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ORIGINAL PAPER

Friction Coefficient Measurement of Hydrogel Materials on Living Epithelial Cells

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Abstract Soft biomaterials are often used in applications that involve contact and relative motion against biological tissues, as well as complicated and variable environments. The friction coefficient of these contacts involving living human cells is of key importance in the analysis and success of these devices. This work measures the contacting friction coefficient between soft hydrogel biomaterial surfaces against live human corneal epithelial cells using a custom micro-tribometer. The friction coefficients were of the order of $\mu = 0.03$ for contacts that did not cause gross destruction of the cell layer. Damage to the confluent cell layer was assessed using a Trypan blue stain with optical microscopy. This damage was quantified statistically using image-processing software. The damage was also correlated to in situ friction measurements, with the lowest friction values seen on undamaged cells and higher friction on damaged regions.

Keywords Biotribology · Hydrogel · Friction

1 Introduction

Hydrogel materials are used in many capacities in the human body, perhaps most commonly as optical correction devices. Hydrogels are polymer networks that contain substantial fractions of water, and they are typically hydrophilic (this depends on the hydration level). Hydrogels are the primary material for soft contact lenses. These lenses move against both the cornea (epithelium) and the inner eyelid (palpebral conjunctiva); this is shown schematically in Fig. 1. Though these soft materials are well suited to the wet environment of the eye, many patients suffer from extensive eye irritation after implantation due to mechanical friction, physiological factors, or both [1].

The mechanical properties of hydrogels are extremely sensitive to water content and humidity, and contact pressure is known to cause redistribution and/or reduction of the water content of hydrogel materials [2, 3]. As such, any tribological testing of hydrogels must be done under relatively low contact pressure conditions, known solution, and controlled humidity environments [4]. The surface topography of these devices is a function of the material parameters and of molding conditions but have a typical R_q of 5–10 nm [5]. Estimates of the contact pressure created by the eyelid during blinking range from 3.5 to 4.0 kPa and blinking speed average around 12 cm/s [6–9]. This combination of low contact pressure and moderate sliding speed are challenging to match with tribological instruments.

Epithelial cells are of particular interest due to their functionality and quantity; the epithelium is one of the four primary body tissues, and its functions include secretion, absorption, protection, sensation detection, and selective permeability. The response of these cells to mechanical stimuli has been investigated in a variety of ways including cultured substrate stretch and in vivo animal studies. There is a stiffening of human alveolar epithelial cells when strained in an equibiaxial fashion as reported by Trepat et al. [10]. In addition, the application of mechanical pressure to oral epithelia of rats with orthodontic elastic bands causes the cells to proliferate more slowly, possibly a mechanism to

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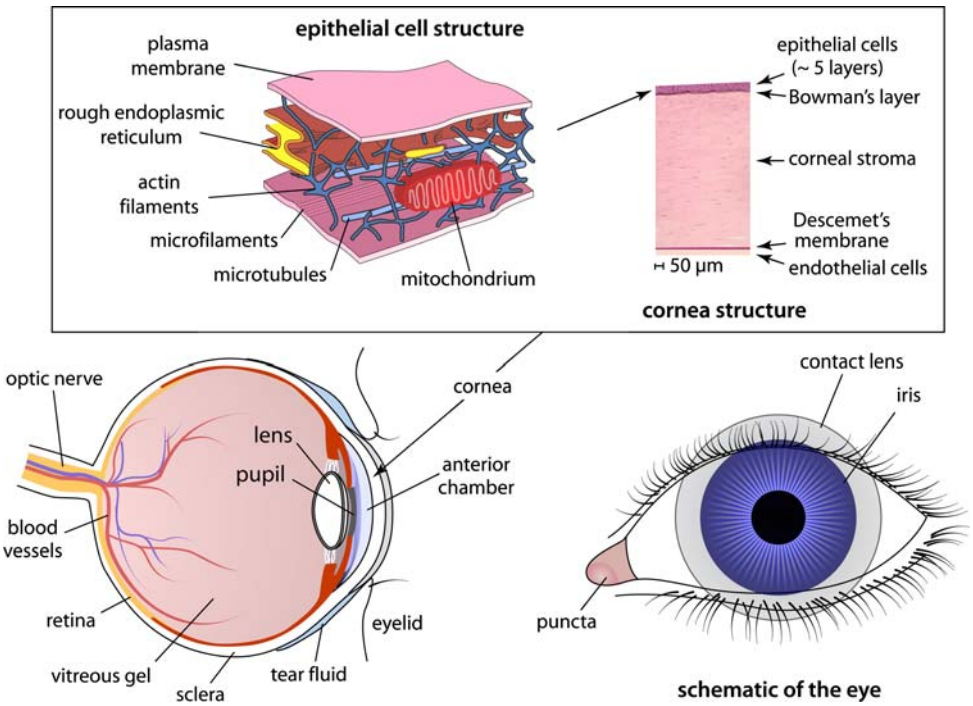

