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Proteoglycan 4 and hyaluronan as boundary lubricants for model contact lens hydrogels

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Abstract: Clinical data show that in vitro contact lens friction is related to in vivo comfort. Solutions of biological lubricants hyaluronan (HA) and proteoglycan 4 (PRG4, also known as lubricin) reduce friction at a cornea–polydimethylsiloxane (PDMS) interface. The purpose of this study was to (1) determine if PRG4 can sorb to and lubricate model contact lens materials and (2) assess the boundary lubricating ability of PRG4 and HA compared to saline on model contact lens materials. PRG4 was obtained from bovine cartilage culture and suspended in saline at 300 mg/mL. N,N-Dimethylacrylamidetris (trimethylsiloxy) silane, (DMAA/TRIS) and methacryloxypropyltris (trimethylsiloxy) silane (pHEMA/TRIS) silicone hydrogels were prepared. A previously described in vitro eyelid–hydrogel and cornea–hydrogel biomechanical friction test was used to determine boundary lubricant effect. PRG4 sorption to the hydrogels was assessed using a soak-rinse protocol and western blotting. PRG4 effectively lubricated both silicone hydrogel materials and HA effectively lubricated pHEMA/TRIS, as indicated by a statistically significant reduction in friction compared to the saline control lubricant. An HA and PRG4 combination showed a synergistic effect for pHEMA/TRIS and effectively lubricated DMAA/TRIS. Biological boundary lubricants HA and PRG4 were shown to effectively lubricate silicone hydrogels when in solution. Additionally, HA and PRG4 showed synergistic lubrication for pHEMA/TRIS. The purpose of this study was not to replicate the friction coefficients of contact lenses, but rather to investigate lubricant–surface interactions for common contact lens constituents. These findings contribute to the potential development of biomolecule based lubricant drops for contact lens wearers.

Key Words: biotribology, hydrogel, lubrication, hyaluronan, proteoglycan 4

INTRODUCTION

Dry eye and discomfort is a significant performance issue for contact lenses, leading to 25% of wearers discontinuing use and 26% of wearers reducing their use of these devices. An important part of the research effort in improving contact lens biomaterials is identifying testable material properties that correlate to their in vivo performance. At the time of writing, the friction of contact lenses is the only property that has been significantly correlated to subjective in vivo comfort of contact lenses.[1,2] In addition to this, contact lens wear has been correlated to lid wiper epitheliopathy, a condition in which visible wear damage occurs in the epithelium of the lid wiper in a patients eye lid.[3,4] Wear damage is often indicative of high friction, which therefore suggests that contact lenses heighten friction in the in vivo ocular environment.

A typical strategy for reducing friction in a mechanical system is to introduce a lubricant. Proteoglycan 4 (PRG4) and hyaluronan (HA) are molecules naturally produced by the body that exhibit boundary lubricating properties. Boundary lubricants function in a regime typically described as "dry sliding" where the two sliding surfaces are in close contact and lubricants function by modifying surfaces rather that creating an entrained fluid film. In a biological context with fully or partially hydrated and porous surfaces, fully dry sliding likely does not occur. During close sliding, high pressures or slow sliding velocities these biological boundary lubricants still must operate by modifying surfaces rather that creating a viscous fluid layer.[5,6] PRG4 is a glycoprotein produced by many glandular tissues including tear secreting tissues found in the eye (the concentrations of which remain to be determined). PRG4 is thought to function by adsorbing to biological surfaces from a solution for example, synovial fluid or tear fluid, and form a hydrated, low friction boundary layer,[7] more specifically, the Nor Cterminals of the protein domains of the PRG4 molecule are thought to anchor the molecule to a surface, leaving a negatively charged, hydrophilic mucin layer that creates electrostatic and steric repulsion from other surfaces. HA is a naturally occurring repeating disaccharide known to lubricate cartilage–cartilage biointerfaces. HA is already used in commercial eye drops and contact lens solutions.[8] A human recombinant PRG4 is also now available (Lubris BioPharma LLC, Framingham, MA, USA) that shows promise for clinical application.[9,10]

Initial studies into PRG4 and HA lubrication investigated their role in synovial fluid of articular joints. In vitro studies at cartilage–cartilage interfaces showed that HA and PRG4 solutions effectively lubricated this interface, as indicated by a statistically significant reduction in friction compared to the control lubricant, in a boundary lubrication regime. When HA and PRG4 were combined in solution, a synergistic lubrication effect was observed, reducing friction to a greater extent compared to PRG4 or HA alone.[11] The presence of PRG4 in the eye motivated additional studies into its function at that interface. PRG4 was shown to lubricate, in vitro, at the cornea–eyelid interface suggesting that it functions as a natural lubricant for the eye.[12] PRG4 was further shown to lubricate a cornea–PDMS interface and exhibited synergistic behavior with HA as well showing that these molecules can function for soft inorganic materials. HA has been shown to adhere to[13] and be absorbed and rereleased[8] by silicone hydrogel contact lenses. This inspired investigation into applying these molecules as natural lubricants for commercial contact lenses while in solution in the form of an eye drop or contact lens solution. In a preliminary study, PRG4 has been shown to lubricate certain commercial contact lens–cornea and commercial

contact lens– eyelid pairs, however, not all.[14] Specifically, PRG4 was seen to reduce friction for popular silicone hydrogel lenses but not for lenses that included a 5mm thick crosslinked gel layer[15,16] that has been shown to reduce friction.[5] Being boundary lubricants, PRG4 and HA must interact with the surface boundaries of materials and form adsorbed, low friction layers in the proper configuration to be effective. It is possible that certain surfaces may not allow these boundary lubricants to function properly. PRG4 in particular, must be able to adsorb and form a boundary lubricating layer on surfaces to be effective.[17,18]

N,N-Dimethylacrylamidetris (trimethylsiloxy) silane, (DMAA/TRIS) and Methacryloxypropyltris (trimethylsiloxy) silane (pHEMA/TRIS) are common silicone hydrogels that are useful as model contact lens hydrogel materials for study.[19,20] While they are not true contact lenses per se, and do not contain other additives commonly found in contact lenses, many contact lenses currently on the market, do include pHEMA, DMAA, and a siloxane macromere.

