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Surface-Functionalized Model Contact Lenses with a Bioinspired Proteoglycan 4 (PRG4)-Grafted Layer

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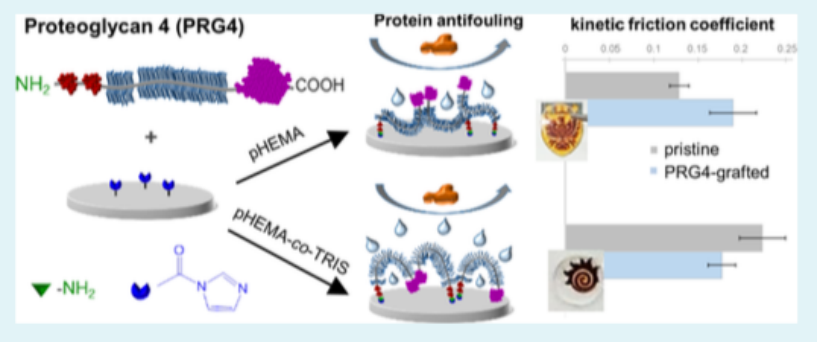
Ocular dryness and discomfort are the primary reasons for the discontinuation of contact lens wear. This is mainly due to poorly hydrated contact lens surfaces and increased friction, particularly at the end of the day and can potentially cause reduced vision or even inflammation. Proteoglycan 4 (PRG4) is a mucinous glycoprotein with boundary lubricating properties, naturally found in the eye, able to prevent tear film evaporation and protect the ocular surface during blinking. Aiming to improve the interfacial interactions between the ocular surface and the contact lens, the synthesis and characterization of surface-modified model contact lenses with PRG4 is described. Full-length recombinant human PRG4 (rhPRG4) was successfully grafted onto the surface of model conventional and silicone hydrogel (SiHy) contact lenses via its somatomedin B-like end-domain using N,N′-carbonyldiimidazole linking chemistry. Grafting was assessed by Fourier transform infrared spectroscopy-attenuated total reflectance, X-ray photoelectron spectroscopy, and radioactive (I^{131}) labeling. Surface immobilization of rhPRG4 led to model conventional and SiHy materials with improved antifouling properties, without impacting optical transparency or causing any toxic effects to human corneal epithelial cells in vitro. The surface wettability and the boundary friction against human corneal tissue were found to be substrate-dependent, with only the rhPRG4-grafted model SiHy exhibiting a reduced contact angle and kinetic friction coefficient compared to the unmodified surfaces. Hence, clinical grade rhPRG4 can be an attractive candidate for the development of novel bioinspired SiHy contact lenses, providing improved comfort and overall lens performance.



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INTRODUCTION:

Despite years of research, soft contact lens materials still suffer from significant limitations. Conventional hydrogel contact lenses, comprised of 2-hydroxyethyl methacrylate (HEMA) as well as methacrylic acid, N-vinylpyrrolidone, vinyl alcohol, or synthetic analogues, such as phosphorylcholine or glycerol methacrylate to obtain high water content and low modulus.[1] However, even the highest water content conventional hydrogel contact lens materials do not possess sufficient oxygen permeability for extended wear. Moreover, high water content hydrogel contact lenses exhibit low tear strength, increased protein deposits, and high dehydration rates, especially in dry environments.[2] Therefore, silicone hydrogel (SiHy) contact lenses were developed to overcome the oxygen permeability limitations of conventional hydrogel materials containing siloxane and/or fluorine polymer segments, these materials were designed to allow overnight wear.[3] The biggest issue with these materials, though, is the surface mobility of the hydrophobic siloxane components which make surface modification necessary to increase wettability and decrease the fouling that occurs in biological environments.[4]

Yet, ocular dryness and discomfort remain significant problems for wearers of both contact lens types, especially towards the end of the day,[5] despite the progress made to improve the composition and surface characteristics of commercial contact lenses. Such symptoms are a major factor limiting their use or even leading to the discontinuation of contact lens wear.[6,7] Upon contact lens insertion into the eye, there is a dynamic interaction between the ocular surface, the tear film, and the contact lens surface that plays an important role for the overall contact lens performance.[8,9] Therefore, tear-related factors such as changes in biophysical and biochemical properties[10] as well as lens-related properties, including surface wettability, effective lens modulus and lubricity[11] must be considered. Clinical signs of the ocular surface, such as lid-wiper epitheliopathy and lid parallel conjunctival folds, that are associated with dryness and discomfort during contact lens wear[12,13] have been studied to determine their relationship with the contact lens

properties, including friction. Interestingly, contact lens lubricity was found in some studies to be the principal lens property associated with contact lens discomfort.[11,14–16]

In this study, surface modification was used to create an interface inspired by the corneal surface and tear film using a lubricating mucin-like glycoprotein that is naturally found on the ocular surface. Proteoglycan 4 (PRG4), also known as lubricin, is an extended polymeric nanostructured protein with a molecular weight (MW) of approximately 230 kDa. It has a hydrophobic globular somatomedin B (SMB)-like (N- terminus) and a hemopexin (PEX)-like (C-terminus) end- domain, and a central mucinous region that is extensively glycosylated with β(1–3)Gal-GalNAc and partially capped with sialic acid (NeuAc).[17] The abundant negatively charged and hydrated sugars of the central domain generate strong steric forces as well as repulsive forces through hydration that play an essential role in lubricin's lubricating properties, while providing high degree of hydrophilicity.[18] PRG4 is able to also lubricate in the absence of a thick fluid film and hence acts as a boundary lubricant. Under physiological conditions (pH 7.4), PRG4 has a small net positive charge, with a reported isoelectric point in the range of 7.8–8.1.[19]

Lubricin is a major component of synovial fluid and as a boundary lubricant in the joints plays a critical role in the protection of cartilaginous surfaces against frictional forces, cell adhesion, and protein deposition.[20] In the eye, PRG4 was found to be transcribed, translated, and expressed by corneal and conjunctival epithelial cells[21] and in human meibomian gland secretions,[22] suggesting that this molecule is responsible for preventing tear film evaporation and for protecting the ocular surface against the significant shear forces generated during blinking. Recently, a 2 week, randomized double- masked study showed that lubricin supplementation rapidly led to significantly reduced signs and symptoms of dry eye disease without causing any adverse events, by improving the damage of the ocular surface epithelium, reducing inflammation on the eyelid and the conjunctiva as well as restoring a competent tear film.[23] PRG4 has also been previously shown to reduce the friction between the cornea–eyelid interface in vitro, further supporting its role as a natural boundary lubricant.[21] Interestingly, when the lubrication properties of PRG4 were examined for a hydrogel–cornea biointerface the results observed varied with the contact lenses, depending on the nature of the hydrogel material and more specifically its surface properties.[24–26] Despite the good boundary lubrication of PRG4 for SiHy, the surface lubricity of conventional hydrogel contact lenses with hydrophilic surfaces was not improved by the presence of physiosorbed PRG4.[26,27] Therefore, it is suggested that not all surfaces are good candidates for PRG4 to function as a boundary lubricant. Depending on the surface chemistry and charge, the conformational and lubricating properties of PRG4 differ.[17,28,29] The lubricating properties of PRG4 are postulated to occur due to glycoprotein's ability to strongly adhere to the surface in an appropriate conformation. It has been shown that the physical sorption of PRG4 on a hydrophobic or negatively charged surfaces results in the formation of a loop-like conformation with friction-lowering behavior, whereas on

hydrophilic surfaces the molecule adopts an extended tail-like conformation mitigating its lubricating properties.[17,28,29] For positively charged surfaces, the con- formation was speculated to be more complicated as both the central mucin domain and the end domain can be adsorbed.[17,28] Overall, PRG4 adhesion to the substrate is thought to occur by hydrogen bonding, hydrophobic as well as electrostatic interactions.[17,28,29]

In this work, full-length recombinant human PRG4 (rhPRG4) was covalently tethered to model conventional and SiHy contact lens materials. We hypothesize that the covalent attachment of rhPRG4 on the surface would improve the surface wettability, lubricity, and resistance to protein deposition under physiological conditions, without compromising the bulk properties of the materials. PRG4 was grafted to the surface via its somatomedin B (SMB)-like (N-terminus) end-domain using N,N′-carbonyldiimidazole (CDI) linking chemistry. Full-length rhPRG4 was selected as it is characterized by an appropriate high order structure, O-linked glycosylation, and boundary lubricating properties consistent with those of native PRG4 found on the ocular surface.[25] Recently, clinically tested full-length rhPRG4 was found to effectively reduce dry eye signs and symptoms, when applied in the form of eye drops.[23] In addition, using CDI linking chemistry allowed for the activity of surface tethered biological molecules to be maintained[30,31] without causing any adverse effect on human corneal epithelial cells (HCECs).[32] It is therefore expected that these coated hydrogel materials may show better ocular compatibility than previous surface- modified contact lenses, providing insight into the nature of surfaces necessary for end-of-day comfort in contact lens wearers. To the best of our knowledge, this is the first time that PRG4 has been grafted to the surface of soft polymeric materials.

