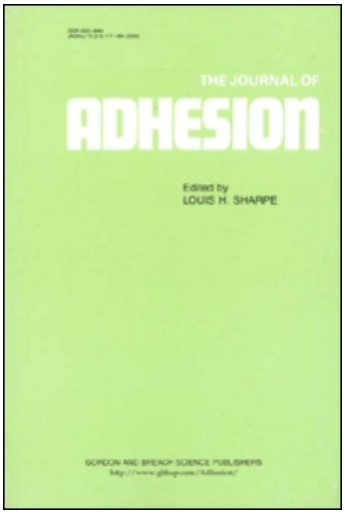

The Journal of Adhesion
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713453635

Tissue-on-Tissue Testing of Dry Eye Formulations for Reduction of Bioadhesion

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To cite this Article Meyer, Anne E. , Baier, Robert E. , Chen, Huagang and Chowhan, Masood(2006) 'Tissue-on-Tissue Testing of Dry Eye Formulations for Reduction of Bioadhesion', The Journal of Adhesion, 82: 6, 607 — 627
To link to this Article: DOI: 10.1080/00218460600766673
URL: http://dx.doi.org/10.1080/00218460600766673

The Journal of Adhesion, 82:607–627, 2006

ISSN: 0021-8464 print/1545-5823 online
DOI: 10.1080/00218460600766673

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Tissue-on-Tissue Testing of Dry Eye Formulations for Reduction of Bioadhesion

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Glutaraldehyde-preserved, human umbilical cord vein graft (UCVG) was selected as a stable surrogate tissue source for testing of bioadhesion-reducing lubricants. Bioadhesion, as manifested in tissue-on-tissue friction coefficients of 0.2–0.4 for saline-lubricated UCVG, was quantitatively and persistently reduced after the instillation of a single aliquot of an ophthalmic "artificial tears" formulation containing active demulcents polyethylene glycol (PEG400) and propylene glycol (PG), as well as a gellable hydroxypropyl guar (HP Guar) in a borate-buffered solution between the "blinking" tissues. Reduced adhesion was maintained (was "substantive"), even after rinsing excess lubricant from the surfaces. Comparative tests with tissue-on-solid, and solid-on-solid, similarly lubricated couples point to a potentially unique mechanism that involves macromolecules modifying the tissue phases to provide rinse-resistant lubricity and surface protection in articulated tissue-to-tissue interfaces. Results for tissue-on-tissue couples were obtained in laboratory trials utilizing a reciprocating pin-on-disc type friction/wear test device articulating preserved human umbilical cord vein segments under increasing loads, and again after saline rinsing to determine persistence of the friction-reducing effects. A single confirmatory test using donated human cornea against vein graft tissue showed the lowest coefficient of friction, below 0.05, for the "artificial tears" formulation. Mechanistic studies employing the same test device and protocol for metal oxide (germanium)-on-metal oxide couples, as well as for metal oxide-on-tissue couples, indicated that simple increases in viscosity were not the likely

Received 18 October 2005; in final form 13 April 2006.

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sources of friction reduction, and revealed frictional values higher than measured for the similarly lubricated tissue-on-tissue couples. Thus, formulation development to minimize bioadhesion requires that appropriate simulations be used to obtain clinically predictive data for circumstances of liquid uptake into the tissues, resultant tissue swelling, and binding to impermeable adjacent materials.

Keywords: Cornea; Friction; Guar; Lubrication; Tears; Tissue

INTRODUCTION

A large portion of the biomedical literature on friction and lubrication addresses articular (joint) cartilage and total joint replacement applications. In addition to reports on complex joint simulator studies, preclinical orthopaedic and maxillofacial joint research continue to utilize simplified model systems such as pin-on-disk apparatus [1,2], pendulum-type friction apparatus [3,4], surface forces apparatus [SFA] [5–7], and atomic force microscopy [AFM] [8]. Tribological techniques also are relevant, but less frequently reported, for applications such as vascular [9] and urological [10] catheter insertion, and dentistry [11].

In the ophthalmologic field, friction and lubrication conditions usually are implied through clinical studies of patient-reported "comfort" [12–15] and/or microscopic inspections of corneal damage [12,16,17]. A large segment of the eye-care industry has grown to deliver eye drops that address discomfort and other complications of contact lens wear [18,19], side-effects of health conditions such as diabetes [20], Sjogren's Syndrome [21], and ophthalmologic surgery [22–24]. Reports of pre-clinical *in-vitro* research on friction and lubrication, and the development of *in-vitro* models, are less prevalent in the ophthalmologic literature. In the pre-clinical stage, simple measurements of water-wettability of contact lenses often are put forth as predictors of *in-vivo* comfort. Some investigators, however, have applied tribological concepts to *in-vitro* studies of cornea/contact lens interactions [25] and eyelid/contact lens interactions [18]. Application of AFM, SFA, and more recently, sum-frequency-generation vibrational spectroscopy [SFG] to simple model systems of lubricant/contact lens interactions have demonstrated the importance of lubricant adsorption to achieve reduced friction [26,27]. Using more complex models, Ubels *et al*. [28] reported results of "artificial tears" formulations to reduce corneal desiccation in *in-vitro* cell culture and *in-vivo* animal experiments. Lenton and Albietz [23] used a modified tensile testing apparatus to evaluate the frictional properties of "artificial tears" solutions that are used as lubricants during laser *in-situ* keratomileusis (LASIK) surgery.

