

# A novel method for low load friction testing on living cells

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**Abstract** A low load tribology technique for studying the effects of friction on living cells was developed. Results show a direct relationship between the coefficient of friction (COF) and the extent of cell damage. The COF,  $\mu$ , for a glass pin on an intact layer of human corneal epithelial cells is determined to be on the order of  $\mu = 0.05 \pm 0.02$  ( $n = 16$ ). The correlations between applied normal load and extent of cell damage, as well as between number of reciprocation cycles and cell damage, are reported. It is also found that cell damage can occur when a loading force as low as 0.5 mN is applied, although the cells appear to be intact.

**Keywords** Cell · Friction · Human corneal epithelial cell · Tribology

## Introduction

Understanding the physical interaction between biomaterials and cells within the body is critical for the development of medical devices. Frictional forces between the two play a crucial role in the safety and life span of devices [e.g. bone cements (Frazier et al. 1997) and vascular stents (Ho et al. 2003)] as well as in patient comfort [e.g. contact lenses (Nairn and Jiang 1995)]. Therefore, a method is needed for quantifying contact friction between materials and living cells, and assessing the effects of friction not only at the cellular level but also at the sub-cellular level. This is critical because damage due to mechanical loading is not always apparent at the tissue level (Needham et al. 1991). Morita et al. (2006) previously reported a method for friction testing on living, engineered tissue (i.e. cartilage constructs). However, the effects of friction on viability at the cellular level were not evaluated in that study. In addition, the apparatus that was used was limited to minimum applied loads in the Newton range, making it difficult to mimic conditions in vivo. In the present work submerged friction testing was performed on monolayers of live human corneal epithelial (HCE-T) cells, and the effects of friction on cell viability were examined. The method reported here employs applied loads of two orders of magnitude less than those reported by Morita et al. (2006).

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## Materials and methods

### Cell culture

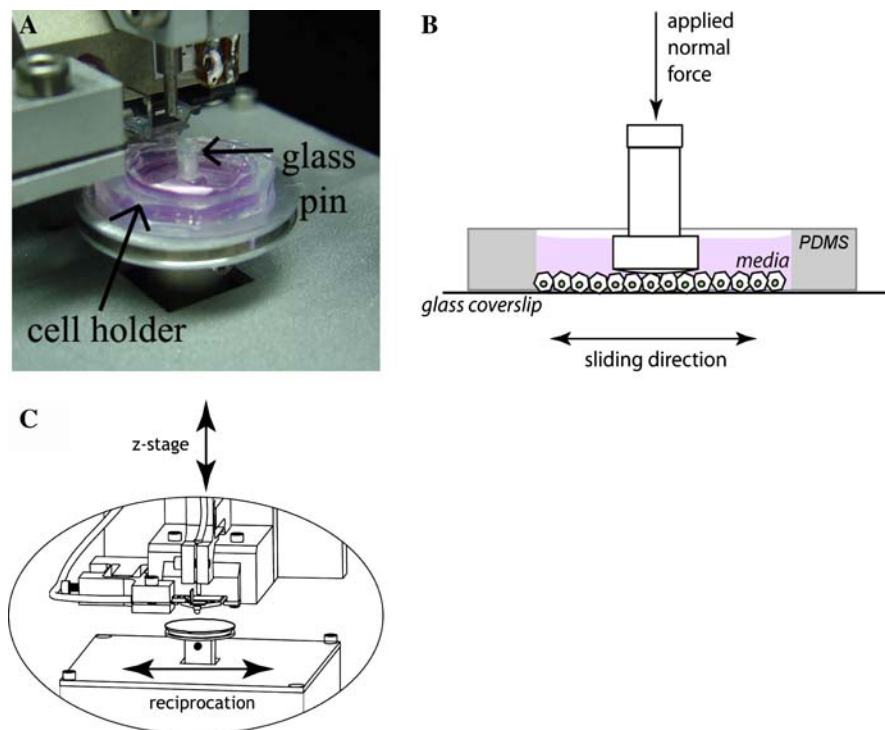
The SV-40 immortalized human corneal epithelial cell line (HCE-T, RCB1384) was provided by Dr. Stephen Sugrue from the Department of Anatomy and Cell Biology, University of Florida College of Medicine. HCE-Ts were cultured in DMEM/F12 media containing 200 U/ml each of penicillin and streptomycin, 5% (v/v) FBS, 0.1  $\mu\text{g}$  cholera toxin/ml, 0.5% (v/v) dimethyl sulfoxide, 5  $\mu\text{g}$  insulin/ml, and 10 ng human epidermal growth factor/ml (Joo et al. 2005). 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  $5 \times 10^4$  and  $10^5$  cells/cm<sup>2</sup>. The cells were allowed to attach and replicate within the holder for approximately 24 h, at which point the level of cell layer confluence was visually assessed by phase contrast microscopy. Holders that had achieved a 100% confluent layer were used for frictional testing, while any holders that were more than 100% confluent were discarded.