The objectives of this study were therefore to:

1. Determine if PRG4 can sorb to model contact lens materials pHEMA/TRIS and DMAA/TRIS and assess the boundary lubricating abilities PRG4 on these materials at eyelid and cornea interfaces.

2. Assess the boundary lubricating ability and synergistic relationship of PRG4 and HA compared to saline on popular contact lens polymer constituents pHEMA/TRIS and DMAA/TRIS at eyelid and cornea interfaces.

MATERIALS AND METHODS

Materials

Human corneas (age: 54–81) were provided by the Southern Alberta Lions Eye Bank. Corneas were harvested and stored in Optisol-GS (Bausch & Lomb, Rochester, NY) at 48C prior to testing. Corneas were tested within 2 weeks of harvest. Human eyelids (age: 69–91) were excised from fresh cadavers, which were donated for scientific research to the University of Calgary body donation program as previously described.[9,12,14,21] Approval for use of these tissues in this study was granted by the University of Calgary Conjoint Health Research Ethics Board.

The model silicone hydrogel materials were prepared according to previously published methods.[22,23] Polydimethylsiloxane (PDMS) and curing agent for the controls was purchased and mixed at 10:1 (Sylgard 184, Dow Corning, Midland, MI, USA). Hydrogel materials 2-Hydroxethyl methacrylate (HEMA), dimethyl methacrylic acid (DMAA) and ethylene glycol dimethacrylate (EGDMA) were purchased from Sigma-Aldrich (Oakville, ON, Canada). Methacryloxy propyl tris (trimethylsiloxy) silane (TRIS) was purchased from Gelest (Gelest, Morrisville, PA, USA). The photoinitiator IGRACURE was purchased from CIBA (Mississauga, ON, Canada).

All saline used in this study was Bausch & Lomb Sensitive Eyes^{VR} Plus Saline Solution (Bausch & Lomb, Rochester, NY, USA). This product is a sterile, isotonic, buffered solution that contains boric acid, sodium borate, potassium chloride, sodium chloride, preserved with polyaminopropyl

biguanide (0.00003%) and edetate disodium (0.025%).[24] HA was from Blink Contacts® lubricating eye drops (Abbott Medical Optics, Santa Ana, CA, USA) in solution at 1.5 mg/ mL.[25] This product is a sterile, buffered, isotonic, preserved solution, with an aqueous formulation that includes purified water, HA, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, boric acid. It is preserved with OcuPure® preservative (stabilized oxychloro complex 0.005%).[26] PRG4 was purified from conditioned media from bovine cartilage explant culture, using previously published methods.[21,27] Purity was confirmed to be ≥95% by SDSPAGE and protein stain, and the concentration of the purified solution of PRG4 was determined by bicinchoninic acid (BCA) assay.[28] Aliquots of the purified PRG4 stock solution were either diluted in saline or speed-vac dried then resuspended in the HA-containing Blink Contacts® isotonic solution, to a final PRG4 concentration of 300 mg/mL, as described previously.[21,29]

pHEMA hydrogel preparation

Model hydrogel materials pHEMA was prepared by mixing HEMA with EDGMA crosslinker (1 wt %) along with IGRACURE (0.5 wt %). The mixture was poured into aluminum molds and cured in a UV chamber (20 min, 340 nm). The materials were then placed in an incubator overnight at 508C to ensure polymerization.[20] Disks were rinsed in distilled H_2O, and then saline, then fully hydrated overnight before use in both sorption and friction experiments.

Silicone hydrogel preparation: pHEMA/TRIS

Model silicone hydrogel pHEMA/TRIS was prepared by mixing HEMA and TRIS at a 9:1 mass ratio, then EDGMA crosslinker (5 wt %) and IGRACURE (0.5 wt %) were added. The material was then cured and polymerized as described above.[22] Disks were rinsed and hydrated as described above.

Silicone hydrogel preparation: DMAA/TRIS

Model silicone hydrogel DMAA/TRIS was prepared by mixing DMAA and TRIS (1:1 mass ratio), followed by the addition of EDGMA crosslinker (5 wt %) and photoinitiator (0.5 wt %). The material was then cured and polymerized, again as described above.[23] Disks were rinsed and hydrated as described above.

PRG4 sorbtion assay via Western blot

PRG4 sorbtion to the model silicone hydrogels was determined using previously published methods.[14,30] 7 mm diameter disks were cut from fully hydrated pHEMA, pHEMA/ TRIS, and DMAA/TRIS samples. Disks were also cut from the control material PDMS. Sample disks were soaked in 80 mL of 500 mg/mL PRG4 overnight at room temperature. The sample disks were the removed and put through three washing steps. In each step the disk was transferred to a fresh 80 mL bath of saline and gently agitated for 5 min. After the wash, the disks were transferred into 80 mL of

NuPage LDS sample buffer and heated to 708C for 20 min to remove sorbed PRG4. The heated sample buffer was then loaded on to a Novex 3–8% Tris–acetate gel for electrophoresis and

Western immunoblotting.[31] Three control samples of 1, 2, and 3 mg of PRG4 were also loaded on to the gel. PRG4 was identified using primary anti-PRG4 mAb 4D6.[32] Relative sorption of PRG4 was assessed qualitatively by densitometry of the blot using ImageJ to quantify the relative intensity of the immunoreactivity PRG4 bands in the sorbed samples.