From a long series of prior investigations of adhesion-resistant natural tissue surfaces, before and after their chemical preservation by glutaraldehyde crosslinking [29–36], it has been recognized that the intimal surfaces of glutaraldehyde-fixed human umbilical cord vein grafts (UCVG) display the same wetting, spreading and critical surface tension for wetting qualities as the natural low adhesion tissues [33,37,38]. Therefore, UCVG segments were selected from laboratory quality-control graft specimens as surrogate tissues for testing of potential adhesion-reducing substances that might be introduced into natural tissue-to-tissue couples. Among many potential applications of friction-reducing formulations in biological systems, the development of "artificial tears" for maintenance of eye comfort is prominent. This investigation utilized a recently introduced formulation ["SYS," Systane®, Alcon Laboratories, Inc., Fort Worth, TX] containing active demulcents (polyethylene glycol [PEG400] and polypropylene glycol [PG]), as well as a gellable polymer hydroxypropyl guar [HP Guar] in a borate-buffered solution [28] that shows good clinical performance [12,13], to evaluate the prospects for UCVG testing as an improved approach to characterize, predict, and better understand the mechanism of tissue-on-tissue lubrication. The formulations for "blinking" friction reduction were first studied in pin-on-disc frictional trials, utilized as lubricants for glow-discharge-treated polystyrene-on-polystyrene couples. The concern was that this conventional solid-on-solid testing, as with earlier work using polymethylmethacrylate couples [18], would not be relevant to the biological settings in which bioadhesion is to be reduced. Trials with metallic and polymeric couples assist with viscosity-based composition selection, but cannot adequately account for liquid uptake into the tissues, resultant tissue swelling, and tissue surface modification. Confirmation of the tissue-on-tissue lubricity of the SYS formulation was extended to tests with a donated human cornea as one member of the tissue couple.

Since the surface properties of cornea [34] and eyelid tissues [35], determined by a combination of contact angle and infrared spectroscopic methods, were found to be remarkably well-matched by the intimal wall properties of glutaraldehyde-preserved human umbilical cord vein grafts (UCVG) used for peripheral arterial surgery [33,37,38], the relevance of this surrogate tissue system is supported. The experimental approach and results are comparable with those developed in tissue-on-tissue testing of rabbit visceral pleura sliding against parietal pleura, lubricated with pleural liquid, as occurs in normal breathing [39].

The following pages describe how segments of laboratory control specimens of UCVG were used in tissue-based test protocols where

coefficients of friction were monitored under reciprocating motion and increasing loads. Ancillary inspection techniques included multiple attenuated internal reflection infrared (MAIR-IR) spectroscopy for lubricant and tissue residues, and microscopy of the articulated tissue surfaces for tissue-on-germanium MAIR-IR prism tests as well as tissue-on-tissue tests of both saline-lubricated and SYS formulation lubricated systems. Germanium-on-germanium couples were used to determine intrinsic lubricity of the formulations and substantivity of components bound to the impermeable materials.

EXPERIMENTAL

Reciprocating Friction

Testing was carried out with a reciprocating pin-on-disc device constructed and donated by Spire Corporation (Bedford, MA, USA), that allowed controlled relative motions for different surface-to-surface couples (Figure 1). One test surface was always attached to a vertically loaded "pin", and the opposing test surface fixed horizontally to a "disk" that oscillated through an arc length of 25 mm at a 1 Hz cycle. Friction between the two surfaces was monitored *via* a strain gauge and strip chart recorder system. The strain gauge received direct frictional (drag) forces transferred from two mutually perpendicular rigid rods intersecting at a pivot point. The frictional forces, transferred to the end of one of the rods connected to the strain gauge,

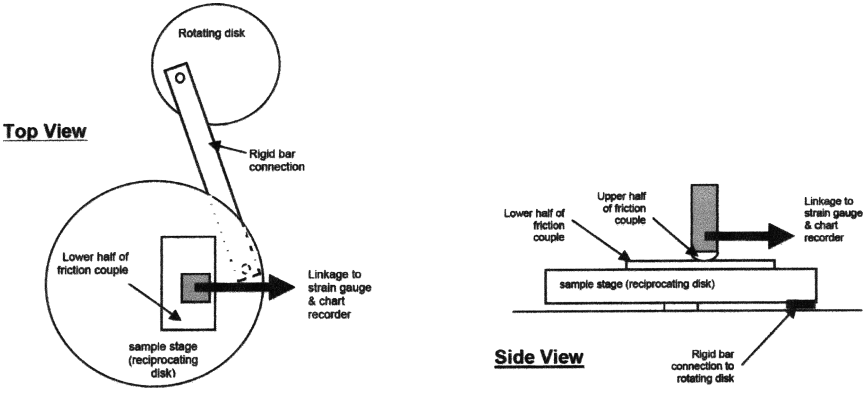

FIGURE 1 Schematic of apparatus used to evaluate tissue-on-tissue, tissue-on-Ge, Ge-on-Ge, and cornea-on-tissue friction. Both a top view and side view are shown.

introduced compression or tension to the strain gauge and caused a consequent deflection of the strip chart recorder pen. Calibrations with known weights allowed experimental correlation of the deflection of the strip chart pen to the frictional force between the two articulating surfaces.

Lubricity measurements were accomplished using this reciprocating friction device to evaluate tissue-on-tissue couples and tissue-on-synthetic [tissue-on-germanium] substratum couples. Synthetic-on-synthetic [germanium-on-germanium] couples also were used. All experiments were conducted at ambient laboratory temperature (approximately 21°C) in a Class 100 clean room. All experiments were performed in triplicate, except for single confirming tests using the donated cornea.