Fig. 1 A schematic of a human eye including a contact lens. The lens sits on the epithelial cells of the cornea and under the eyelid. The structure of the cornea and an illustration of the structural components of the cell are also shown

ensure survival under adverse conditions [11]. Though the epithelium of human corneas is designed to thrive under a variety of environmental changes, these cells can be abraded away by response to contact lens design and materials, as well as tear fluid and immune response. The experiments reported here aim to provide insight as to how biological surfaces (cell layers) react to direct contact and sliding against contact lens hydrogel materials. This differs from prior proof-of-concept experiments carried out by Dunn et al. [12] with endothelial cells that were tested against glass pins [12], in that the contacting counter material is compliant and hydrated. Under similar loads the contact pressures should be substantially lower, but may not be as low as the forecasted pressures during blinking.

2 Experimental Protocol

The tribology experiments were performed on a dual flexure-based reciprocating pin-on-disk tribometer that is more fully described by Dickrell et al. [13, 14], Rennie et al. [4], Dunn et al. [12], and Cobb et al. [15]. The contact schematic is shown in Fig. 2. The flexure assembly and the pin holder for the hydrogels are shown in Fig. 3. The applied normal loads and sliding cycles were varied from either 500 µN or 2 mN and 2–20 cycles, respectively. This is more fully outlined in Table 1. Additionally, cell culture methods, hydrogel materials, and fixation methods are described in detail in the Methods Section at the end of this letter.

The experimental protocol for performing the tribological test is as follows:

(1) Cell chambers are removed from incubator, the growth media is refreshed, the cells are then transferred to a transporting dish in a sterile manner and sterility was maintained until the samples reached the tribology laboratory.
(2) The hydrogel sample is prepared as illustrated in Fig. 2 and examined to confirm that the mounted pin sample is free of damage or gross defects.

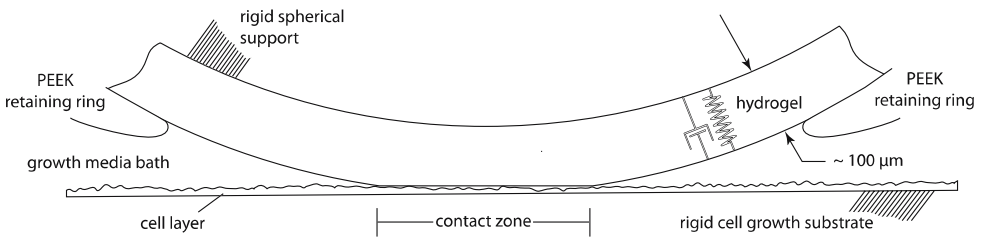

Fig. 2 A schematic of the contact zone. The hydrogel material is located on the pin surface and slides against a single layer of epithelial cells that are grown on a rigid substrate (as compared to the hydrogel). The entire experiment is run submerged in a bath of growth media