In vitro friction measurement: sample preparation Friction was evaluated using a previously described in vitro ocular friction test.[9,12,14,21] Cornea–model contact lens and eyelid–model contact lens tests were done using a BOSE ELF3200 with axial and rotation actuators and a torqueaxial load cell combination (Figure 1). For cornea–model contact lens material tests, model silicone hydrogel samples were cut into annuli (outer radius r_o 5 3.2 mm, inner radius r_i 5 1.5 mm) while fully hydrated and fixed to annular sample holders using silicone adhesive. This holder was then fixed to the linear actuator–sensor complex located at the top part of the BOSE ELF3200. Resected corneas were fixed to spherical silicone rubber sample holder (radius 5 6 mm) by applying cyanoacrylate adhesive to the sclera. This was then fit on to a cylindrical high molecular weight polyethylene (HMWPE) cylinder (diameter 5 11 mm, height 5 19 mm). A silicone rubber sleeve was fitted over the cornea–sample holder to hold a lubricant bath. The cornea plug was then fitted to the rotational actuator at the bottom of the BOSE ELF3200. The linear actuator was used to articulate the model material with the cornea and the rotational actuator was used to slide the cornea against the material. Torque and axial load are collected to calculate the friction coefficients. Eyelid–model contact lens testing samples were prepared by cutting annuli (r_o 5 3.5 mm, r_i 5 1.5 mm) were from cadaver eyelids and fixed to annular sample holders using cyanoacrylate adhesive. This sample holder was fixed to the linear actuator-sensor complex at the top of the BOSE ELF3200. Fully hydrated model silicone hydrogels were cut into disks (radius 5 10 mm), fixed to the HMWPE cylindrical sample holders using silicone adhesive and subsequently fixed to the rotational actuator located at the bottom of the BOSE ELF3200. A silicone sleeve was fitted over the model material–cylinder to hold a lubricant bath. With this setup, the linear actuator was used to articulate the eyelid sample to the model material and the rotational actuator slides the material against the eyelid. As above, torque and axial load are collected to calculate the friction coefficients.

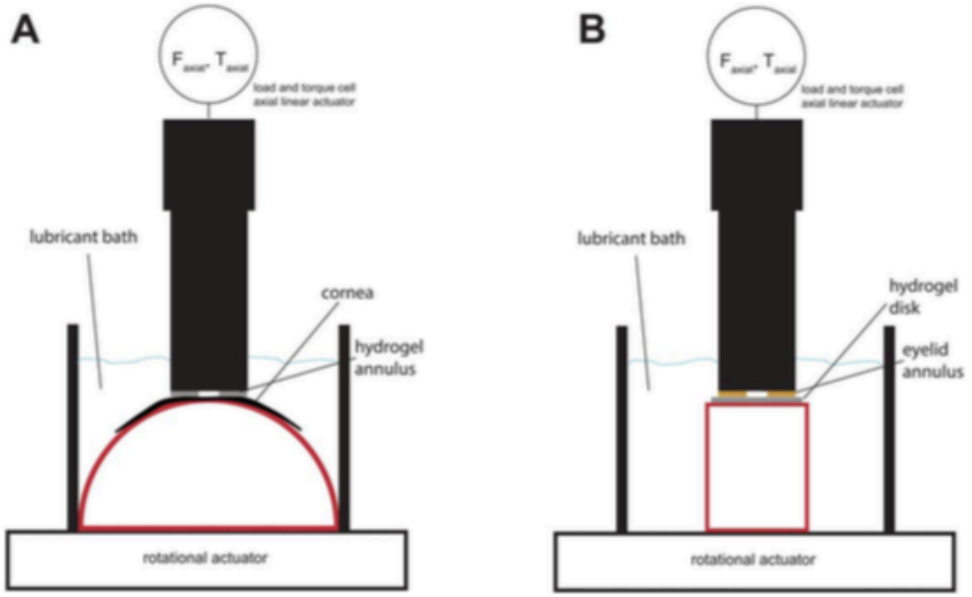

FIGURE 1. Schematic of the *in vitro* human cornea–hydrogel (**A**) and human eyelid–hydrogel (**B**) biomechanical friction test setup.

In vitro friction measurement: test protocol After the samples were mounted, 300 mL of lubricant was placed in the bath (saline, 300 mg/mL PRG4 in saline, HA, or 300 mg/mL PRG4 in HA). The linear actuator was then used to bring the samples mm apart so that both samples were submerged in lubricant. Lubricant and samples were allowed to equilibrate for 5 min. The linear actuator was then used to bring tissue samples into contact at three manually determined axial positions to correspond with axial loads of 0.3 6 0.03, 0.5 6 0.03, and 0.7 6 0.03 N (normal pressures: 10.1–27.3 kPa based on an apparent contact area of 24.6 mm^2). Once in contact at a given axial position, a 12 s dwell time preceded four revolutions in both rotation directions (positive was defined to be counter-clockwise and negative clockwise) directions at four different effective linear velocities (sliding velocities (m_{eff} 5 30, 10, 1.0, 0.3 mm/s) where m_{eff} 5 x $_{eff}$ and r_{eff} 5 2/3[$(r_0^3 - r_i^3)/(r_o^2 - r_i^2)$].[33] Torque and axial load data from each test was collected at 20 Hz. All tests were performed at room temperature (20–218C). Tests were organized according to a repeated measures design in five separate sequences, with a preconditioning run using PDMS in the place of a model contact lens material. The five test sequences were as follows (initial precondition in PDMS not shown):

1. pHEMA (cornea): saline, PRG4 2. pHEMA/TRIS (cornea and eyelid): saline, PRG4 3. DMAA/TRIS (cornea and eyelid): saline, PRG4 4. pHEMA/TRIS (cornea): saline, HA, PRG4, PRG4 6 HA 5. pHEMA/TRIS (cornea): saline, HA, PRG4, PRG4 6 HA

One full sequence was done on a single tissue sample; this was considered to be one repeat when performing statistical analysis. Each test sequence had n 5 6–7 repeats.