Cell holders were made from a glass coverslip of diameter  $D = 25$  mm, fitted with a polydimethylsiloxane ring which maintained growth media on top of the cell layer to provide nutrients during the culture period and lubrication during friction testing (Fig. 1a, b).

### Tribology equipment

Friction testing was performed with a reciprocating micro-tribometer (CSM Instruments, Switzerland) that was adapted for submersion testing (Fig. 1a, b) (Rennie et al. 2005). The apparatus was situated on a vibration-isolation granite table and housed in a class 10,000 clean room. Tests were run and data was collected using a custom program written in Lab-View. The normal load was applied by motion of the z-stage, which was controlled with a piezoelectric cell (Fig. 1c). The working range of applied loads for the apparatus was 0.1 mN to 1 N, the maximum track length that could be imposed was 600  $\mu\text{m}$ , and the prescribed velocity ranged from 0.01 to 18 mm/s (Liu and Bhushan 2003). The frictional force was applied with a plano-convex glass lens (Edmund Optics,

**Fig. 1** (a) Cell holder in place on the micro-tribometer with the pin lowered onto the cell surface; (b) Schematic of pin running across cell surface within the cell holder; (c) Schematic of micro-tribometer showing the axis of applied load, z, and the direction of reciprocation



Barrington, NJ) with radius  $R = 7.78$  mm (which will be referred to as ‘the pin’ from this point on) that was attached to a two-beam cantilever flexure. Normal and tangential forces were continually measured via optical sensors that measured the stiffness and displacement of the flexure. For each test the z-stage was manually lowered until the pin made contact with the cell layer, as indicated by a sudden increase in the force reading. The z-stage was then raised until the force value returned to the value just prior to the sudden increase. Any forces acting on the pin at this point were capillary forces from the culture media; therefore, the reading was zeroed to disregard these forces. The desired load was then applied to the cell layer. The applied normal load was controlled by force feedback and the adjustable, vertical piezoelectric cell. Position and corresponding average normal force,  $F_n$ , and friction force,  $F_t$ , data was recorded at 1.2 kHz for each test. Kinetic coefficients of friction (COFs) were calculated as the ratio of  $F_t$  to  $F_n$ .

### Tribology testing

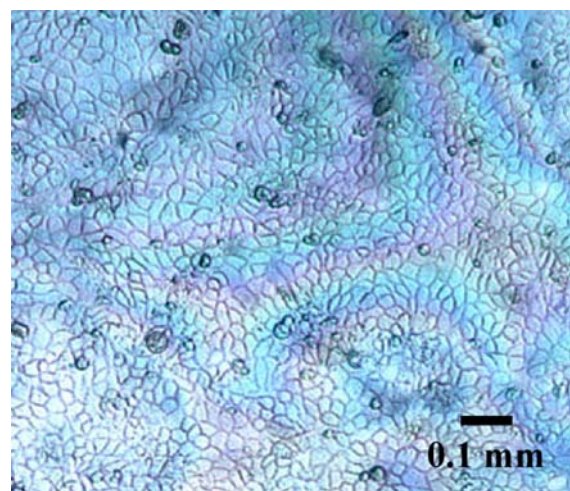
Friction tests were performed with a sliding speed of  $300 \mu\text{m/s}$ , normal loads ranging from 0.5 mN to 6 mN, and cycle number ranging from 2 to 20 cycles. The pin was cleaned with 100% methanol between each test. The procedure for frictional testing and damage assessment was as follows: (1) Culture media in the cell holders was removed and the cell layer was rinsed with fresh media. (2) The rinsing media was removed and replaced with a 1:1 dilution of a 0.4% (w/v) Trypan Blue solution which remained in the chamber for 1 min at room temperature. (3) The Trypan Blue solution was removed from the holder, the cell layer was rinsed twice with fresh media, and then replaced with fresh media. (4) Cells were observed via differential interference contrast microscopy (Leica DMLM, McBain Instruments, Chatsworth, CA) to check for a confluent monolayer of viable cells. (5) The cell holder was attached with double-sided tape to a base stub which was then mounted onto the stage of the micro-tribometer. (6) The pin was manually lowered until it made contact with the cell layer, and capillary forces were zeroed. (7) The applied load, sliding speed, and number of cycles were specified and force data was collected for each cycle. (8) The pin was lifted and the holder was

removed from the tribometer. (9) The Trypan Blue assay described in steps (1) through (3) was repeated. (10) Integrity of the cell monolayer and individual cell damage was viewed with the microscope and images were recorded with an attached SPOT Insight 4 MP Color Mosaic Camera (Diagnostic Instruments, Sterling Heights, MI).