Supporting analyses included multiple-attenuated internal reflection infrared [MAIR-IR] spectroscopy and comprehensive contact angle measurements, performed in accord with previously published methodology [40]. Light microscopy and scanning electron microscopy were used to determine surface morphologies.

Tissue Sources

The tissues used as surrogates for the corneal and conjunctival surfaces of the eye were segments of stabilized human umbilical vein grafts, ("Biograft," UCVG, Meadox Medicals, Oakland, NJ, USA), with established surface and micro-architectural features [29,30,36]. A confirming experiment with a donated human cornea against the vein graft tissue also was performed. The cornea was obtained from the Central Florida Lions Eye & Tissue Bank after approval for research use of the tissue by the appropriate regulatory review process.

Characterization of the Formulations

Table 1 describes the formulations tested. The test formulations were characterized before use, by a series of infrared spectroscopic measurements of their dried resides on germanium MAIR-IR prisms. The saline used for all rinsing purposes and as one of the tested formulations was borate-buffered sodium chloride solution ("saline control;" Unisol®, Alcon Laboratories, Inc., Ft. Worth, TX). The "artificial tear" formulation ("SYS") contained active demulcent (PEG400 and PG), as well as the gellable HP-Guar in a borate-buffered solution of the chlorides of potassium, calcium, magnesium, zinc, and sodium, preserved with polyquaternium-1 (Polyquad®, Alcon Laboratories, Inc.). Prior

TABLE 1 Formulations Used

Formulation	Composition [concentrations in %]	Lot number & other label information
Saline control	Sodium Chloride: 0.66 Other: Boric Acid; Sodium Borate; Sodium Hydroxide (adjust pH to 7.0); Hydrochloric Acid (adjust pH to 7.0) Purified Water: to 100%	Lot K2A013; Alcon; Unisol® 4; preservative-free pH-balanced saline sln; 120 ml/bottle; exp 2005/01
SYS	PEG 400: 0.4 Propylene Glycol: 0.3 Other: AL-12355 (HP Guar); Boric Acid; Potassium Chloride; Sodium Chloride; Calcium Chloride; Magnesium Chloride; Zinc Chloride; Sodium Hydroxide (adjust pH to 7.0); Hydrochloric Acid (adjust pH to 7.0); Purified Water: to 100%	Lot 45803 F; Alcon; Systane® Lubricant Eye Drops; 15 ml/bottle; exp 2004/08

testing showed that the SYS formulation without the gellable HP Guar did not exhibit protective properties for ocular tissues [28].

Tissue-on-Tissue Test Protocol

The protocol used preserved umbilical cord vein graft [UCVG] tissue segments as both members of the friction couple. A hemispherical support was used for the upper UCVG tissue. This support was the round bottom of a smooth, polymeric centrifuge tube (12 mm outside diameter; 6 mm radius of curvature). For each experiment, the bottom portion of the centrifuge tube was secured to the vertical "pin" of the friction apparatus, using "super glue" (Instant Krazy® Glue, Elmer's Products, Columbus, OH, USA). The round-bottom centrifuge tubes were selected because they were light in weight (thus being able to use the same loading protocol as with the Ge-on-Ge experiment described later in this section) and, when glued to the post, they resisted loosening due to the forces/stresses created during the experiments. Also, by using these supports, minimal alteration to the instrument set-up was required for transition through Ge-Ge, Tissue-Ge, Tissue-Tissue, and Cornea-Tissue experiments. The hemispherical supports did not deform at the loadings used in the experiments. For each experiment, with each formulation, a new piece of tissue was glued onto an individual support using "super glue" beneath the entire area of the tissue, which was approximately

1 mm thick. The contact area for all experiments was approximately 1 square centimeter. Prior research with these preserved tissue segments, in a similar frictional model (temporomandibular joint disc [41]), demonstrated that there was no show-through of the glue to the surface of the tissue, at the loadings used in this study. The bottom portion of each couple was a flat piece of the UCVG tissue, fixed in place ("super glue" beneath the entire area of tissue) on the flat stage of the reciprocating device. For each test formulation and for each experimental set, new tissue segments were used.

The contacting surfaces in the friction-testing device first were lubricated with a 125 μl aliquot of saline control solution and moved against one another in a reciprocating arc path for 5 minutes at a normal load of 20–25 grams at a 1 Hz "blinking" rate. Frictional forces were recorded for 5 seconds at 1-minute intervals. After the initial 5-minute period with saline, a 125 μl aliquot of test formulation [saline control or SYS] was applied, and the 5-minute test cycle was repeated (20–25 gram load). The normal load then was increased by 10 grams, and the measurements were repeated for 5 minutes (1-minute intervals). The load was increased by another 25 grams (to a total of 55–60 grams), and a third set of measurements was obtained (1-minute intervals).

After 5 minutes at the 55–60 gram load, the device was stopped, and the two surfaces were separated from each other and rinsed (in place) with saline control solution. The volume and application force of the saline was controlled and repeated for each experiment. While still wet, the surfaces were brought back into contact with each other and testing at 1 Hz resumed for another 5 minutes (55–60-gram load, measurements at 1-minute intervals). This protocol is similar to the on/off, stop/start features used in a recent study of the frictional properties of articular cartilage [42].