In vitro friction measurement: analysis

To evaluate the effectiveness of the PRG4 and HA lubricant solutions, static friction coefficient

μ_{static} and kinetic friction coefficients [$\mu_{kinetic}$] ([]denotes the kinetic equilibrium mean) were calculated from measured torque and axial forces. $\mu_{kinetic}$ was calculated from mean data during the final 2 of 4 rotations. Friction coefficients (l) were calculated as l 5 s/(r_{eff}N), where N is normal load, s is torque, and r_{eff} is described above. l_{static} was calculated by the following equation, l_{static} 5 $|s_{max}|/(r_{eff}N_{eq})$ where the absolute values bars (|x|) denote |x| 5 (|x| 5 [($x^1 - x^-$)/2]) and s_{max} was the maximum torque measured within the first 0.17 rad of rotation in the positive and negative directions. N_{eq} was the equilibrium load measured the instant prior to initiation of spinning. $hl_{kinetic}i$ was calculated as $hl_{kinetic}i$ 5 $|hs_{kinetic}i|/(r_{eff}hN_{kinetic}i|)$ where $|hs_{kinetic}i|$ and $|hN_{kinetic}i|$ were the mean equilibrium torque and axial load, respectively.[14]

Statistical analysis

Data are shown as the mean 6 SEM. Lubricant effects of PRG4, HA, and PRG4 1 HA, velocity effects were assessed by repeated measures 2-way ANOVA with Sidak post hoc test using Systat 12 statistical analysis software (version 12.00.08, SYSTAT Inc., Chicago, IL).

RESULTS

PRG4 sorbtion assay

The Western blot sorption assay (Figure 2) and semiquantitative densitometry analysis showed that PRG4 persisted on the pHEMA, pHEMA/TRIS, DMAA/TRIS, and the PDMS control samples after the wash protocol. (The signal for DMAA/ TRIS was feint compared to others, but was detectable via densitometry analysis.) This indicates that the PRG4 was sorbed to the sample disks during the overnight soak period. Relative sorption showed that pHEMA/TRIS sorbed the most PRG4, sorbing approximately 73% compared to the 1 mg control. The pHEMA samples had similar sorption compared to the 1 mg controls sorbing ~71%. The PDMS sorbed ~50%. The DMAA/TRIS sorbed the least amount of PRG4, ~29% compared to the 1 mg control.

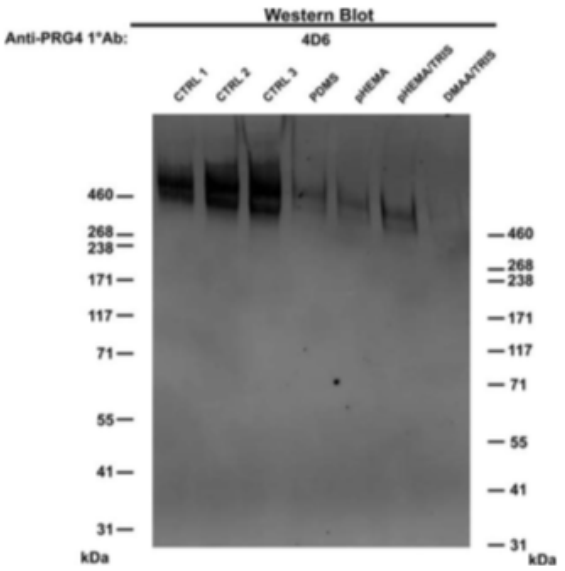

FIGURE 2. SDS-PAGE Western blotting adhesion assay for biomaterials PDMS, pHEMA, pHEMA/TRIS, and DMAA/TRIS. Disks of biomaterials were soaked in 500 μg/mL PRG4 solutions and washed. Washed disks were boiled in SDS buffer to remove sorbed PRG4. SDS buffer was loaded to SDS-PAGE gel and imaged with anti-PRG4 antibody 4D6. CTRL 1, 2, and 3 are 1, 2, and 3 μg of PRG4 loaded into gel.

PRG4 lubrication effect on pHEMA

Friction coefficients were not significantly affected by PRG4 at the pHEMA–cornea biointerface (l_{static} p 5 0.99, $hl_{kinetic}i$ p 5 0.69) (Figure 3). There was a significant effect of velocity on l_{static} ($p < 0.01$), with values of l_{static} increasing with velocity.

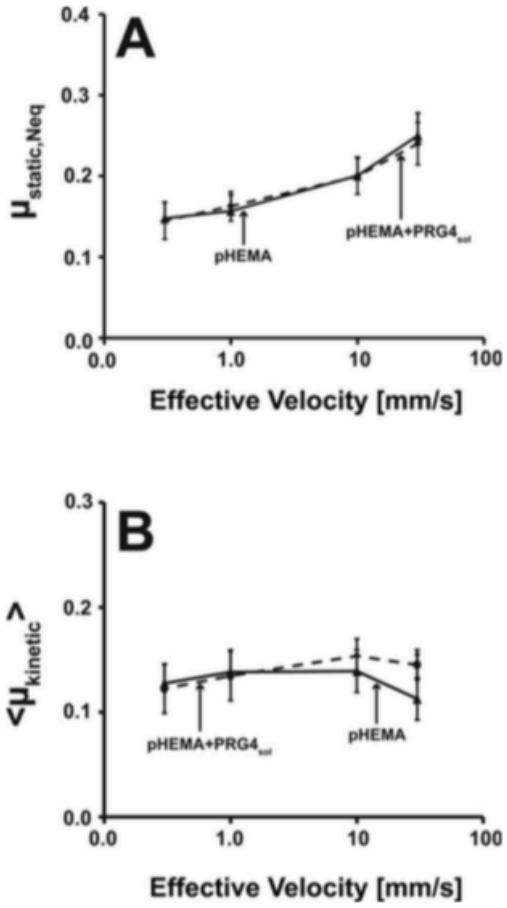

FIGURE 3. Effect of PRG4 on boundary lubrication at a human cornea–pHEMA biointerface. Static, μ_{static} (**A**), and kinetic, $\langle\mu_{kinetic}\rangle$ (**B**). Friction coefficients measured in baths of saline and PRG4 at 300 μg/mL in saline. Values are mean ± SEM (n = 6 cornea, n = 6 eyelid) with an average normal stress of 21.3 ± 0.6 Pa for cornea. Lubricant effect (p = 0.99 A; p = 0.69), velocity effect ($p < 0.01$ A; B p = 0.86).