EXPERIMENTAL SECTION

2.1. Chemicals and Reagents. 3-Methacryloxypropyl-tris- (trimethylsiloxy)silane (TRIS, ≥95%) was supplied by Gelest (Morrisville, PA). The photoinitiator 1-hydroxy-cyclohexyl-phenyl-ketone (Irgacure 184) was generously donated by BASF Chemical Company (Vandalia, IL). Keratinocyte serum-free medium (K-SFM) supplemented with human recombinant epidermal growth factor 1– 53 (EGF 1–53) and bovine pituitary extract (BPE) were purchased from Thermo Fisher Scientific (Burlington, ON, Canada). The human corneal epithelial cell line (HCE-2 [50.B1] ATCC CRL- 11135) was purchased from the American Type Culture Collection (ATCC, Rockville, MD). Full-length recombinant human PRG4 (rhPRG4) was a kind gift of Lubris BioPharma LLC (Boston, MA).[25,33] Human corneas (age: 63–86) that were harvested and stored in OptiSol-GS (Bausch & Lomb, Rochester, NY) at 4°C prior to testing[24] were provided by the Southern Alberta Lions Eye Bank. These tissues were tested within 2 weeks of harvest. Approval for tissue use was granted by the University of Calgary Conjoint Health Research Ethics Board. All other chemicals, reagents, and proteins used were obtained from Sigma Aldrich (Oakville, ON, Canada).

2.2. Synthesis of Model Poly(HEMA) (pHEMA) and pHEMA- co-TRIS Hydrogel Materials. Prior to synthesis, 2-hydroxyethyl methacylate (HEMA), TRIS, and ethylene glycol

dimethacrylate (EGDMA) were passed through a custom-made column filled with an inhibitor remover for the removal of monomethyl ether hydro- quinone. Model pHEMA hydrogels were generated by mixing the HEMA monomer, the EGDMA crosslinker (2 mol %), and the photoinitiator Irgacure 184 (0.5 wt %) together for 15 min. For the synthesis of model SiHy pHEMA-co-TRIS, HEMA and TRIS were added in a 90:10 weight ratio and mixed with EGDMA (3.5 mol %) under vigorous stirring conditions for 30 min, followed by the dissolution of the photoinitiator Irgacure 184 (0.5 wt %). The prepolymer mixture of either pHEMA or pHEMA-co-TRIS was then injected into a custom-made UV-transparent acrylic mold which was in turn placed into a 400 W UV chamber (365 nm, Cure Zone 2 Con- Trol-Cure, Chicago, IL) for 10 min for the polymerization reaction. The thickness of the resulting disks could be controlled by using an adjustable spacer in the acrylic mold. Following initiation of polymerization, the hydrogel materials were postcured overnight at room temperature, demolded, and immersed in Milli-Q water for 18 h. Swollen hydrogels were cut using a cork borer into disks with a diameter of 6.35 mm (1/4 in.) and a thickness of 0.5 mm and used for all the experiments with the exception of the friction measurement where the disks used were 7.94 mm (5/16 in.) in diameter and 1 mm thick due to experimental restrictions. For the extraction of unreacted chemicals, disks were initially placed into a 1:1 vol % methanol/water solution for 12 h and then into Milli-Q water for 36 h. Disks were dried at room temperature until further use.

2.3. Synthesis of CDI-Activated pHEMA and pHEMA-co-TRIS Hydrogels. Prior to the CDI activation step, both pHEMA and pHEMA-co-TRIS disks were dried under vacuum overnight at 25 °C, while the vials and pipettes used were rinsed with acetone and dried overnight at 70 °C to eliminate any trace of humidity. For the surface activation reaction, CDI in anhydrous 1,4-dioxane (40 mM/disc) was added dropwise in a 20 mL glass vial that contained the pHEMA or pHEMA-co-TRIS disks, under a dry N_2 atmosphere. After stirring for 3 h at 37 °C, samples were rinsed three times with anhydrous 1,4- dioxane, with a 5 min sonication between each rinse, to remove any unreacted CDI. The disks were dried under N_2 and used immediately for the immobilization reaction of rhPRG4.

2.4. Synthesis of Surface rhPRG4-Grafted pHEMA and pHEMA-co-TRIS Hydrogels. For the surface grafting reaction, each disc was placed vertically into an Eppendorf tube filled with 1 mL of rhPRG4 aqueous solution (0.3 mg/mL, pH 9.2). The Eppendorf tubes were vortexed (800 rpm) for 24 h at 4 °C for the completion of the immobilization procedure. The disks were then washed with an excess of phosphate-buffered saline (PBS) (pH 7.4) to remove ungrafted rhPRG4. Samples were stored in Milli-Q water at 4 °C until further use.

2.5. Surface Characterization. 2.5.1. Fourier Transform Infra- red (FTIR) Spectroscopy-Attenuated Total Reflectance (ATR). The surface chemistry of dehydrated unmodified, CDI-activated, and surface rhPRG4-grafted pHEMA and pHEMA-co-TRIS disks was determined using Fourier transform infrared (FTIR) spectroscopy in attenuated total reflectance (ATR) mode (Vertex 70 FTIR spectrometer, Bruker Instruments, Billerica, MA) equipped with a diamond ATR cell. Measurements on dehydrated samples were performed in the frequency range of 600–3000 cm^{-1} (64 scans, 4 cm^{-1} resolution) at room temperature.

2.5.2. X-ray Photoelectron Spectroscopy (XPS). The elemental composition of unmodified and rhPRG4-grafted pHEMA and pHEMA-co-TRIS surfaces was determined on vacuum-dried disks

using the PHI Quantera II XPS scanning spectrometer (Physical Electronics (PHI), Chanhassen, MN) equipped with a monochro- matic anode Al Kα X-ray source (1486.7 eV) operating at 50 W 15 kV. The survey spectra used for elemental analysis were collected from scans taken over a binding energy range of 0–1100 eV with the photoelectron take-off angle set at 45°. An analyzer pass energy of 280 eV allowed for rapid data acquisition and accurate quantitative analysis. A dual beam charge compensation system was used for neutralization of all samples (beam diameter 200 μm). The instrument was calibrated using a sputter-cleaned piece of Ag, where the $Ag_{3d_{5/2}}$ peak had a binding energy of 368.3 ± 0.1 eV and full width at half-maximum for the $Ag_{3d_{5/2}}$ peak was at least 0.52 e. High- resolution N_{1s} scans were obtained with a pass energy set at 55 eV for higher energy resolution. The binding energy scale was referenced to the C_{1s} peak set at 285.0 eV. The operating pressure did not exceed 2×10^{-8} Torr. Data analysis of low-resolution spectra was performed using the PHI MultiPak version 9.4.0.7 software to calculate the elemental compositions of the surfaces from the integrated intensities of the XPS peaks and to peak fit the high-resolution spectra. At least two different spots per disc were examined for each sample.

