berg, T. J.; Spekreijse, H. Near Infrared Light Absorption in the Human Eye Media. Vision Res. 1997, 37, 249–253.

(67) Shibata, I.; Isogai, A. Depolymerization of Cellouronic Acid During Tempo-Mediated Oxidation. Cellulose 2003, 10, 151–158.

(68) Tranoudis, I.; Efron, N. Tensile Properties of Soft Contact Lens Materials. Cont. Lens. Anterior. Eye 2004, 27, 177–191.

(69) Gore, A.; Horwitz, V.; Gutman, H.; Tveria, L.; Cohen, L.; Cohen-Jacob, O.; Turetz, J.; McNutt, P. M.; Dachir, S.; Kadar, T. Cultivation and Characterization of Limbal Epithelial Stem Cells on Contact Lenses with a Feeder Layer: Toward the Treatment of Limbal Stem Cell Deficiency. Cornea 2014, 33, 65–71.