PRG4 lubrication effect on pHEMA/TRIS. PRG4 had a significant effect on l_{static} and $hl_{kinetic}i$ for the pHEMA/TRIS– cornea and pHEMA/TRIS eyelid biointerfaces (Figure 4). Values of $hl_{kinetic}i$ (Table I) were significantly reduced in PRG4 compared to saline for both the pHEMA/TRIS–cornea biointerface [p 0.01, mean over all velocities; Figure 4(B)] and the pHEMA/TRIS–eyelid biointerface [p 0.05, mean over all velocities; Figure 4(D)]. A similar reduction of l_{static} by PRG4 was observed, with a significant effect of velocity on l_{static} for both the cornea [$p < 0.01$, Figure 4(A)] and eyelid [$p < 0.05$, Figure 4(C)].

TABLE I. Values of $\langle \mu_{kinetic} \rangle$, Mean Over All Velocities, for the Various *In Vitro* Eyelid–Hydrogel and Cornea–Hydrogel Friction Tests Conducted to Assess the Lubricating Properties of PRG4 and/or HA

		$\langle \mu_{kinetic} \rangle$			
Lubrication Effect	Biointerface	Saline	HA	PRG4	PRG4+HA
PRG4 on pHEMA/TRIS	Cornea–hydrogel	0.21 ± 0.03	-	0.18 ± 0.03	-
	Eyelid–hydrogel	0.19 ± 0.03	-	0.13 ± 0.03	-
PRG4 on DMAA/TRIS	Cornea–hydrogel	0.17 ± 0.03	-	0.13 ± 0.03	-
	Eyelid–hydrogel	0.13 ± 0.04	-	0.09 ± 0.03	-
PRG4, HA on pHEMA/TRIS	Cornea–hydrogel	0.31 ± 0.03	0.23 ± 0.04	0.26 ± 0.05	0.17 ± 0.04
PRG4, HA on DMAA/TRIS	Cornea–hydrogel	0.15 ± 0.02	0.16 ± 0.02	0.12 ± 0.02	0.10 ± 0.02

Data is mean ± SEM.

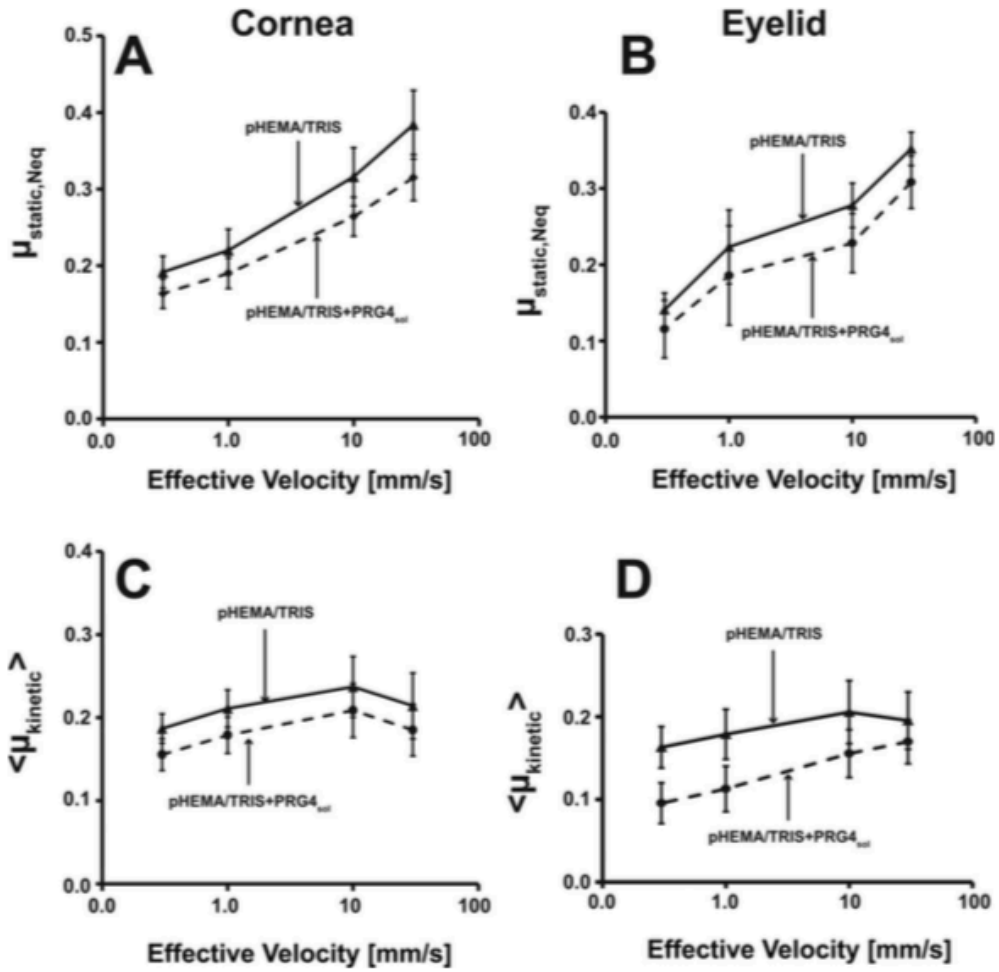

FIGURE 4. Effect of PRG4 on boundary lubrication at a human cornea–pHEMA/TRIS biointerface. Static, μ_{static} **(A)**, and kinetic, $\langle \mu_{kinetic} \rangle$ **(C)**, and at a human eyelid–pHEMA/TRIS biointerface. Static, μ_{static} **(B)**, and kinetic, $\langle \mu_{kinetic} \rangle$ **(D)**. Friction coefficients measured in baths of saline and PRG4 at 300 μg/mL in saline. Values are mean ± SEM (n = 7 cornea, n = 6 eyelid) with an average normal stress of 22.1 ± 0.7 Pa for cornea and 9.2 ± 0.7 Pa for eyelid. Lubricant effect: ($p < 0.01$ A, C, D; $p < 0.05$ D), velocity effect: ($p < 0.01$ A, C; $p < 0.05$).