2.6. Quantification of Surface-Grafted rhPRG4 I^{131}- rhPRG4. For the quantification of rhPRG4 that was present on the surface of the pHEMA and pHEMA-co-TRIS materials, rhPRG4 was radiolabeled with $Na^{131}I$ using the iodine monochloride (ICl) method.[34] The glycine buffer (2 M, pH 8.8) that was used during the radiolabeling procedure contains primary amines that can react with CDI-target groups, impeding the surface grafting of rhPRG4. Hence, after the radiolabeling reaction the I^{131}-rhPRG4 solution was dialyzed extensively against PBS (pH 7.4) for 2 days using Slide-A- Lyzer Dialysis Cassette (Thermo Fisher Scientific, Burlington, ON, Canada) to remove glycine as well as unreacted iodide. The percentage of free iodine, determined by the trichloroacetic acid precipitation assay, was less than 3% of the total radioactivity of I^{131}-rhPRG4. For the reaction incubation solution, 5% I^{131}-rhPRG4 was mixed with rhPRG4 solution to give a final concentration of 0.3 mg/ mL. All other conditions for the surface grafting reaction were the same as above. At the end of the reaction, the samples were washed with PBS (pH 7.4) (5 mL/disc for 12 h, two cycles). The disks were further stored in PBS (pH 7.4, 2 mL/disc) for the next 7 days at room temperature, with PBS being changed daily. For the quantification of the chemically attached rhPRG4 on the surface of CDI-activated pHEMA and pHEMA-co-TRIS disks, the radioactivity of the disks was measured immediately after the immobilization reaction, after the first two PBS wash steps (wash 1: 12 h and wash 2: day 1) and on the 2nd, 4th, and 7th day of incubation using a γ counter (PerkinElmer Wallac Wizard 1470 Automatic γ Counter, Wellesley, MA). Before each reading, the disks were gently blotted with a Kimwipe and placed in a counting vial (5 mL nonpyrogenic, polypropylene round-bottom tube). At the end of the reading, the disks were then placed back in the medium for the next measurement. A standard calibration curve was used to convert the measured radioactivity (cpm) into the surface density of rhPRG4 ($\mu g/cm^2$). The decay of the isotope over time was taken into consideration by measuring the radioactivity of the standard solutions at each time point. The surface density of physically sorbed rhPRG4 on the surface of unmodified pHEMA and pHEMA-co-TRIS disks was also determined, following the protocol above.

2.7. Contact Angle Measurements. The contact angles of unmodified and rhPRG4-grafted pHEMA and pHEMA-co-TRIS surfaces were measured using static captive bubbles on an optical contact angle analyzer, OCA 35 (DataPhysics Instruments, Germany). Briefly, an air

bubble with a volume of 10 μL was dispensed on the surface of a fully hydrated disc (5 μL/s) which had been previously immersed into a chamber filled with Milli-Q water. The contact angle of both sides of each disc was measured. After a single drop measurement, the disc was placed back in Milli-Q water for rehydration. The Milli-Q water in the chamber was replaced every time a new set of samples was assessed. All measurements were done at room temperature.

2.8. Quantification of Protein Deposition Lysozyme and Human Serum Albumin (HSA). Radiolabeled lysozyme (from chicken egg white) and human serum albumin (HSA) were used for the quantification of the amount of protein sorbed on the surface of the pHEMA and pHEMA-co-TRIS hydrogels. The proteins were radiolabeled with $Na^{125}I$ using the iodine monochloride (ICl) method; the iodination procedure was that same as was used with I^{131}-rhPRG4. The protein deposition protocol and data analysis were as previously described.[35] The percentage of free iodine determined by the trichloroacetic acid precipitation assay was less than 1% of total radioactivity for both proteins.

For the quantification of the deposited protein, a single protein solution (1 mg/mL, PBS pH 7.4) was prepared, containing 5% I^{125}- lysozyme or I^{125}-HSA. Initially, fully hydrated unmodified and rhPPRG4-grafted pHEMA and pHEMA-co-TRIS hydrogel disks were individually incubated in 250 μL of the single protein-containing solution at room temperature for 6 h. Disks were placed vertically in 96-well plates to ensure that both surfaces were exposed to solution. At the end of the incubation time, the disks gently blotted with a Kimwipe and then rinsed three times with PBS (pH 7.4) (10 min wash intervals) to remove any loosely bound protein. The rest of the procedure for the determination of the surface density of each protein sorbed was the same as that followed for the quantification of rhPRG4 surface density above.

2.9. In Vitro Friction Measurement Boundary Lubrication Test Setup. The boundary lubricating ability of the rhPRG4-grafted layer was investigated using an in vitro ocular friction test at a human cornea–hydrogel disc biointerface. The experimental setup and protocol used herein were as previously described.[24,36,37] Briefly, samples were tested using the BOSE ELF3200 biomechanical testing machine equipped with axial and rotational actuators, and axial load (N) and torque (τ) sensors. The resected cornea was prepared and placed on the bottom of the rotational actuator compartment. The annulus of fully hydrated in Milli-Q water unmodified and rhPRG4- grafted pHEMA and pHEMA-co-TRIS disks was firmly fixed to a custom holder which in turn was placed on the linear actuator, forming the upper articulating surface. The linear actuator was used to articulate the hydrogel disc with the cornea and to control axial load, while the rotational actuator was used to slide the cornea against the material. Axial load (N) and torque (τ) were collected during sliding to calculate the friction coefficients.

After mounting the samples, the two surfaces were immersed and allowed to equilibrate in a chamber containing 300 μL of Bausch & Lomb Saline Plus contact lens solution (Bausch & Lomb, Rochester, NY) at room temperature. A three-position axial compression was followed so as to achieve entire contact of the cornea–disc interface. Once the entire contact was achieved in each axial position, a 12 s time interval (dwell time) allowed for stress relaxation of the corneal tissues under load. The cornea–disc samples were subjected to relative articulations (four revolutions in both rotation directions) at four effective sliding velocities (v_{eff}) at 0.3, 1.0, 10, and 30 mm/s, using a repeated measure sequence under pressure of approximately 18–25 kPa.

Normal load (N) (axial forces) and torque (τ) were recorded at 20 Hz during rotations to calculate the friction coefficients (μ). The boundary lubrication properties of the hydrogels were evaluated by calculating the static (μ_{static}, N_{eq}) (N_{eq}: equilibrium axial load measured the instant after the 12 s stress relaxation duration) and kinetic ($\langle\mu_{kinetic}\rangle$) friction coefficients ($\langle\rangle$ denotes the kinetic equilibrium mean) of all the samples as previously described.[24,25,27] The static friction coefficient (μ_{static}, N_{eq}) measures the force to initiate surface movement while kinetic friction coefficient ($\langle\mu_{kinetic}\rangle$) measures the force to maintain the steady state movement. In each test, a preconditioning run using a poly(dimethylsiloxane) (PDMS) disk in the place of a model hydrogel disc was followed by the unmodified and then the rhPRG4-grafted disks. A full test sequence was done on a single corneal tissue sample and was considered to be one repeat when performing statistical analysis.

2.10. Optical Transparency. The impact of the surface immobilization of rhPRG4 on the optical transparency of the pHEMA and pHEMA-co-TRIS hydrogels was assessed by measuring the light transmittance (%) of fully hydrated hydrogel disks in the range of 400−750 nm at ambient temperature, using a UV−vis spectrophotometer (SpectraMax Plus 384, Molecular Devices, Corp., Sunnyvale, CA). The disks were immersed in 100 μL of Milli-Q water in a 96-well plate during the measurement.

2.11. Equilibrium Water Content (EWC). The hydrogel disks were soaked in Milli-Q water for 24 h, under stirring conditions at ambient temperature to achieve equilibrium. The fully hydrated disks were removed, blotted with a Kimwipe to remove any excess water and weighed (W_{wet}). The samples were then placed in a 37 °C oven overnight and then into a vacuum oven for 12 h to completely remove any traces of water and subsequently weighed (W_{dry}) again. The equilibrium water content (EWC) of the hydrogels was determined using the equation below

$$\mathrm{EWC}\% = \frac{W_{wet} - W_{dry}}{W_{wet}} \times 100\% \qquad (1)$$

2.12. Cell Viability 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphe- nyltetrazolium Bromide (MTT) Assay. A study was conducted to assess the effect of rhPRG4 grafting on the pHEMA and pHEMA-co- TRIS substrates via CDI chemistry on cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, immortalized human corneal epithelial cells (HCECs) were cultured in keratinocyte serum-free medium (K-SFM) supplemented with human recombinant epidermal growth factor 1–53 (EGF 1–53) and bovine pituitary extract (BPE) for 1 week in a 5% CO_2 atmosphere at 37 °C. The HCEC were then subcultured and seeded in a flat bottom 96-well polystyrene culture plate (Corning, Costar, NY) (1.5×10^4 HCEC/well) with 100 μL culture medium and incubated in 5% CO_2 atmosphere at 37 °C. Following a 24 h incubation period to ensure adhesion of the cells, the cell medium was replaced with fresh K-SFM (250 μL). The hydrogel disks, previously extensively washed with sterilized PBS (pH 7.4) for 6 h, were then placed vertically in the cell-containing well without touching the bottom and incubated in the cell culture incubator for 24 h. At the end of the incubation period, the hydrogel disks were discarded and the medium was aspirated. Each well was gently rinsed with PBS (pH 7.4), and 10 μL of MTT solution (5 mg/mL in PBS pH 7.4) with