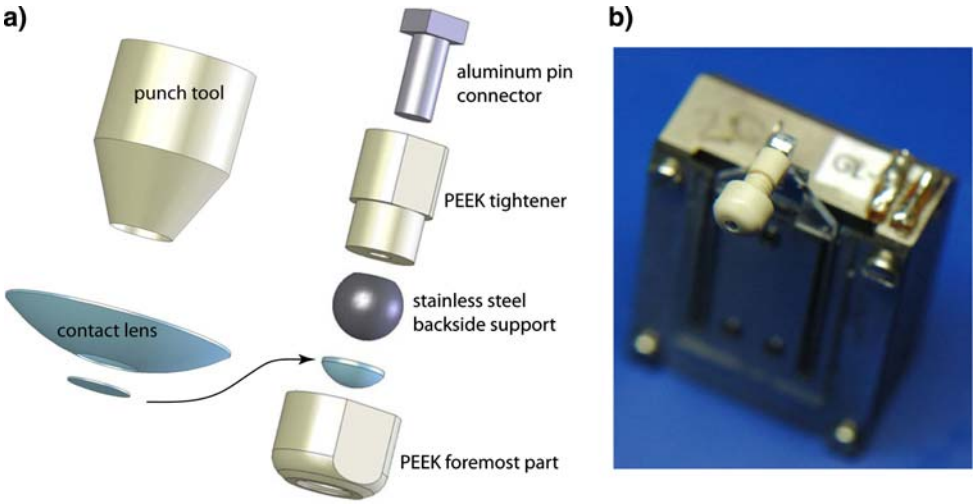

Fig. 3 A schematic (**a**) and photograph (**b**) of the pin holder designed to hold a thin circular section of hydrogel material. The sample is taken from the central region of commercially available contact lenses. The sample holder is mounted onto a calibrated glass flexure that is used to make lateral and normal force measurements

Table 1 A matrix of experiments along with a populated list of hydrogel material properties and contact geometry including the associate nomenclature. The average sliding speed for these experiments was 300 μm/s

Contact conditions	Hydrogel materials	F_n	Number of cycles	Number of tests
Radius $R = 1$ mm	Poly(2-hydroxyethyl methacrylate) (pHEMA)	500 μN	2	5
Thickness $t = \sim 100$ μm			5	4
Modulus $E = 255$ kPa			10	3
Poisson ratio $v = 0.45$			20	5
		2 mN	5	2
			10	3
			20	3
	Silicone-based hydrogel	500 μN	2	3
			5	3
			10	3

(3) Growth media is replaced with 100% Trypan blue 0.4% solution for 1 min, rinsed, and replaced with fresh growth media directly before evaluation. The chambers and cells are imaged at 100× magnification to confirm a single layer of viable confluent cells.
(4) The chamber is secured to the reciprocating stage through an adhesive mounted sample holder.
(5) The hydrogel pin sample is mounted into the tribometer, and lowered into the growth media bath without contacting the cells and allowed to soak for 5 min. After soaking the hydrogel sample is then brought into contact with the cells and a normal load is slowly applied (~0.1 mN/s) until the target load is reached.
(6) Motion is initiated over a 500-μm stroke length, and force and displacement data is collected continuously at 1.2 kHz for the duration of the experiment.
(7) After unloading the pin the chamber and cells are removed and transported back to the microscope where steps 2 and 3 are repeated. The Trypan blue necrosis assay and imaging are then compared to the original images to quantify cell damage.

3 Results

The results from an experiment with silicon-based hydrogel against a layer of human corneal epithelial cells under a load of 500 μN is shown in Fig. 4, along with the microscopy image of the cells after testing and staining with the Trypan blue. The friction coefficient loops at 5, 10, 15, and 20 cycles show a systematic increase in friction coefficient and a statistically significant spatially resolved region of high friction in the middle of the path at cycle 20. This is correlated to the removal of cells from this part of the wear track and substantial cell death throughout the remaining portion of the wear track. The average friction coefficients are computed from the friction coefficient loops according to Eq. 1.

$$\bar{\mu} = \frac{\bar{\mu}_{\text{forward}} - \bar{\mu}_{\text{reverse}}}{2} \quad (1)$$

This trend of higher friction coefficient along the wear tracks in regions where cells were detached from the substrate was consistent. For experiments that showed limited cell death (the early cycles of experiments operated under