PRG4 lubrication effect on DMAA/TRIS. PRG4 also had a significant effect on l_{static} and $hl_{kinetic}i$ for the DMAA/TRIS– cornea and DMAA/TRIS–eyelid biointerfaces (Figure 5). Values of $hl_{kinetic}i$ (Table I) were significantly reduced in PRG4 compared to saline for both the DMAA/TRIS–cornea biointerface [$p < 0.05$, mean over all velocities; Figure 5(B)]. and the

DMAA/TRIS–eyelid biointerface [p < 0.05, mean over all velocities; Figure 5(D)]. PRG4 also significantly reduced values of l_{static} compared to saline for both the cornea (p 0.05) and eyelid (p 0.05) counterfaces, with a significant effect of velocity for both the cornea [p < 0.01, Figure 5(A)] and eyelid [p < 0.05, Figure 5(C)] as well.

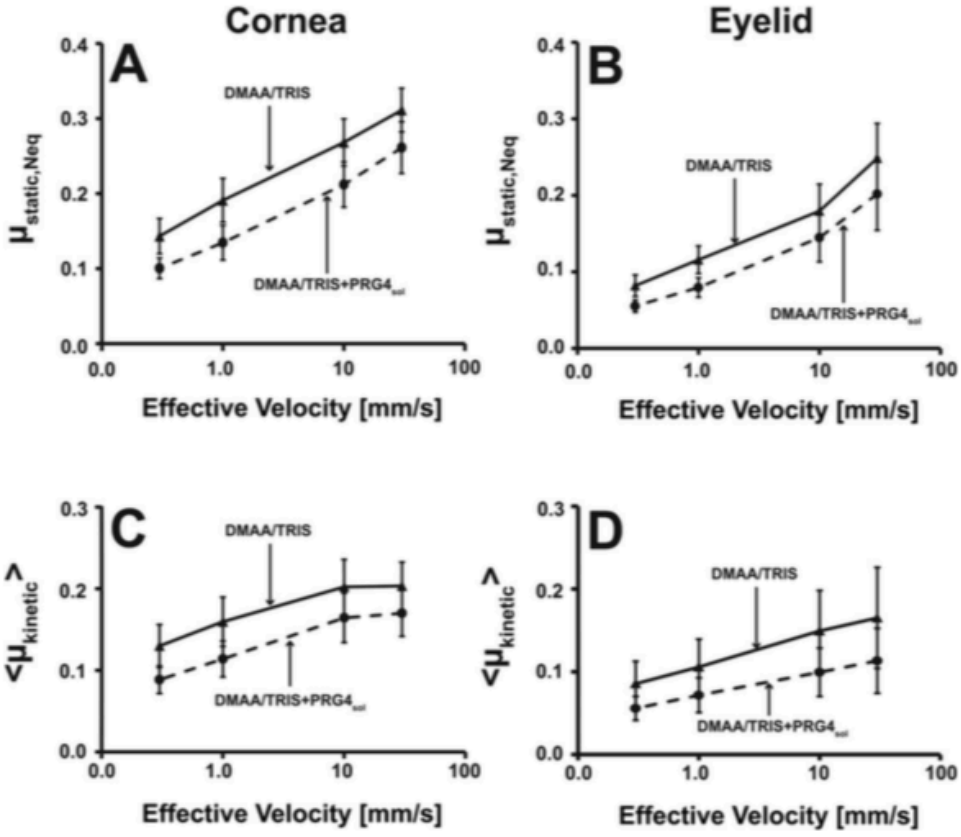

FIGURE 5. Effect of PRG4 on boundary lubrication at a human cornea–DMAA/TRIS biointerface. Static, μ_{static} **(A)**, and kinetic, $\langle\mu_{kinetic}\rangle$ **(C)**, and at a human eyelid–DMAA/TRIS biointerface. Static, μ_{static} **(B)**, and kinetic, $\langle\mu_{kinetic}\rangle$ **(D)**. Friction coefficients measured in baths of saline and PRG4 at 300 μg/mL in saline. Values are mean ± SEM (n = 7 cornea, n = 6 eyelid) with an average normal stress of 22.1 ± 0.7 Pa for cornea and 9.2 ± 0.7 Pa for eyelid. Lubricant effect: ($p < 0.01$ A, B, C; $p < 0.05$ D), velocity effect: ($p < 0.01$ all; $p < 0.05$ D).

PRG4 and HA lubrication effect on pHEMA/ TRIS. PRG4 6 HA significantly reduced values of $hl_{kinetic}i$ compared to either PRG4 or HA alone (p 0.01), both of which reduced friction to similar extents compared to saline (p 0.05), at the pHEMA-TRIS–cornea biointerfaces [Figure 6(B), values summarized in Table I]. Values of l_{static} showed similar trends with PRG4 (p < 0.05) and HA (p < 0.01) both significantly reducing friction compared to saline, although PRG4 appeared to reduce l_{static} to a greater extent, with values in PRG4 6 HA again being further reduced in a significant manner (p 0.01) [Figure 6(A)]. Velocity also had a significant effect on l_{static} (p < 0.01).