Cornea-on-Tissue Test Protocol

A flat piece of UCVG was used as the bottom portion of the couple, as described for the tissue-on-tissue test protocol. The cornea was glued to a polymeric round-bottom tube in the same manner as described for the earlier tissue-on-tissue protocol with UCVG tissue. In the cornea-on-tissue experiments, the diameter of the polymeric support was slightly larger (approximately 16 mm diameter; approximately 8.5 mm radius of curvature) than was used for the tissue-on-tissue and tissue-on-Ge experiments, to accommodate the natural radius of curvature of the cornea tissue. A different UCVG segment was used for each test formulation, but–due to the preliminary nature

of this experiment and the limited available cornea supply – the same cornea was used for both test formulations.

The contacting surfaces in the friction-testing device first were lubricated with a 125 μl aliquot of saline control solution and moved against one another in a reciprocating arc path for 3 minutes [rather than the 5 minutes used in all other experiments] at a normal load of 20–25 grams and 1 Hz rate. Frictional forces were recorded for 5 seconds at 1-minute intervals. After the initial 3-minute period with saline, a 125 μl aliquot of test formulation (saline control solution or SYS) was applied, and the 3-minute test cycle was repeated (20–25 gram load). The normal load then was increased by 10 grams, and the measurements were repeated for 3 minutes (1-minute intervals). The load was increased by another 25 grams (to a total of 55–60 grams), and a third set of measurements was obtained over 3 minutes (1-minute intervals).

After 3 minutes at the 55–60 gram load, the device was stopped, and the two surfaces were separated from each other and rinsed (in place) with saline control solution. The volume and application force of the saline was controlled. While still wet, the surfaces were brought back into contact with each other and testing at 1 Hz resumed for another 3 minutes (55–60-gram load, measurements at 1-minute intervals).

After each experiment with a different test formulation, the cornea was rinsed thoroughly with saline control solution for 30 minutes before beginning another cornea-on-tissue experiment.

Tissue-on-Germanium Test Protocol

The protocol for this phase of the task was the same as that described for the Tissue-on-Tissue couple, with the exception that tissue represented only the upper half of the friction couple. A flat, optically-polished germanium plate served as the lower half of the couple. Upon conclusion of each experiment, the lower half of the couple (germanium substratum) was gently leached with distilled water, drained, and air-dried prior to analysis of any retained lubricant or transferred tissue by MAIR-IR spectroscopy and contact angle measurements. Tissue surfaces were examined microscopically.

Germanium-on-Germanium Test Protocol

High-surface-energy, highly water-wettable germanium coupons [top and bottom portions of couple] were mounted in the friction-testing device. The protocol for this phase of the project was the same as that described for the Tissue-on-Tissue experiments.

Upon conclusion of each experiment, the germanium substrata were gently leached with distilled water, drained, and air-dried prior

to analysis of retained lubricant constituents by MAIR-IR spectroscopy and contact angle measurements. Individual, replicate germanium substrata were used for experiments with the different formulations.

RESULTS

Figure 2 illustrates that the SYS formulation is immediately and persistently capable of reducing the tissue-on-tissue coefficient of friction by nearly 80% from the values displayed when only saline is used as the liquid lubricant.

Table 2 provides these data in a format allowing comparisons among the individual pairs of tissues articulated, through each of the 4 stages of fluid addition, loading, increased loading, and sustained loading after rinsing with the saline control solution. The latter values of friction coefficients are compared in Table 3, indicating a significant maintenance of the friction-reducing components from the SYS "artificial tear" system, even after rinsing away of the original formulation with saline alone. This residual effect of the formulation is called "substantivity" in the pharmaceutical field. Figure 3 plots

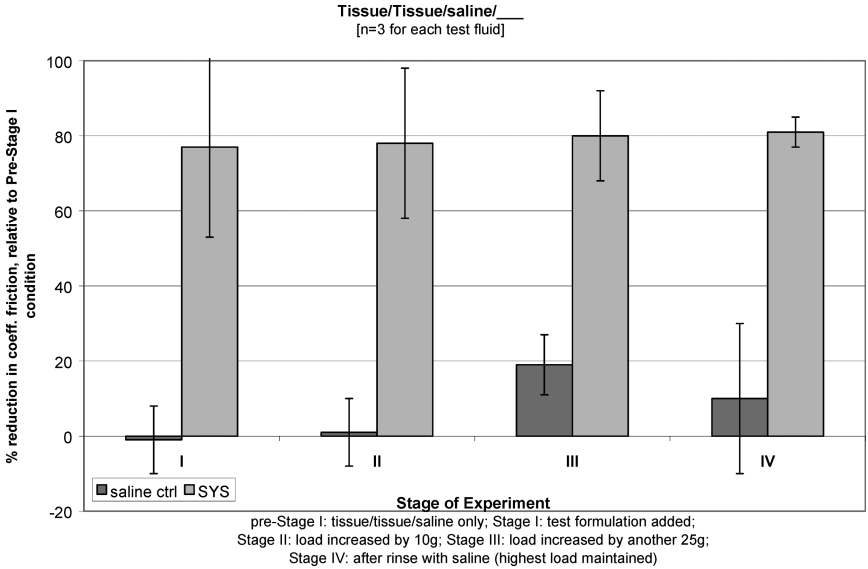

FIGURE 2 Comparison of coefficient of friction reductions (tissue on tissue) for 3 replicate experiments. Data are presented for each stage of the tests, relative to the coefficients of friction determined for the "pre-Stage I" condition.