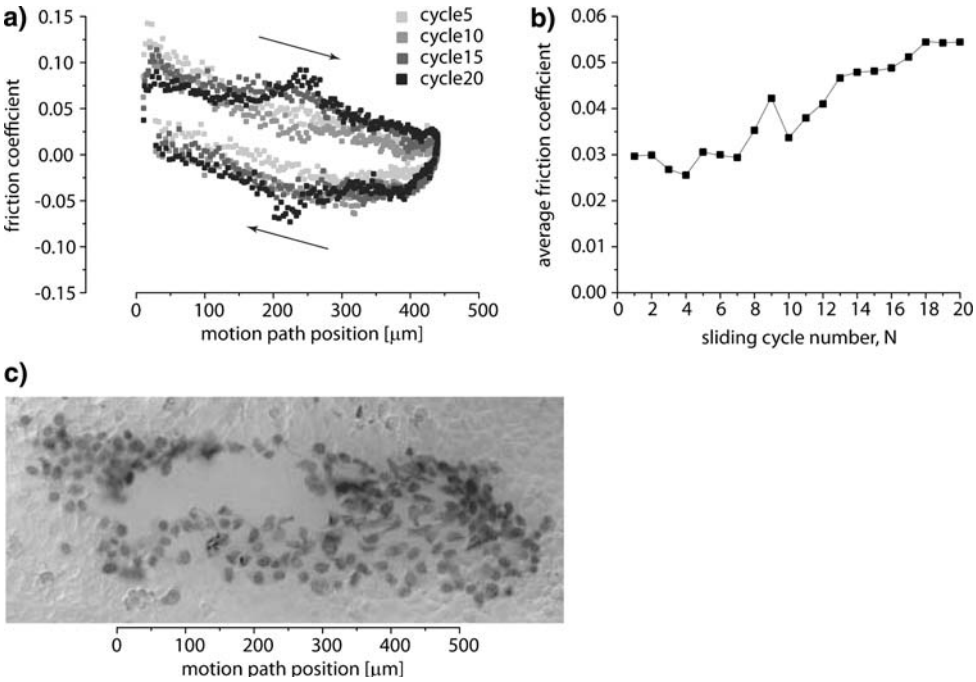

Fig. 4 (**a**) Friction loops for cycles 5, 10, 15, and 20 of a silicone-based hydrogel running on a layer of human corneal epithelial cells (HCE-T) under a normal load of 500 μN. (**b**) Average values of friction coefficient over the 20 cycles show a monotonic increase presumably correlated with a monotonic increase in cell damage. (**c**) A post-test micrograph reveals gross cell damage in the center of the wear track and cell death throughout

500 μN loads) the friction coefficients were of the order of $\mu = 0.03$. For experiments conducted at normal loads of 2.0 mN, 88% of the experiments showed cell removal with no discernable trend.

In those experiments run under a normal load of 500 μN, there was not a clear trend in the number of cells that were damaged (indicated by the Trypan blue staining) as a function of the number of reciprocating cycles. This is shown graphically in Fig. 5, where approximately 50 cells were ruptured regardless of sliding distance. However, for those experiments that underwent 20 cycles 4/5 experiments showed regions of gross cell detachment over the majority of the sliding track.

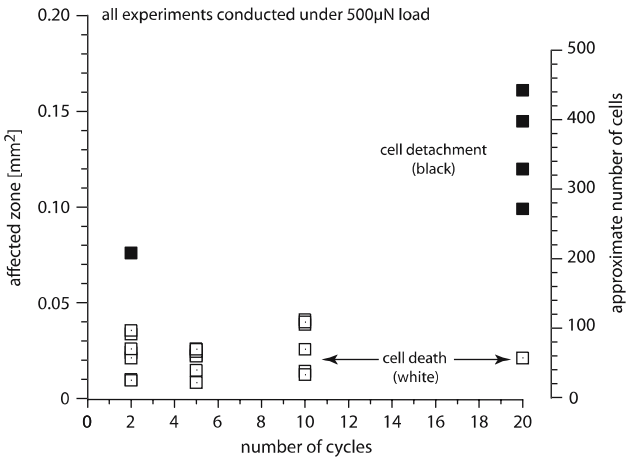

Fig. 5 Affected area on the cell layer in square millimeters as a function of the number of cycles run during each experiments under a load of 500 μN. The open points indicate tests where all cells remained on the substrate, but were dyed by the Trypan blue, showing some cell wall rupture, while solid points are areas of gross cell detachment, which was very common for tests over 10 cycles. The hydrogel material was pHEMA

4 Discussion

Previously it was reported that there was a threshold normal load of about 0.7 mN above which the endothelial cells would detach [12], presumably, due to the friction forces across this interface. One of the challenges with this work is the difficulty in maintaining low contact pressures on the cell layer; this motivated the use of a compliant hydrogel pin as the countersurface to the cell layer. Previous work modeling hydrogels in tribology has used a viscous damped elastic foundation [4] that was necessary due to the cyclic deformation of a hydrogel foundation. Here the hydrogel pin is statically compressed, and a relatively simple elastic foundation solution to the maximum pressure (P_{max}), the average pressure (P_0), and the contact half width (a) are given in the following equations. The radius of the hydrogel pin (R) is assumed to conform to the stainless steel back support, and the thickness of the hydrogel (t) is of the order of 100 μm. Any additional compliance from the cell layer is neglected in this analysis; thus the expectation is that the computed pressures represent an upper bound and the computed areas a lower. The equation for the pressure distribution of the elastic foundation is given by Eq. 2, where the effective elastic modulus (E') is given by Eq. 3, and the symbol δ represents the local deformation.