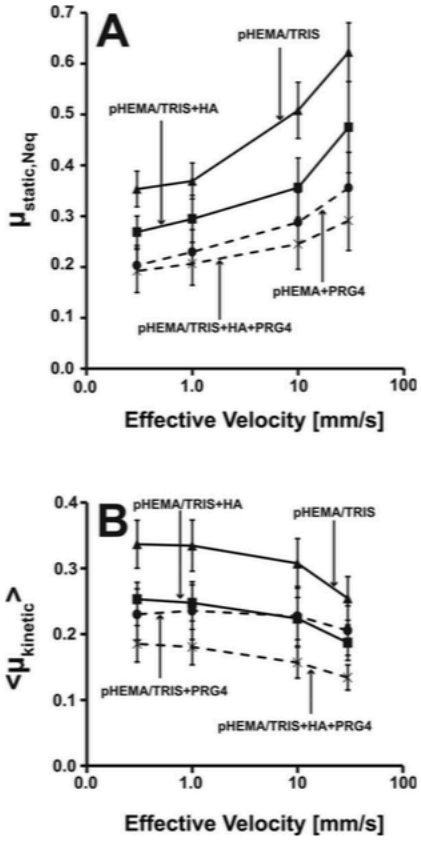

FIGURE 6. Effect of PRG4 and HA on boundary lubrication at a human cornea-pHEMA/TRIS biointerface. Static, μ_{static} (A), and kinetic, $\langle\mu_{kinetic}\rangle$ (B). Friction coefficients measured in baths of saline, PRG4 at 300 μg/mL in saline, HA at 1.2 mg/mL in saline or HA and PRG4 in saline at these concentrations. Values are mean ± SEM ($n = 6$) with an average normal stress of 20.5 ± 0.6 Pa. Lubricant effect: ($p < 0.01$ A, B), velocity effect: ($p < 0.01$ A).

PRG4 and HA lubrication effect on DMAA/TRIS. Values of $hl_{kinetic}i$ were significantly reduced in by both PRG4 and PRG4 1 HA compared to saline at the DMAA/TRIS–cornea biointerface ($p < 0.05$ for both), while HA did not [p 5 0.71, Figure 7(B), values summarized in Table I]. Furthermore, PRG41HA did not appear to reduce values of $hl_{kinetic}i$ further than the PRG4 alone. Values of l_{static} showed similar trends with both PRG4 and PRG4 1 HA significantly reduced friction compared to saline ($p > 0.01$), the combination of PRG4 1 HA not appearing to further reduce friction compared to PRG4, and HA not significantly reducing friction compared to PRG4 [Figure 7(A)]. Velocity also had a significant effect on l_{static} ($p < 0.05$).

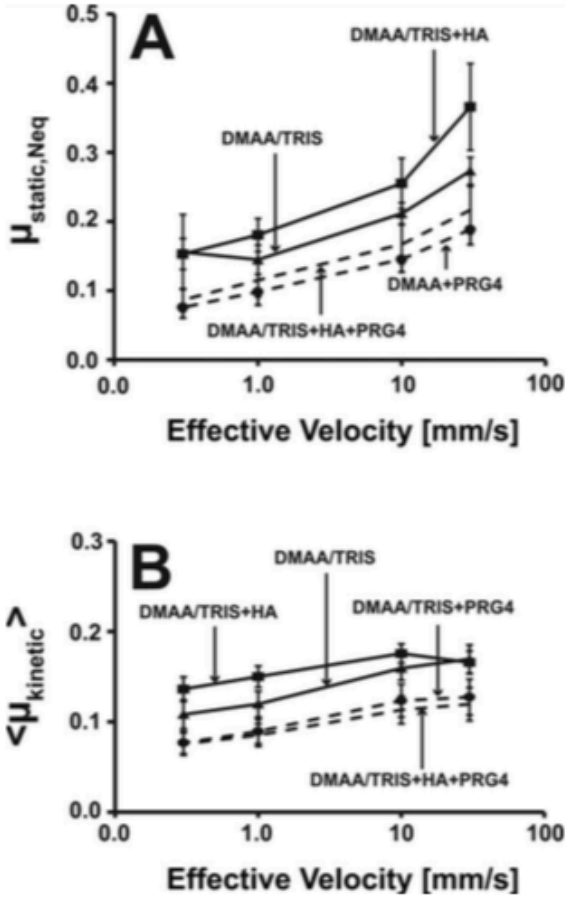

FIGURE 7. Effect of PRG4 and HA on boundary lubrication at a human cornea–DMAA/TRIS biointerface. Static, μ_{static} (**A**), and kinetic, $\langle\mu_{kinetic}\rangle$ (**B**). Friction coefficients measured in baths of saline, PRG4 at 300 μg/mL in saline, HA at 1.2 mg/mL in saline or HA and PRG4 in saline at these concentrations. Values are mean ± SEM ($n = 6$) with an average normal stress of 17.1 ± 0.6 Pa. Lubricant effect: ($p < 0.01$ A; $p < 0.05$ B), velocity effect ($p < 0.01$ A; $p < 0.05$ B).

DISCUSSION

The objectives of this study were (1) to determine if PRG4 can sorb to and lubricate model contact lens materials and (2) to assess the boundary lubricating ability and synergism of PRG4 and HA on model contact lens materials. Sorption data show that PRG4 can adhere to pHEMA, pHEMA/TRIS and DMAA/TRIS. Boundary friction data for pHEMA showed that PRG4 and HA were not effective lubricants for this interface compared to saline. Data for DMAA/TRIS showed that PRG4 was an effective lubricant for this material, and HA and PRG4 were effective for pHEMA/TRIS, as indicated by a statistically significant reduction in friction compared to the saline control lubricant. PRG4 and HA only showed evidence of synergistic lubrication for pHEMA/TRIS. These data indicate that PRG4 and HA could be effective lubricants for some contact lens materials, which could potentially help improve comfort. PRG4 and HA boundary lubrication is, however, surface specific and therefore cannot be used effectively for all contact lens materials.