TABLE 2 Percent Change* in Coefficient of Friction Relative to Initial Tissue/Saline/Tissue Coefficient (minutes 1–5) for that Specific Tissue/Test Fluid/Tissue Couple

Test fluid	After addition of test fluid (minutes 6–10)	After adding 10 g normal load (minutes 11–15)	After adding 25 g normal load (minutes 16–20)	After rinsing with saline control sln (minutes 21–25)	Overall Avg ± s.d
Saline control					
SET 1	+2%	−5%	−24%	−27%	−7
SET 2	+10%	+9%	−10%	+12%	±13
SET 3	−8%	−8%	−22%	−15%	
SYS					
SET 1	−51%	−57%	−66%	−76%	−78
SET 2	−83%	−81%	−85%	−82%	±14
SET 3	−97%	−89%	−89%	−84%	

*negative value → reduction in friction relative to initial condition.
positive value → increase in friction relative to initial condition.

TABLE 3 Comparison of Post-Rinse Coefficients of Friction (Tissue-on-Tissue Experiments)

Formulation	Final average coefficient for sets 1, 2, 3	Average (s.d.)
Saline control	0.384, 0.263, 0.223	0.290 (0.068)
SYS	0.051, 0.057, 0.047	0.052 (0.004)

these data, indicating the large standard deviations for the starting tissue qualities.

Figures 4 and 5 show representative photographs of the surfaces of the tissue specimens before and after frictional testing with the SYS lubricant system. These photographs confirm the impression from gross physical inspection that very little tissue damage occurred during loaded articulations in the SYS-lubricated couples. There did appear to be some superficial swelling, however, suggestive of uptake and retention of formulation ingredients.

Table 4 reports the much less effective lubrication for both the formulations tested, when tissue was articulated against smooth

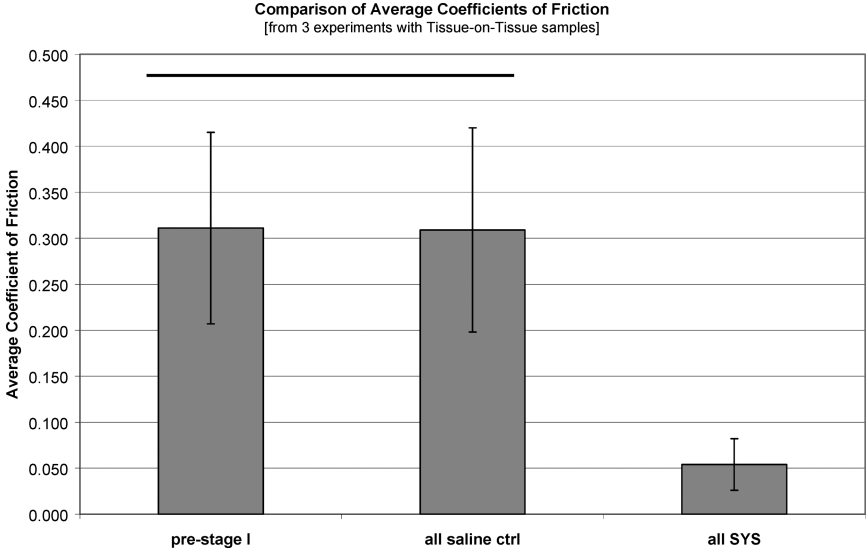

FIGURE 3 Summary of tissue-on-tissue results, as averages and standard deviations for data from 3 replicate experiments, combining data for Stages I through IV. The solid line above the first 2 bars indicates absence of statistical difference between these two conditions.

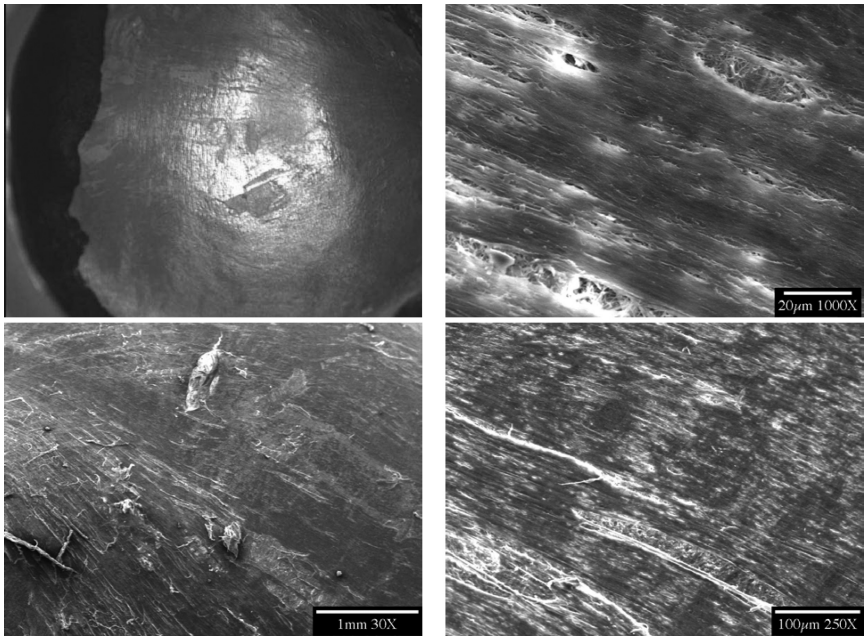

FIGURE 4 Light microscopic (upper left) and scanning electron microscopic views of a tissue control (no friction).

germanium plates rather than against other tissue. Table 5 summarizes the results for germanium-on-germanium when lubricated by these same two formulations, indicating lower coefficients of friction associated with the retained aqueous layers between the two hydrophilic plates. The combined results of Tables 4 and 5 suggest that tissue uptake of the aqueous formulations led to direct tissue contact with the high-surface-energy germanium substratum, and the formation of a transfer film from tissue to plate. Post-friction critical surface tension for wetting values and infrared spectra of the germanium prism residues are consistent with this interpretation. Consequently, the high coefficients of friction recorded in tissue-on-germanium couples are indicative of bioadhesion and surface damage that does not occur in tissue-to-tissue couples lubricated with the same formulations.