$$P = \frac{E}{(1-\nu^2)}\frac{\delta}{t} \tag{2}$$

$$E' = \frac{E}{(1-\nu^2)} \tag{3}$$

The contact geometry as shown roughly to scale in Fig. 2 has a maximum central deformation (δ_{max}) approximated by Eq. 4, which assumes that the half width of the contact area (a) is less than the radius of the spherical support (R).

$$\delta_{max} \cong \frac{a^2}{2R} \tag{4}$$

The relationship between the deformation and the normal load (F_n) for this type of a contact can be found by integrating the pressure distribution given in Eq. 2, and is given by Eq. 5, along with the approximate solution.

$$F_n = \int_0^{2\pi}\int_0^{a} P\ r\ \mathrm{d}r\ \mathrm{d}\theta = \int_0^{2\pi}\int_0^{a} \frac{E'\delta}{t} r\ \mathrm{d}r\ \mathrm{d}\theta \cong \frac{\pi E' a^4}{4tR} \tag{5}$$

Using these approximate solutions, the contact half width (a), the maximum contact pressure (P_{max}), and the average contact pressure (P_0) are given in Eqs. 6–8, respectively.

$$a = \sqrt[4]{\frac{4F_n tR}{\pi E'}} \tag{6}$$

$$P_{max} = \sqrt{\frac{F_n E'}{\pi tR}} \tag{7}$$

$$P_0 = \frac{1}{2}P_{max} = \frac{1}{2}\sqrt{\frac{F_n E'}{\pi tR}} \tag{8}$$

Using the material and geometry conditions given in Table 1, the maximum contact pressures at normal loads of 0.5 and 2 mN are 23 and 45 kPa, respectively. These contacts have corresponding predicted contact widths of 240 and 340 μm for the 0.5 and 2 mN loads. Using a measured cell density of 2,750 cells/mm^2 calculations suggests that ~125 cells are under the contact at a load of 0.5 mN. These computations agree favorably with the measured damage zones observed on the cell layers as shown in Fig. 6.

Here the tribology of a well-described epithelial system against a hydrogel pin is described during sliding. Mechanics of the cell layer were not thoroughly investigated. The hydrogel pin was soaked in growth media for 5 min prior to testing to provide equilibrium with respect to swelling and protein adsorption, which is known to occur rapidly on submerged material surfaces [16–18]. In fact, material surface properties affect composition and functional activity of adsorbed proteins, modulating cellular adhesive responses [19–21]. Friction tests were carried out in complete growth medium that contains 5% serum and

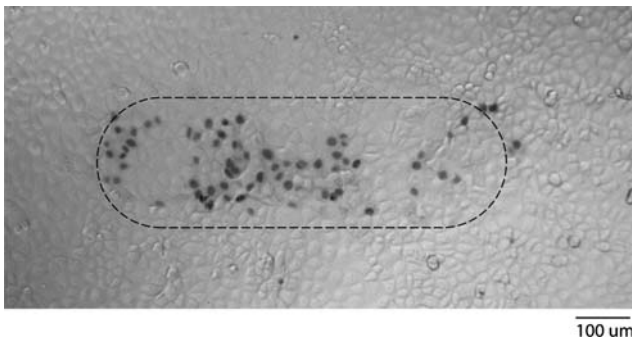

Fig. 6 A microscope image of a wear track after five cycles of sliding under a silicone-based hydrogel at 500 μN of load. The dark cells were stained by Trypan blue and indicate cells that have been damaged during the experiment. The analytical solution to the contact problem and a 550 μm sliding track gives a dashed region of contact area that corresponds to an average contact pressure of approximately 12 kPa

the hydrogel pin used in this study is expected to be coated with serum proteins (e.g., vitronectin, albumin, and fibronectin). While protein adsorption would be expected to differ if carried out in the presence of human tear fluid, standard culturing conditions were used for these preliminary experiments. Future developments will include a light CO_2 gas partial pressure for continued cell viability over long testing times and provide regulated temperature for uninterrupted culture during testing. Additionally, the contact pressure must be further reduced to reach clinically relevant levels.