This study used in vitro friction testing with human cadaver tissues. These tissues can have high variability and low availability; which was why the repeated measures test setup was chosen where control variables (lubricant) were tested on a single tissue. The materials in this study were restricted to common contact lens hydrogels pHEMA, DMAA and TRIS. These do not encompass all contact lens hydrogel materials contact lenses can commonly include other additives such as vinyl-pyrrolidone and methacrylic acid. These were intentionally chosen as many contact lenses currently on the market, however, do include pHEMA, DMAA, and a siloxane macromer. The model materials were not synthesized to replicate the geometries of commercially available contact lenses, being flat and much thicker, which gives them different bulk material properties such as ultimate strength and may alter shearing behavior. The purpose of this study was not to replicate the friction coefficients of contact lenses but rather to investigate lubricant–surface interactions for common contact lens constituents.

Sorption data (Figure 2) show that PRG4 adheres to pHEMA, pHEMA/TRIS, and DMAA/TRIS suggesting that it could function as a boundary lubricant for these surfaces. Indeed, friction results indicate that PRG4 alone is an effective boundary lubricant for the model silicone hydrogel materials (Figures 4–7) but not the conventional hydrogel

pHEMA (Figure 3), despite sorption that is comparable to the PDMS control. However, the location of adsorbed protein cannot be inferred using the Western blot method. Due to this limitation, it is also unclear whether the protein adsorbed differently to the cut edges versus the surfaces of the hydrogel disks. This data would be valuable in guiding the future development of materials designed to interact with PRG4.

PRG4 is an amphiphilic molecule, having both hydrophobic and hydrophilic domains that serve different functions in vivo. For proper lubricating function, it is thought that the hydrophobic Nand/or C-termini of the protein domains of the molecule are anchors that adhere to surfaces and that the hydrophilic mucin domains create a hydrated, low friction layer.[17,34] The lubrication behavior of PRG4 on artificially controlled hydrophilic and hydrophobic surfaces shows that PRG4 did not lubricate a hydrophilic surface.[35] Silicone macromers such as TRIS are hydrophobic, and the addition of these constituents to hydrogels is known to increase their water contact angle and therefore their hydrophobicity.[36] Based on our current knowledge, it follows that the addition of the silicone macromer TRIS to a hydrogel creates hydrophobic bonding points for the Nand/or C-termini of the PRG4 molecule allowing it to function as a boundary lubricant. In the case of the more hydrophilic pHEMA, it may be that the surface of the pHEMA has a more favorable attraction to the hydrophilic mucin domain of PRG4. This could create an unfavorable tail-like conformation, where the mucin domains are adherent to the surface leaving the protein domains to interact with other surfaces.[35] Another scenario that could explain this failure in lubrication is the hydrophilic surface attracting the mucin domain and partially collapsing the lubricating brush layer into a “mushroom-like” configuration.[37] This reduces the effectiveness of PRG4 by reducing the radius of gyration of the mucin brush layer and therefore its steric repulsion.

In experiments looking at both a material–cornea and material–eyelid interface (Figures 4 and 5) the relative lubrication of PRG4 does not appear to be drastically different. This suggests the choice of ocular tissue does not affect PRG4 lubrication to a large extent. This, combined with

previous data showing that PRG4 effectively lubricates an eyelid–cornea interface[9,12] suggest that PRG4 lubricates both eyelid and cornea tissue to a similar degree, at least in the experimental setup employed here.

HA was an effective boundary lubricant for pHEMA/ TRIS and PRG4–HA synergism appeared to exist for pHEMA/TRIS (Figure 6). HA did not effectively lubricate the DMAA/TRIS model silicone hydrogel (Figure 7). The mechanisms of HA lubrication and HA–PRG4 synergy for these biomaterials are not well understood currently. HA is an effective boundary lubricant at a cartilage–cartilage interface,[38,39] and a cornea–PDMS interface,[21] but not a cartilage–glass interface.[40–42] Like PRG4, HA normally functions in biological systems which are typically soft, porous elastic materials with complex surface chemistries. This has been speculated to be due to poor adsorption behavior. A similar effect could be seen here with DMAA/TRIS exhibiting poor HA adsorption. Another possible difference could be due to the fact that DMAA/TRIS is considerably stiffer than pHEMA/TRIS[43] which parallels the cartilage–glass results from literature indicating a possible link between the stiffness of the interfaces and HA boundary lubrication. According to Newtonian mechanical contact models, given the same surface roughness and adhesive properties, a stiffer material will experience a lower real contact area compared to a softer material, which will result in reduced adhesive interaction and reduced friction.[44] A softer hydrogel may also experience greater hysterysis than stiffer ones, which can have a significant effect on friction.[45] However, there is insufficient data on the lubrication mechanism to speculate further.

The results of this study agree with and extend previous studies looking at PRG4 and HA lubrication at biointerfaces. HA and PRG4 lubrication and synergism was observed at a pHEMA/TRIS–cornea interface which parallels data observed at a cornea–PDMS interface.[21] PRG4 was also observed to lubricate more hydrophobic silicone hydrogel materials and not hydrophilic pHEMA. Collectively these results showed different sorption and friction behavior for PRG4, and differing friction behavior for HA and PRG4 1 HA solutions depending on the hydrogel material. Because boundary lubricants must interact/bind with surfaces to be able to reduce friction, and PRG4 was observed to reduce friction on some but not all materials tested here, these data indicate one must carefully test and select a lubricant for a given biomaterial. Future studies will examine at linking PRG4 and HA to the surface of other biomaterials, nonsilicone hydrogels as well as additional commercial contact lenses, and consider strategies for prolonged persistence/release of PRG4 in the ocular environment.

Overall, the results of this study contribute to our understanding of PRG4 and HA interactions with contact lens materials pHEMA/TRIS and DMAA/TRIS. Such enhanced understanding, combined with the existence of HA containing solutions and clinical grade PRG4, could aid in the development of improved biocompatibility and comfort of contact lens materials by the merging biological and artificial materials with lubricants.

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