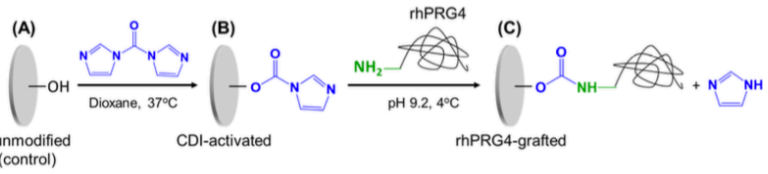
100 μL of PBS were added. The plate was then covered with foil and placed in the cell incubator for 4 h (5% CO_2, 37 °C) to allow the soluble yellow MTT to be reduced into dark blue insoluble formazan crystals by the metabolically active cells. The formazan crystals were dissolved into 50 μL of dimethyl sulfoxide per well, and after 5 min of shaking at room temperature under dark conditions, the absorbance was measured spectrophotometrically at 540 nm (SpectraMax Plus 384, Molecular Devices, Corp., Sunnyvale, CA). All of the results were normalized to cells grown in wells without hydrogel disks which were assumed to possess 100% cell viability.

2.13. Statistical Analysis. Experiments were performed with a minimum of four repeats (disks) and data were verified in at least two independent experiments, unless otherwise stated. For comparative studies, statistical analysis was conducted using a one-way analysis of variance and Tukey's honestly significant difference for post hoc comparisons using Statistica 10 (StatSoft Inc. Tulsa, OK). A p value of 0.05 was set as the threshold of statistical significance. All data were expressed as mean ± standard deviation (SD) with the exception of friction coefficients where data were expressed as mean ± standard error of the mean (SEM).

RESULTS AND DISCUSSION

3.1. Synthesis of Surface rhPRG4-Grafted pHEMA and pHEMA-co-TRIS Hydrogels via CDI Chemistry. The reaction scheme illustrating the surface CDI linking chemistry for rhPRG4 immobilization on pHEMA and pHEMA-co-TRIS hydrogels is depicted in Scheme 1. CDI functionalization promotes the condensation between the hydroxyl (–OH) and amine ($–NH_2$) groups to covalently attach the glycoprotein onto the model hydrogel contact lens surfaces via covalent bonds.[38]

Scheme 1. Schematic Illustration of the Synthesis of rhPRG4-Grafted pHEMA and pHEMA-*co*-TRIS Hydrogels via CDI Linking Chemistry



The chemistry of the surfaces was initially determined by FTIR-ATR. The observed decrease in the broad band of the –OH groups at approximately 3405 cm^{-1} in combination with the appearance of two new peaks at approximately 1765 and 1475 cm^{-1} which were attributed to the asymmetric stretch of the CDI carbonyl groups and the imidazole cycle characteristic bands respectively,[39] indicated successful incorporation of imidazolyl-carbamate groups on the surface of both hydrogels (Figure 1A,B, graph b). The peaks at 3130 and 1530 cm^{-1} which were due to the presence of amide II of the carbamate in activated surfaces as well as the disappearance of the typical HEMA stretching band of the alcohol group (C–O) at 1074 cm^{-1} upon CDI reaction, also confirmed the successful attachment of the intermediate linkage. For the pHEMA-co-TRIS hydrogels, the additional adsorption peaks at approx- imately 1276, 1025, and 876 cm^{-1} were attributed to Si–CH_3 and Si–O groups of the SiHy materials[40] (Figure 1B).

Finally, successful covalent attachment of rhPRG4 on both pHEMA and pHEMA-co-TRIS surfaces was indicated by the broad peak of 3600–3250 cm^{-1} which was reinforced by hydrogen stretching vibrations of O–H and N–H from the hydroxyl, carboxyl, amine, and amide groups of rhPRG4,[38] while the new peak at approximately 1640 cm^{-1} was assigned to the amide groups of the glycoprotein (Figure 1A,B, graph c).

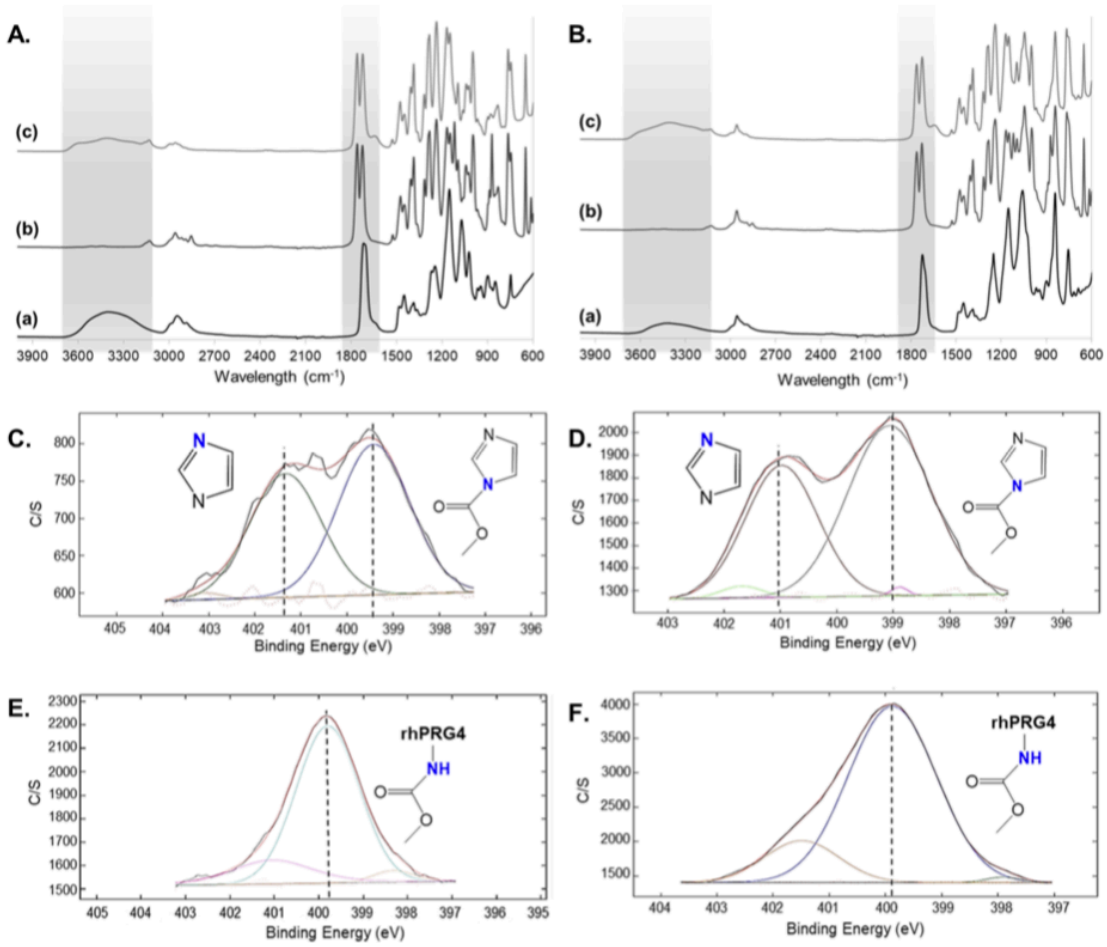


Figure 1. Characterization of surface chemistry. ATR-FTIR adsorption spectra of (A) pHEMA and (B) pHEMA-*co*-TRIS for (a) unmodified (control), (b) CDI-activated, and (c) rhPRG4-grafted hydrogel surfaces (600–4000 cm^{-1}). High-resolution N_{1s} XPS spectra for (C, D) CDI-activated and (E, F) rhPRG4-grafted pHEMA and pHEMA-*co*-TRIS hydrogel surfaces, respectively (395–405 eV).