5 Closure

Micro-friction tests were run between HEMA- and silicone-based hydrogel materials and a confluent HCE-T layer in a customized micro-tribometer under loads of 500 μN to 2 mN. Cells were analyzed post-testing with respect to the amount of cell necrosis as determined by Trypan blue stain, the size of any holes ripped in the cell layer, and contact pressures estimated from micrographs. Overall friction coefficient for hydrogel sliding against cells is of the order of $\mu = 0.03$. The more sliding cycles run, the larger the affected zone on the cell layer, in terms of discernable damage. There is a direct correlation between detachment of the cell layer during sliding and the acquired friction coefficient; the hydrogel-on-cell configuration provides the lowest friction environment.

6 Cell Culture Methods

Human corneal epithelial cells, SV40 immortalized (HCE-T, RCB1384), were cultured in a 1:1 blend of Dulbecco's

modified Eagle's medium and Ham's F12 media (DMEM/F12) containing 200 U/mL each of penicillin and streptomycin, 5% (v/v) fetal bovine serum (FBS), 0.1 μg cholera toxin/mL, 0.5% (v/v) dimethyl sulfoxide, 5 μg insulin/mL, and 10 ng human epidermal growth factor/mL [22]. Confluent HCE-Ts were rinsed in Hank's balanced salt solution and detached with 0.25% (w/v) trypsin–EDTA, and subsequently seeded into the specialized cell holders at a density between 5×10^4 and 1×10^5 cells/cm^2. The cells were subcultured within the holder for approximately 24 h so that 100% confluency was reached before frictional testing was performed. The cultured cell density as measured optically was 2,750 cells/mm^2.

Cell holders were constructed using a polydimethylsiloxane (PDMS) ring fitted onto a glass coverslip of diameter $D = 25$ mm (Electron Microscopy Sciences, 1560 Industry Road, Hatfield, PA 19440), which provided a small bath of ~ 2 mL growth media on top of the cell layer during the culture period and lubrication during frictional testing. Detailed information on the construction of the cell holders and methods for culturing cells in the holders can be found in Cobb et al. [15].

Before and after testing, cells are submerged in 10% Trypan blue for 1 min and then observed under a 10× objective in a Leica DMLM microscope using the DIC/Nomarski mode [15]. Damage is assessed immediately before testing and after testing to assure that only cells affected by friction tests are included in the analysis. In all cases, no cells were damaged in transit between labs. The hydrogel pins were assessed before and after testing at 20× to assure that no ripping or bunching of the lens material occurred during setup or transit. A pristine lens has small surface features that range from 0 to 5 μm in size. Adobe Photoshop was used to quantify confluent cell density, amount of cell necrosis (number of cells), and areas where cells detached. Amira software (Mercury Computer Systems) was used to quantify cell detachment regions.

7 Hydrogel Materials and Fixation Methods

Two hydrogel materials were examined. Samples with a base of poly(2-hydroxyethyl methacrylate) (pHEMA) [23] and samples with a base of PDMS were obtained from commercially available contact lenses (Vistakon, Jacksonville, FL). A special holder made of PEEK was manufactured in order to provide a compliant countersurface on the pin. This holder was comprised of three sections and functions to hold a thin circular sample of hydrogel material around the edge while the middle is the frictional contact region. The hydrogel sample was first cut with a custom-machined punch, and was then placed into the collar with a stainless steel ball that acts as a rigid back support. The steel ball was held in place through a threaded back piece. This entire assembly is threaded onto the cantilever flexure, which then fits into the tribometer, and was stored in hydrogel packing solution (Vistakon, Jacksonville, FL) until testing.

8 Tribometer and Data Acquisition Methods

The experimental apparatus used to run these friction tests was a customized micro-tribometer [4]. It was modified to achieve submerged sample capability, lower applied normal loads (100 μN–1 N), and straightforward data acquisition and processing.

The capillary forces from the submerged cylindrical pin are zeroed manually after contact with the cell layer was located. Position-synchronous data was acquired at 1.2 kHz. Kinetic coefficients of friction were calculated as the ratio of F_t to F_n. Normal force feedback was achieved by adjusting a vertical piezoelectric cell according to the difference between the desired average normal load and the average normal load from the previous sliding cycle.

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