Figure 6 contains infrared spectra from an initial analysis (no friction testing) of the formulation composition, for comparison with spectra of any residues from friction tests. When used in the friction testing, however, the SYS formulation residues that temporarily

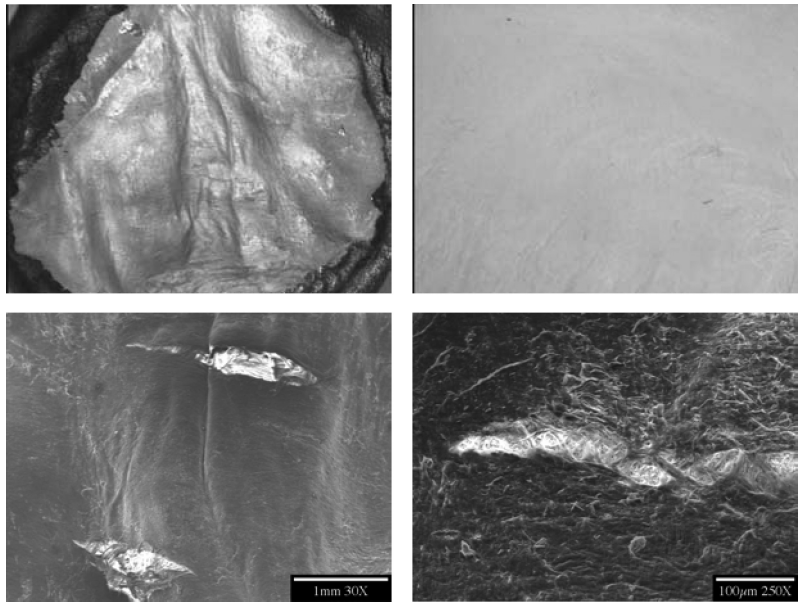

FIGURE 5 Light microscopic (top) and scanning electron microscopic views of tissue from tissue-on-tissue experiment with SYS (set 1). Light microscopic magnification at upper right is approximately 10 times magnification at upper left. Folds in tissue (upper left) occurred after all measurements were made, during processing of the tissue for microscopic analysis.

remained attached to the germanium prism were completely rinsed away by the saline control. It is likely that more persistent binding and retention of the macromolecular components in the articulating tissue interphase zones is responsible for their continuing to display the low frictional coefficients indicative of minimized bioadhesion.

TABLE 4 Results Summary for Tissue-on-Ge Experiments

Test fluid	Post-friction testing residue presence (MAIR-IR)	Final % change in coeff. friction, relative to initial Tissue/saline/Ge	Post-friction critical surface tension for wetting (contact angles)
Saline control	Slight residue	−26% .427 → .314	39.3 mN/m
SYS	Substantial residue	−14% .515 → .444	35.8 mN/m

TABLE 5 Results Summary for Ge-on-Ge Experiments

Test fluid	No-friction substantivity (MAIR-IR)	Post-friction testing residue presence (MAIR-IR)	Final % change in coeff. friction, relative to initial Ge/saline/Ge	Post-friction critical surface tension for wetting (comp. contact angles)
Saline control	Little or no residue	Slight residue	−13% .113 → .098	34.2 mN/m
SYS	Substantial residue (see Figure 6)	Slight residue	+3% .077 → .079	33.6 mN/m

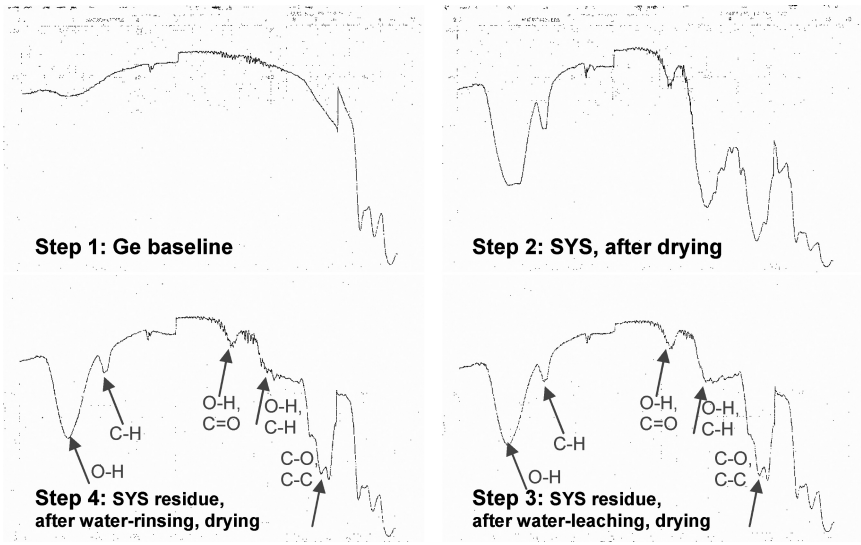

FIGURE 6 Initial characterization of SYS formulation on germanium, using MAIR-infrared spectroscopy. Step 1: obtain Ge "baseline" spectrum. Step 2: 1 drop fluid on Ge → dry → IR spectrum of residue. Step 3: water-leach residue from Step 2 → dry → IR spectrum of any material remaining on Ge. Step 4: water-rinse residue from Step 3 → dry → IR spectrum of any material remaining on Ge. These spectra were compared with spectra following friction testing, to determine whether formulation components were retained on coupled surfaces. Quantitation of residue was determined from calculation of absorbance at selected wavelengths, using percent transmission data (y-axis on spectra) from the baselines and sample spectra.