The atomic composition of the surfaces before and after each modification step was also determined by XPS. Since the unmodified (control) pHEMA and pHEMA-co-TRIS do not contain nitrogen (N) in their structure, the presence of an N_{1s} peak in the low resolution XPS spectra was used to monitor the CDI-tethered to the surfaces (Table 1). The further increase in the N_{1s} signal on the rhPRG4-modified surfaces was attributed to the successful covalent attachment of the glycoprotein on the pretreated surfaces. The decreasing trend of the Si_{2p} percentage for the pHEMA-co-TRIS sample was another indication of successful rhPRG4 grafting, with the glycoprotein layer masking the underlying silicone-based substrate. To further confirm the covalent attachment of the

glycoprotein on the CDI-modified surfaces, high-resolution N_{1s} spectra were collected before and after the rhPRG4 conjugation reaction and compared. Analysis of the N_{1s} high- resolution spectra showed that CDI-activated surfaces had the characteristic imidazole derived-doublet peak with maxima at approximately 399 and 401 eV for pHEMA, and 399.4 and 401.3 eV for pHEMA-co-TRIS (Figure 1C,D), attributed to the two distinct molecular environments of the imidazole- carbamate nitrogens.[30] More precisely, the first peak centered around 399.1 eV included the contribution of the nitrogen of the carbamate groups and likely the nitrogen of unreacted imidazole groups, while the other peak at 400.8 eV was assigned to the inner imidazole ring nitrogen.[41] Upon rhPRG4 grafting reaction, the peak at 401 eV was significantly decreased whereas the other was shifted, forming a more dominant peak at 399.8 eV for both pHEMA and pHEMA-co- TRIS hydrogels (Figure 1E,F). The latter peak corresponded to the replacement of the imidazole ring structure with the amide bond,[42] suggesting the successful covalent attachment of rhPRG4 on the material surface.

Table 1. Atomic Composition (%) of the Surface of Nonmodified, CDI-Activated and Surface rhPRG4-Grafted pHEMA, and pHEMA-*co*-TRIS Hydrogel Disks from Low Resolution XPS Spectra (n = 3)

samples	C_{1s}	O_{1s}	N_{1s}	Si_{2p}	S_{2p}
pHEMA	70.6 ± 0.8	28.2 ± 0.8		1.3 ± 0.04	
CDI-pHEMA	72.2 ± 1.2	21.5 ± 0.8	4.9 ± 0.1	1.4 ± 0.1	
rhPRG4-pHEMA	63.8 ± 0.1	23.8 ± 0.03	10.6 ± 0.3	1.2 ± 0.1	0.3 ± 0.01
pHEMA-*co*-TRIS	67.0 ± 1.8	23.3 ± 2.0		9.8 + 0.6	
CDI-pHEMA-*co*-TRIS	63.8 ± 0.2	23.1 ± 0.4	3.5 ± 0.2	9.6 ± 0.05	
rhPRG4-pHEMA-*co*-TRIS	62.0 ± 0.3	23.2 ± 0.1	5.1 ± 0.2	8.8 ± 0.2	

CDI treatment has been shown to be an easy, effective, and rapid method for surface modification with biomolecules with little effect on the bulk properties of the materials.[30,38] In contrast to other immobilization methods,[43−45] CDI chemistry is advantageous for affinity adsorbent preparation processes because there is no need for an intermediate basic catalyst as the alkyl carbamate linkage formed between the hydroxyl support and the amine-containing ligand is neutral, thus decreasing the chance of nonspecific adsorption by ion exchange.[46] For the surface grafting reaction of rhPRG4 to the model pHEMA and pHEMA-co-TRIS hydrogels, basic pH was chosen so as to increase the degree of deprotonation of the glycoprotein amine groups, allowing for a more efficient reaction with the imidazole ring. The literature suggests that the remaining active CDI groups upon surface grafting reaction can be removed by hydrolysis using carbonate buffer.[46]

3.2. Surface Density of rhPRG4 Quantification of I^{131}-rhPRG4. Figure 2 demonstrates the surface density of rhPRG4 on each sample as a function of time. Immediately after the reaction (24 h), the amount of rhPRG4 on the rhPRG4-grafted pHEMA sample was found to be 857.8 ± 64.5 ng/cm^2 while the surface density of the physically sorbed rhPRG4 for the unmodified (control) pHEMA sample was 577.3 ± 24.5 ng/cm^2, a 33% difference ($p < 0.0003$). In contrast, the surface density of rhPRG4 for the rhPRG4-

grafted pHEMA-co-TRIS hydrogels (727.4 ± 22.9 ng/cm^2) was similar to that of the physically sorbed rhPRG4 for the unmodified (control) pHEMA-co-TRIS sample (751.8 ± 39.8 ng/cm^2) ($p = 0.9$). After the first 24 h of incubation in PBS (pH 7.4), the surface density of rhPRG4 for the surface-modified pHEMA and pHEMA-co-TRIS samples did not change significantly over time (rhPRG4-grafted pHEMA: 582.8 ± 19.7 ng/cm^2 and rhPRG4-grafted pHEMA-co-TRIS: 492.2 ± 10.0 ng/cm^2) ($p > 0.05$), suggesting that the 24 h washing step with PBS was adequate for the effective removal of the loosely bound rhPRG4. However, for the unmodified pHEMA and pHEMA- co-TRIS samples, the amount of physically sorbed rhPRG4 was gradually decreased over the course of the 7 day wash period.

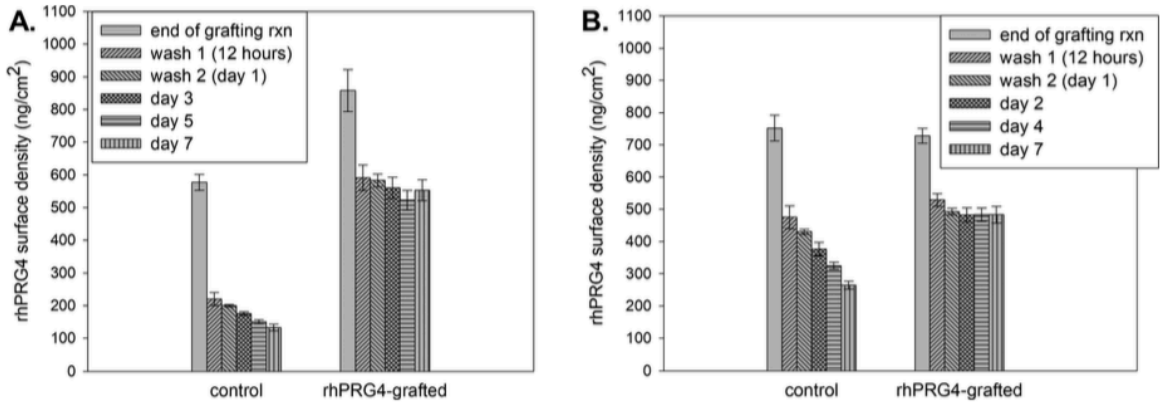


Figure 2. Quantification of the surface-bound rhPRG4. The surface density (±SD) of physisorbed or grafted rhPRG4 for (A) pHEMA and (B) pHEMA-*co*-TRIS hydrogels was determined ($n = 4$).

Quantification of rhPRG4 on the surface-modified hydrogels further confirmed that the surface density of rhPRG4 on the rhPRG4-grafted pHEMA samples was slightly higher than that of the respective model SiHy ($p < 0.01$). Interestingly, the amount of the glycoprotein detected on the unmodified (control) hydrogels was not completely removed over the course of 7 days, suggesting that some of the physically sorbed rhPRG4 was tightly and irreversibly bound on these surfaces. Moreover, the affinity of the amphiphilic rhPRG4 for the unmodified (control) pHEMA-co-TRIS sample was signifi- cantly stronger than that of the respective pHEMA surface ($p < 0.0003$), indicating that partitioning may occur between the silicone domains of the SiHy and the hydrophobic domains of the rhPRG4. A recent study by Samsom et al.[27] demonstrated that rhPRG4 had a higher affinity for PDMS and for commercial SiHy contact lenses (senofilcon A) than for the hydrophilic conventional contact lenses (etafilcon A). In fact, PRG4 is proposed to strongly adsorb on hydrophobic surfaces through the nonpolar amino acid residues of the globular end- domain regions of the glycoprotein via hydrophobic interactions.[17,18,29] On the other hand, the low degree of rhPRG4 physical deposition on pHEMA surfaces may reflect the absence of strong long-range electrostatic or hydrophobic interactions between rhPRG4 molecules and the relatively hydrophilic nonpolar pHEMA surfaces,[47] as well as the potentially weak van der Waals forces or

hydrogen bonding between the pHEMA bonding active sites (–OH and C O groups) and the rich hydrogen bond donor site of the galactose and sialic acid groups of the glycosylation layer of the rhPRG4 mucin domain.[47] It was therefore assumed that the amount of rPRG4 determined for the CDI-activated pHEMA and pHEMA-co-TRIS surfaces was not only covalently attached and but also physically adsorbed. Finally, it is important to note that PRG4 in its native state can form intra- and intermolecular disulfide bonds because of the availability of cysteine in the C- and N-termini domains. Therefore, since rhPRG4 was in its native form and not reduced or alkylated, dimers and possibly oligomers can be present in the reaction solution potentially impeding the degree of surface grafting.

3.3. Surface Wettability Contact Angle Measure- ments. An important property for contact lenses is sufficient surface wettability so that the tear film can spread and remain stable on the surface of the lens, without breaking up prematurely.[11] Contact lenses exhibiting poor wettability have been associated with reduced optical quality, increased surface deposition and discomfort.[3,48] In this work, we utilized the captive-bubble technique to maintain fully hydrated conditions during the contact angle measurement, since this should correlate more realistically with the on-eye conditions. Another advantage of this technique is that neither the air bubble nor the lens surface is susceptible to surrounding atmospheric conditions.

In vitro measurement of the surface wettability was achieved by the measurement of the contact angle using the captive bubble technique. As shown in Figure 3, the contact angle of the rhPRG4-grafted pHEMA sample was slightly higher than that of the unmodified (control) pHEMA sample ($p < 0.002$). However, the contact angle on rhPRG4-grafted pHEMA-co- TRIS was decreased (35%) when compared to the unmodified (control) sample ($p < 0.0002$). Lower contact angles suggest improved surface wettability.

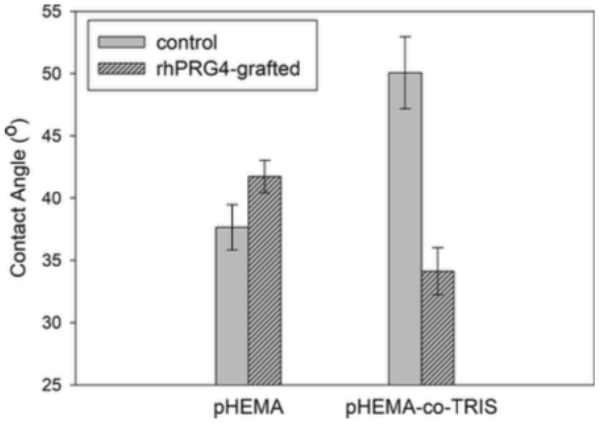


Figure 3. Impact of surface rhPRG4-grafted on the surface wettability. The static water contact angles (±SD) of unmodified (control) and rhPRG4-grafted pHEMA and pHEMA-*co*-TRIS hydrogel surfaces, swollen in Milli-Q water, using the captive bubble technique ($n = 6$).

The immobilization of its hydrophobic and positively charged N-terminus, made the amphiphilic glycoprotein adopt an energetically favorable conformation that rendered the rhPRG4-grafted pHEMA surfaces less wettable compared to the unmodified (control) pHEMA surfaces. On the contrary, the wettability of the pHEMA-co-TRIS surfaces was improved by covalently attaching rhPRG4. Interestingly, the contact angle for the rhPRG4-modifed model SiHy was lower than that of the unmodified (control) pHEMA hydrogels ($p < 0.001$), suggesting better surface wettability. This observation can be explained by considering the structure of the amphiphilic rhPRG4, which mimics that of a surfactant.[29] The contact angle increases for rhPRG4-grafted pHEMA surfaces, and was therefore attributed to presence of free hydrophobic hemopexin-like end-domains, whereas for the rhPRG4-grafted pHEMA-co-TRIS samples, the wettability enhancement was due to the highly glycosylated, and thus highly hydrated mucinous central domain of the glycopro- tein.[17]

3.4. In Vitro Protein Sorption Study. Tear film components, including proteins and lipids, adhere on the contact lens surface within minutes following lens insertion, while the contact lens–tear film interface remains in a dynamic interaction throughout the period of wear. The process of protein deposition on the contact lens surface is complex including both nonspecific adsorption and absorption.[49] Thus, to avoid confusion, protein uptake is herein referred to as protein sorption or deposition.

The materials investigated in this study were incubated in a noncompetitive, single protein solution of either lysozyme or human serum albumin (HSA) to establish their individual nonspecific binding properties (Figure 4). The amount of lysozyme and HSA physically sorbed on the rhPRG4-grafted pHEMA surfaces was reduced by approximately 60 and 45% respectively when compared to that of the unmodified (control) pHEMA hydrogels ($p < 0.0004$ and $p < 0.001$ respectively). For the pHEMA-co-TRIS materials, the presence of the surface-grafted rhPRG4 layer led to a 75% reduction in the nonspecific deposition of both lysozyme and HSA ($p < 0.001$ and $p < 0.0002$ respectively).

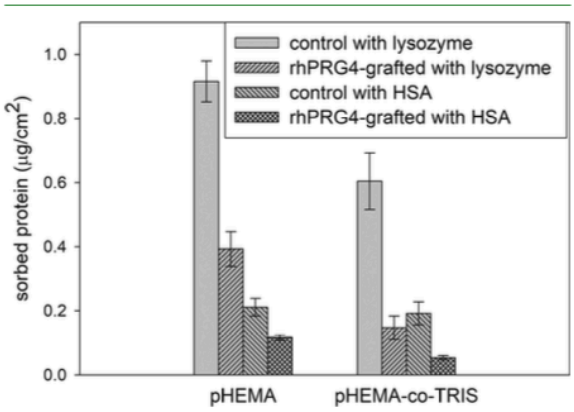


Figure 4. Impact of surface rhPRG4-grafted on protein deposition. The amount (±SD) of physisorbed lysozyme and human serum albumin (HSA) on the surface of unmodified (control) and rhPRG4-grafted pHEMA and pHEMA-*co*-TRIS hydrogels upon a 6 h incubation period at room temperature (n = 6).

The conformation of the adsorbed rhPRG4 layer, as a result of the affinity of the glycoprotein with the respective substrate, was previously found to be responsible for the antifouling properties of rhPRG4.[47] Additionally, the chain flexibility of the surface-grafted layer plays an important role in the development of antifouling properties.[50] Reduction in non- specific immunoglobulin G (IgG) and bovine serum albumin binding to rhPRG4-coated surfaces was previously attributed to the telechelic brush-like layer formed upon rhPRG4 adsorption that effectively can hide the underlying substrate while exposing the heavily glycosylated and low adhesion mucin-like central domain to the surrounding solutions.[47] Therefore, in a manner similar to the high natural resistance of the glycocalyx[51] and other glycocalyx-mimetic peptoids[52] toward nonspecific protein interactions, the observed decrease in protein sorption is believed to be mainly the synergistic outcome of steric repulsion and surface hydration forces provided by the heavily glycosylated mucin-like domain of rhPRG4,[50] thus inhibiting the protein–surface interactions. It is worth noting that the work presented herein is the first, to our knowledge, to examine the behavior of rhPRG4-grafted surfaces toward lysozyme deposition. Despite the expected strong ionic interactions between the highly positively charged and relatively small lysozyme (MW 14.3 kDa, pI 11.35) and the negatively charged mucin-like central domain of rhPRG4, as previously observed between mucin and lysozyme under physiological conditions,[53,54] lysozyme sorption was decreased in all cases with rhPRG4 immobilization. Similarly, interactions between the larger negatively charged albumin (MW 66 kDa, pI 4.9) and the positively charged and hydrophobic end-domains of PRG4, which would also be expected to thermodynamically favor protein deposition, did not overcome the protein repellent properties of the glycoprotein.