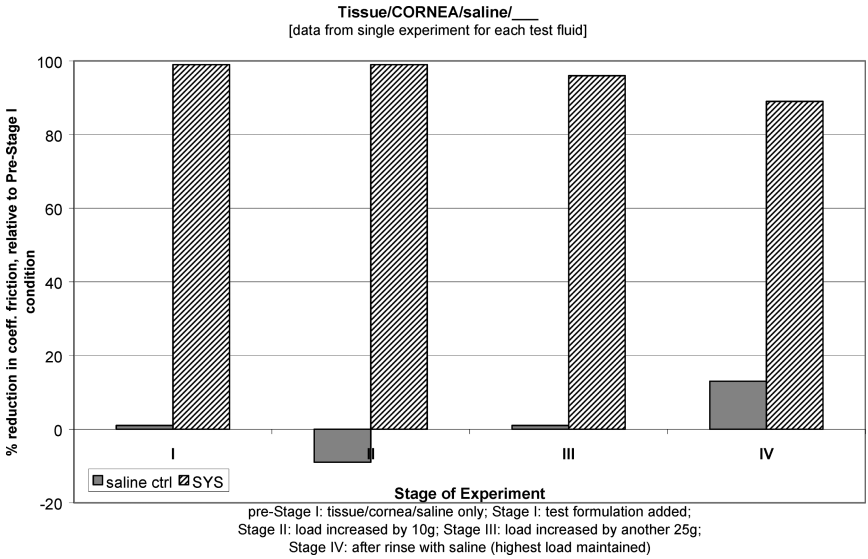

FIGURE 7 Comparison of coefficient of friction reductions (cornea on tissue) for a single experiment with each fluid formulation. Data are presented for each stage of the tests, relative to the coefficients of friction determined for the "pre-Stage I" condition.

Figure 7, providing results for the experiment with a donated human cornea, confirms the potential generality and relevance of these findings to actual in-the-eye situations.

DISCUSSION

The maximum normal pressure on the eye during blinking has been estimated at $35\,g/cm^2$ [43], most likely applied by the "lid wiper" region of the upper eyelid [16,17] during its average excursion of about 9 mm [44]. The maximum load of 55–60 grams used in these tests roughly approximated this pressure over the surface areas in contact. During pre-clinical investigations, it was shown that the ability of the SYS product to protect the ocular surface layers was not manifested by the identical formulation absent the gellable HP Guar component [28], so the emphasis here is on how the gellable HP Guar within the test formulation interacts with tissue surfaces to minimize their mutual adhesion.

The most important outcome from these studies is the ability to provide differential analyses of the experimental results for the

tissue-on-tissue *vs*. tissue-on-solid comparative couples. The straightforward explanation would be that fluid viscosity in the SYS formulation increases as the aqueous electrolyte phase is preferentially taken up into the tissue, increasing from about 10 centiPoise at the original formulation concentration (to nearly 1000 centiPoise at 0.75% concentration, as recently reported [45]). Alternatively, polymer taken up into the tissue could be pressure-released back into the interface in a lubricating mechanism called "weeping." If either or both of these mechanisms were prominent, however, the coefficients of friction for the tissue-on-Ge couples should have been close to those of the tissue-on-tissue couples, rather than the order-of-magnitude higher values actually recorded.

Clearly, dependence on gross coefficient of friction comparisons is too simplistic for tissue-based couples capable of complex interactions with multi-component lubricating fluids. The utility of employing the additional methods of multiple attenuated internal reflection infrared spectroscopy, contact angle measurements, and microscopic inspection was to evaluate bioadhesive tissue transfer *vs*. lubricant residues at the germanium and tissue interfaces. From those measurements, it was found that the tissue-on-Ge couple experienced liquid film starvation followed by tissue adhesion and then cohesive tissue failure—giving higher frictional values—rather than preferential segregation of a higher-viscosity macromolecular concentrate to the interface. The MAIR-IR spectra from the end-of-experiment germanium prisms revealed that very little of the polymer was retained after saline rinsing. The saline-rinsed once SYS-lubricated tissue-on-tissue couples continued to show very low coefficients of friction, however, indicating preferential uptake and retention of the macromolecules (polymer) in the tissues' interfacial zones.

The more likely mechanism for the superior tissue-on-tissue results is that superficial polymer uptake into the adjacent tissues modifies the interfacial water structure to minimize friction based on the inherent drag reducing property of the high-molecular-weight hydrophilic polymer, similar to the Toms' effect of minimizing viscous flow resistance in dilute polymer solutions [46,47]. This interpretation contrasts with the initially proposed mechanism, that rising interfacial fluid viscosity would maintain better separation of the sliding surfaces and diminish bioadhesive wear. The additional control experiments of Ge-on-Ge couples, where separating fluid films were present throughout the test series, did not display coefficients of friction as low as those of tissue-on-tissue couples. This finding also supports a boundary modification mechanism for tissue-on-tissue lubrication rather than one regulated by the viscosity of the fluid.