These results in combination with the ability of PRG4 to also reduce deposition of IgG,[47] another protein that causes adverse effects when sorbed on contact lenses, suggest that rhPRG4 could be an effective antifouling coating useful for contact lens applications.

3.5. In Vitro Friction Coefficient under Boundary Lubrication Conditions. For contact lens applications, friction is considered the principle material-related property shown to be highly correlated to in vivo discomfort.[14–16,55] During contact lens wear, tear film lubrication of the ocular surface is believed to reduce the significant shear forces between the contact lens and the ocular surface developed during blinking, by preventing surface-to-surface contact at the eyelid–lens and lens–cornea biointerfaces.[56] Contact lenses can provide hydrodynamic lubrication during the fastest part of the blinking with continuous tear film maintenance at the eye– lens interface. At lower ocular movement speeds, boundary lubrication occurs and the contact lens is in direct contact with the ocular tissues, especially if the tear film breakup occurs prior to blinking, resulting in significantly higher friction.[57] Increased mechanical interactions between the contact lens and the ocular surface are postulated to be associated clinically with lid-wiper epitheliopathy, lid parallel conjunctival epithelial folds, and contact lens associated papillary conjunctivitis, provoking symptoms of dryness and discomfort.[12,13,58]

The impact of surface immobilization of rhPRG4 on the friction coefficients of pHEMA and pHEMA-co-TRIS samples was determined at a human cornea–disc biointerface, under boundary conditions. The applied sliding velocities and loads during the friction measurement experiments were within the calculated range observed in the eye during blinking.[57] All of the data presented herein were log transformed to improve the uniformity of variance for statistical analysis.[24] The μ_{static} of the rhPRG4-grafted pHEMA sample was similar to that of the unmodified (control) pHEMA hydrogels ($p = 0.14$), whereas the $\langle\mu_{kinetic}\rangle$ of the rhPRG4-grafted pHEMA hydrogels was slightly higher than that of the unmodified (control) pHEMA materials ($p < 0.05$) (Figure 5A,B). For the pHEMA-co-TRIS sample, even though surface immobilization of rhPRG4 did not alter μ_{static}, a friction-lowering effect was observed for $\langle\mu_{kinetic}\rangle$ on the rhPRG4-grafted sample ($p < 0.05$) (Figure 5C,D). For both pHEMA and pHEMA-co-TRIS samples, μ_{static} increased significantly by increasing the sliding velocity ($p < 0.05$), whereas no effect of velocity was observed for $\langle\mu_{kinetic}\rangle$.

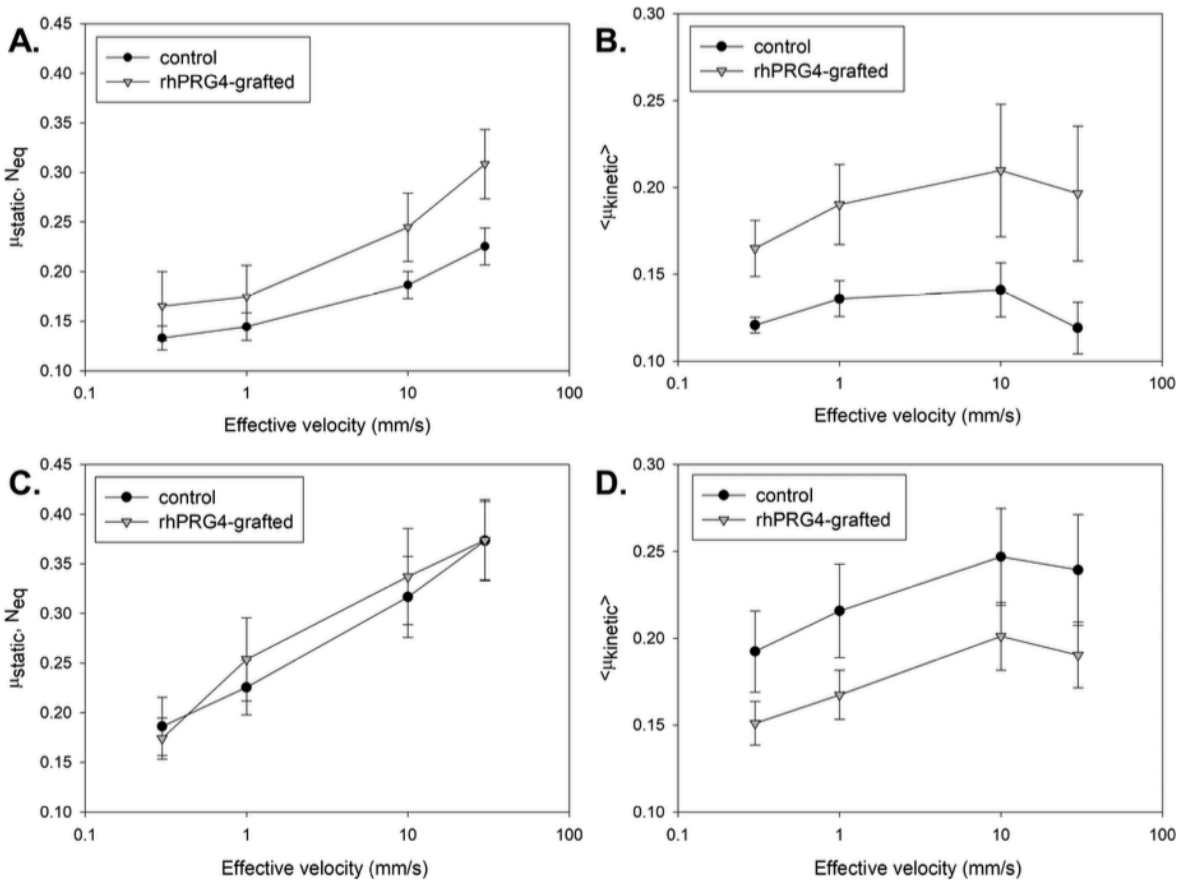


Figure 5. Effect of surface rhPRG4-grafted on boundary lubrication at a human cornea–hydrogel disc biointerface. The static ($\mu_{static,\ N_{eq}}$) and kinetic ($\langle\mu_{kinetic}\rangle$) friction coefficients (±SEM) of unmodified (control) and rhPRG4-grafted for (A, B) pHEMA (n = 3) and (C, D) pHEMA-*co*-TRIS (n = 6) hydrogel surfaces in saline bath at room temperature. The average normal stress (±SD) was 20.6 ± 2.5 kPa for pHEMA and 20.1 ± 1.9 kPa for pHEMA-*co*-TRIS. Sliding velocity values were log transformed to improve the uniformity of variance for statistical analysis.

The rhPRG4-grafted pHEMA hydrogels were characterized by similar (μ_{static}) or slightly higher friction coefficients ($\langle\mu_{kinetic}\rangle$) than the unmodified (control) pHEMA sample. This is not the first time that PRG4 has been reported as not an effective lubricating agent for relatively hydrophilic surfaces. Previous work showed that physically adherent rhPRG4 on the surface of pHEMA hydrogels did not reduce the boundary friction for a human corneal–disc biointerface,[36] while Chang et al.[29,59] found that physically adsorbed PRG4 on hydroxyl- terminated self-assembled monolayer hydrophilic surfaces showed an increase in the overall friction force, in a concentration-dependent manner. Conversely, the reduced friction noted for the rhPRG4-grafted pHEMA-co-TRIS samples was also observed for the same model SiHy when rhPRG4 was physically sorbed,[36] suggesting that covalent attachment of rhPRG4 did not adversely affect its boundary lubricating ability. Likewise, Abubacker et al.[60,61] showed that covalent attachment of aldehyde modified-PRG4 on depleted articular surfaces through its N-terminus, enhanced its binding ability without significantly affecting the structure of the glycoprotein, while also exhibiting a friction reducing cartilage boundary lubricating properties similar to the physisorbed PRG4. The velocity-dependent profile of μ_{static}, which has been previously