As documented in Table 4, since the tissue-on-germanium final coefficients of friction were greater than those for the tissue-on-tissue couples lubricated with the same formulations (Table 3), "imbibation" of the liquid lubricant phase by the tissue, without equal lubricity being imparted to the articulating impervious plate, was not equivalent to drying of the system in a manner that could support tissue-on-Ge separation.

In contrast to the tissue-on-germanium results, Table 5 documents the fluid lubrication of the two hard, smooth, impermeable germanium substrata; the effect being predominantly hydrophilic retention of the aqueous liquid layer between them. When absorbent tissue was present in the frictional couple, there was a transition from fluid lubrication to boundary lubrication as the liquid film became thinner and the number of contacting surface asperities, and potential for bioadhesion, increased. The film of remaining fluid did not exhibit drag reduction, and became so thin that reactions between components of the fluid and the articulating surfaces dictated the nature of the resistance to contact-based adhesion. Since retention of even as little as a monomolecular layer of a lubricant film is sufficient to provide boundary lubrication, it is unlikely that the SYS formulation provided such layers to the tissue faces, but instead formed strong lateral attractions between the tissues and high molecular weight (MW) polymers, reducing viscous drag. This is consistent with the clinical observation that the SYS system provided an "ocular shield" [12] by which corneal tissue was protected from desiccation and corneal epithelium resisted chemical damage, at levels of protection not observed with guar-free formulations in pre-clinical trials [28].

When tissue was articulated against nonretentive substrata, such as the germanium plates used here, points of lubricant starvation occurred that led to bioadhesion of even the SYS-modified tissue. Breakage of the tissue-to-substratum bonds during relative motion was manifested in higher coefficient of friction values and superficial damage to the tissue.

CONCLUSIONS AND RECOMMENDATIONS

The results support the conclusion that the mechanism of SYS lubrication of tissue-on-tissue couples involves uptake and binding of sol-phase macromolecular components to the tissue's superficial layers, and not simply liquid retention or viscometric increases in the interphase zone. Guar is a natural polysaccharide gum based on a linear backbone of mannose units with galactose side chains, in a mannose to galactose ratio of 2 to 1. The SYS formulation tested here is a

neutral, borate-based liquid containing demulcents PEG400 and PG, as well as gellable 0.36–0.42 molar hydroxypropyl-substituted guar (HP-Guar, approximately 20,000,000 MW) capable of rapid crosslinking by borate to a gel as the pH increases above 7 upon insertion in the eye. No pH changes were likely in the testing reported here, so the involvement of the gel phase is considered minor in producing the excellent lubricity recorded for the tissue-on-tissue couples. It is likely that changes in polymer molecular weight and/or degree of substitution, even within this specific system, will have measurable effects on the lubricity results. In this regard, it will be important to choose the relevant articulating pairs for the testing phase, since the current work makes clear that results cannot be simply extrapolated from a tissue-on-synthetic to a tissue-on-tissue system.

Binding of the sol-phase SYS formulation components to the tissue faces may, in addition to providing hydrodynamic lubrication *via* hydrophilic retention of an aqueous film, also "toughen" the tissue faces against abrasive damage. This is a qualitative conclusion drawn mainly from inspection of light- and electron-microscopy views of the post-articulated specimens, where tissue superficial swelling with the SYS formulation was associated with preservation of the original tissue architecture.

Acceptance of the generality of the mechanisms and paths identified in this work for the currently marketed SYS formulation will depend upon validation and extension of the methods and findings. First is the need to confirm these findings at eye conditions, about 34°C and pH 7.5 [48], recognizing all data in this report were obtained in a constant temperature clean room at about 21°C with pH 7 solutions. Second, there is the need to extend this study to include many more donated human corneas for 2 reasons: (a) surrogate ocular tissue, in the form of preserved human umbilical cord vein segments, is not completely equal in all respects to the ophthalmologic tissues to be lubricated, and (b) the single quantitative cornea-on-tissue data set reported here gave results similar to the original qualitative observations made with another human cornea-on-tissue couple, both providing lubricated tissue frictional values for the SYS composition lower than seen with any other lubricious preparation. Inspections of lubricated/articulated tissue and cornea specimens in cross-section, especially after staining for the SYS macromolecular components, could be a key to further isolating the locus of function of the guar macromolecular arrays—either as superficial overlayers or as interpenetrating hydrophilic networks.

It is critical to advancing our understanding of the lubricity of natural cornea-on-conjunctiva couples–in health and disease—that more complete information be developed about the unique interfacial

qualities of the cornea that apparently allow more efficacious binding of the SYS lubricants than do even the surrogate graft intimal wall structures.

For better judgment of the clinical utility and longevity of lubricious agent delivered to the human eye, it will be important to extend these measurements to cases where continuous slow diluent flow, simulating human tear production, is provided during the "blinking" frictional trials. Also, it is recommended that these studies be taken into the domain of contact lens comfort and in-the-eye cleaning trials, recognizing that the synthetic-on-tissue frictional data reported here suggest that formulation modifications might improve lubricity for contact lens-against-tissue blinking, beyond that now experienced.

ACKNOWLEDGMENT

This project was funded by Alcon Research Ltd. The authors thank Robert L. Forsberg and James M. Fick (University at Buffalo) for their technical contributions.

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