observed for similar setups when PRG4 was used in solution,[21,24,27] was attributed to interdigitations between the two soft material surfaces of the biointerface prior to sliding One of the basic requirements for rhPRG4 to act as a boundary lubricant is the development of strong interactions with the substrate through its hydrophobic end-domains, allowing its central highly glycosylated mucinous domain to create a low friction layer.[29] Since the surface density of grafted rhPRG4 to pHEMA and pHEMA-co-TRIS surfaces was found to be similar, the difference in the friction profile of these two samples is thought to derive from the difference in the surface chemistry between pHEMA and pHEMA-co-TRIS materials. The increase in the friction coefficients of rhPRG4-grafted pHEMA samples could be due to weak adhesive interactions (hydrogen bonding) between the mucinous domain of rhPRG4 and pHEMA[47] or an unfavorable hydrophobic interaction between PRG4 end-domains and pHEMA substrates, resulting in a poorly formed extended tail-like steric mucin layer[17] with the free hydrophobic hemopexin-like end-domains (C-termini) exposed to the sliding biointerface. On the other hand, the presence of the hydrophobic TRIS domains in the model SiHy presumably resulted in a conformation that allowed rhPRG4 to present as an effective friction-lowering boundary lubricant. More specifically, sur- face-grafted rhPRG4 on pHEMA-co-TRIS surfaces was presumably able to organize into a telechelic brush-like layer (loop-like) conformation where the central mucinous domain is exposed to the biointerface providing strong repulsion through steric and hydration forces.[18,47,62,63] This speculated conformation of the grafted rhPRG4 layer on the pHEMA and pHEMA-co-TRIS surfaces was also supported by the contact angle results presented above. Moreover, the friction behavior would not be expected to be significantly different for eyelid– hydrogel biointerface as the choice of ocular tissue was not found to affect PRG4 lubrication to a large extent.[27,36]

3.6. Optical Transparency and Equilibrium Water Content (EWC). The optical properties of unmodified (control) and rhPRG4-grafted pHEMA and pHEMA-co- TRIS disks are shown in Figure 6. Surface immobilization of rhPRG4 led to a minor reduction in the optical acuity of the materials (approximately 4% for both cases). The model SiHy used in this study was found to be slightly more opaque than the pHEMA due to microphase separation between the hydrophilic HEMA and hydrophobic TRIS domains. The optical transparency is an important parameter that needs to be considered for the design of contact lens-based applications. Although the rhPRG4-grafted pHEMA-co-TRIS samples had transmittance values that were slightly lower than 90%, it should be noted that the all disks used in this study were almost 5 times thicker than commercially available contact lenses (0.5 mm thickness). Therefore, despite the decrease observed in the optical transmittance upon the surface modification step, grafting rhPRG4 to the surface of contact lenses would not be expected to have clinical impact on their optical transparency.

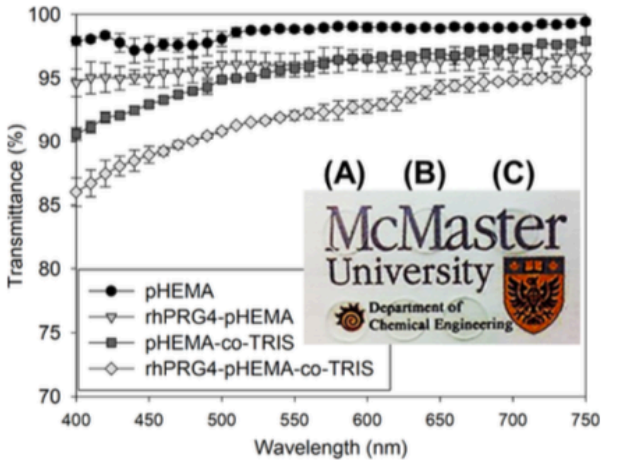


Figure 6. Impact of surface modification on optical transparency. Transmittance spectrum (±SD) for the unmodified (control) and rhPRG4-grafted pHEMA and pHEMA-*co*-TRIS hydrogel disks (n = 6). Inset: a photograph of (A) unmodified, (B) CDI-activated and (C) rhPRG4-grafted pHEMA (top) and pHEMA-*co*-TRIS (bottom) hydrogel materials.

Water content is another influential property for both conventional and SiHy contact lenses, playing a role in oxygen permeability, ion transport, and mechanical properties.[64] The equilibrium water content (EWC) of the pHEMA and pHEMA-co-TRIS hydrogel materials was determined using eq 1. The results, presented in Table 2, demonstrate that the modification procedure did not change the EWC of the surface rhPRG4-grafted hydrogel materials when compared to the unmodified (control) samples ($p > 0.05$). The EWC of the hydrogels remained within an acceptable range for contact lens wear. In general, the bulk properties of the surface-modified materials are not expected to differ from those of the pristine samples because the 1,4-dioxane used as the solvent for the intermediate CDI activation reaction did not cause any swelling of the hydrogels.

Table 2. Equilibrium Water Content (EWC) (%) (±SD) of the Unmodified (Control) and rhPRG4-Grafted pHEMA and pHEMA-*co*-TRIS Hydrogel Disks (n = 6)

samples	EWC (%)
pHEMA—control	34.2 ± 0.78
rhPRG4-grated pHEMA	34.73 ± 0.94
pHEMA-*co*-TRIS—control	26.9 ± 2.4
rhPRG4-grafted pHEMA-*co*-TRIS	28.35 ± 1.51

3.7. In Vitro Cytotoxicity Study MTT Assay. The cytotoxicity of potentially leachable components arising from the surface modification procedure was assessed using an MTT assay with human corneal epithelial cells (HCECs). Immortalized HCEC have been shown to be an appropriate in vitro model of the human ocular surface for assessing

toxicity of front-of-the-eye biomaterials, such as contact lenses.[65] As shown in Figure 7, there was no statistical difference in the cell viability of HCEC cultured in the presence of unmodified (control) or surface rhPRG4-grafted pHEMA and pHEMA-co-TRIS hydrogels.

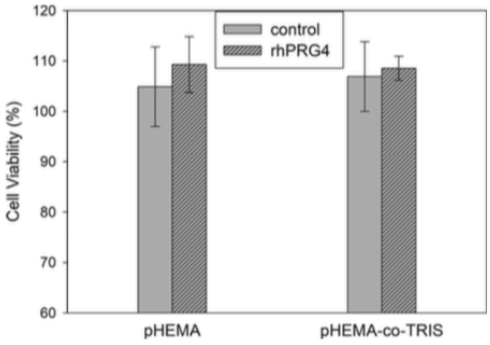


Figure 7. Cytotoxicity of rhPRG4-grafted hydrogel materials. Cell viability (%) (±SD) of the human corneal epithelial cells (HCECs) upon incubation with unmodified (control) and surface rhPRG4-grafted pHEMA and pHEMA-*co*-TRIS disks for 24 h. Results expressed relative cell viability with respect to cells grown in the absence of hydrogel disks (n = 4).

These results demonstrate that the rhPRG4-grafted pHEMA and pHEMA-co-TRIS hydrogel materials were not cytotoxic and the washing steps following synthesis as well as after each modification step were sufficient for the removal of any leachable component that would affect the HCEC viability. According to previous work, CDI as a linking agent did not exhibit any significant toxic effect in vitro on human corneal epithelial cells[32] and in vivo,[66–68] while rhPRG4-containing eye drops were used successfully in a clinical trial for the treatment of dry eye symptoms.[23]

CONCLUSIONS

In conclusion, in this study full-length human recombinant rhPRG4 was successfully covalently attached to the surface of model pHEMA and pHEMA-co-TRIS hydrogel contact lenses from its somatomedin B-like N-terminus via CDI linking chemistry. The rhPRG4-grafted model contact lenses remained optically clear and were found to be noncytotoxic. Quantification of the rhPRG4 on the surfaces indicated that the grafting density of rhPRG4 on pHEMA substrates was slightly higher than that on the pHEMA-co-TRIS, while a strong interaction was observed between the physically sorbed amphiphilic glycoprotein and the hydrophobic TRIS domains of the unmodified model SiHy. Surface immobilized rhPRG4 was found to acquire a substrate-specific conformation, which was dependent on the composition substrate materials. Even though covalently bound rhPRG4 formed a protein protective layer against lysozyme and albumin sorption for both pHEMA and pHEMA-co-TRIS hydrogels, it was found to be more effective as a wetting and boundary lubricating agent only for the model SiHy used in this study. These results suggest that the full-length rhPRG4 tested herein is a good candidate for the development of novel bioinspired SiHy contact lenses. Future studies

should examine the impact of sterilization and shelf-life on the properties of the modified model contact lens materials.

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NOTES

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