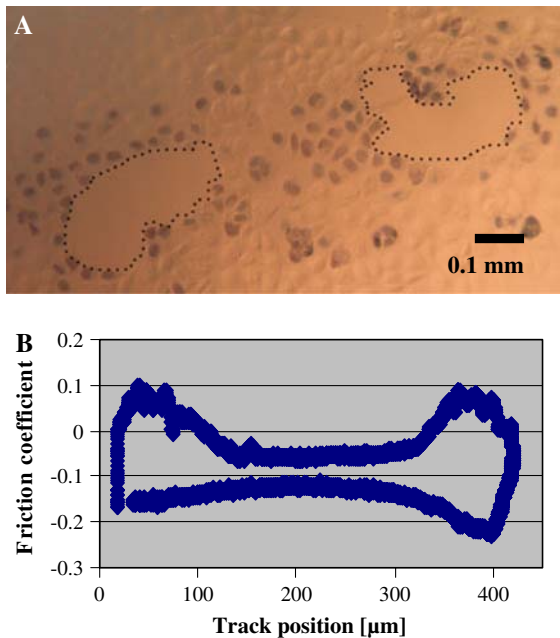
### Results and discussion

HCE-T monolayers were successfully cultured in the cell holders. They exhibited the typical cobblestone morphology characteristic of epithelial cells, as shown in Fig. 2.

In addition, protocols for assessing cell damage within the cell holders were developed. Damage to the cell monolayer, as well as to individual cells was examined. Integrity of the monolayer after frictional testing was examined via microscopy. In several tests the shear forces acting on the cells overcame cell–cell and cell–substrate adhesion, thereby stripping cells off the glass coverslip and creating holes within the monolayer. These holes were obvious indications of damage, as shown in Fig. 3a. However, the absence of a hole does not indicate that no damage occurred. Cell membranes can be ruptured by contact shear stresses from the pin while maintaining cell–cell and cell–substrate contacts. A protocol was developed in which Trypan Blue was used to stain the corneal cells



**Fig. 2** HCE-T cell monolayer grown in the cell holder. Scale bar is 0.1 mm



**Fig. 3** (a) HCE-T layer subjected to a 2 mN normal load for 5 cycles. Holes within cell monolayer are outlined. (b) Friction data loop for cycle 5 of this test. The friction coefficient data is not centered around  $\mu = 0$  due to an un-level sample and/or asymmetry of the cantilever which results in unequal forces measured for the forward and reverse sliding directions. This is corrected in the software by taking the difference between the forces measured for each direction and dividing by 2. Scale bar is 0.1 mm

within the cell holders. After staining, non-viable cells appeared opaque blue. As shown in Fig. 3a, this identified any cells that suffered membrane damage during testing but remained attached to the coverslip.

Friction data was obtained for the glass pin of the micro-tribometer rubbing on a confluent layer of cultured HCE-Ts. The coefficient of friction (COF) values obtained directly correlated with the degree of cell damage (i.e. larger friction coefficients corresponded with more damage), as depicted in Fig. 3. The cells shown in Fig. 3a underwent a 2 mN normal force load for a total of 5 cycles. The holes that were created on each end of the wear track indicate areas where the glass pin stripped the cells off and began rubbing directly against the glass coverslip. This is reflected in the COF values obtained at both ends of the track ( $\mu \geq 0.1$ ), as shown in the friction data loop in Fig. 3b. In addition to holes within the cell layer, there was an area of intact cells in the middle of the wear track. Trypan Blue staining indicates that there

are some damaged cells in this area; however, they were not damaged to the point that they were stripped off the coverslip. The COF values in the middle of the data loop are low ( $\mu \leq 0.05$ ). This value agreed with other tests where the friction between the pin and an intact cell layer was found to be  $0.05 \pm 0.02$  ( $n = 16$ ). Tests in which the measured COF exceeded this value typically compromised the integrity of the cell layer and/or produced large patches of damaged cells.

The image in Fig. 3a depicts a wear track with holes in the monolayer at each end. This phenomenon was seen in several tests. The micro-tribometer stage reciprocates, creating a wear track in which the pin contacts the monolayer. At each end of the track the stage velocity slows down, stops, and increases again when the direction of motion is reversed. As a result, the cells at both ends of the track are subjected to the applied normal load for a longer duration than the cells in the middle of the track, and consequently, sustain more damage.

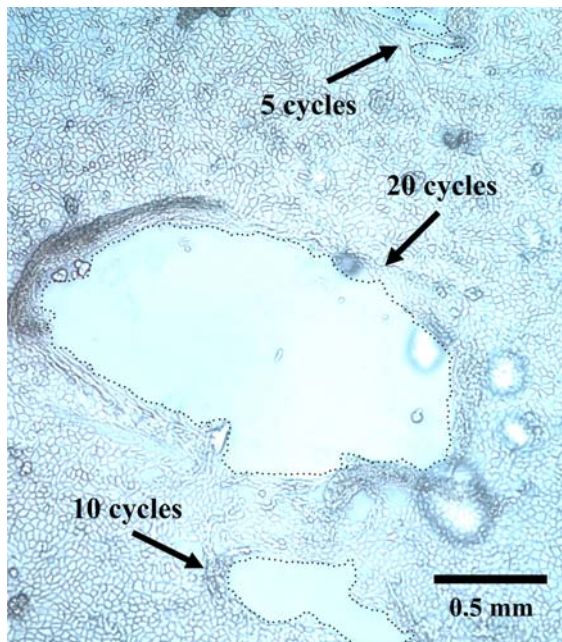
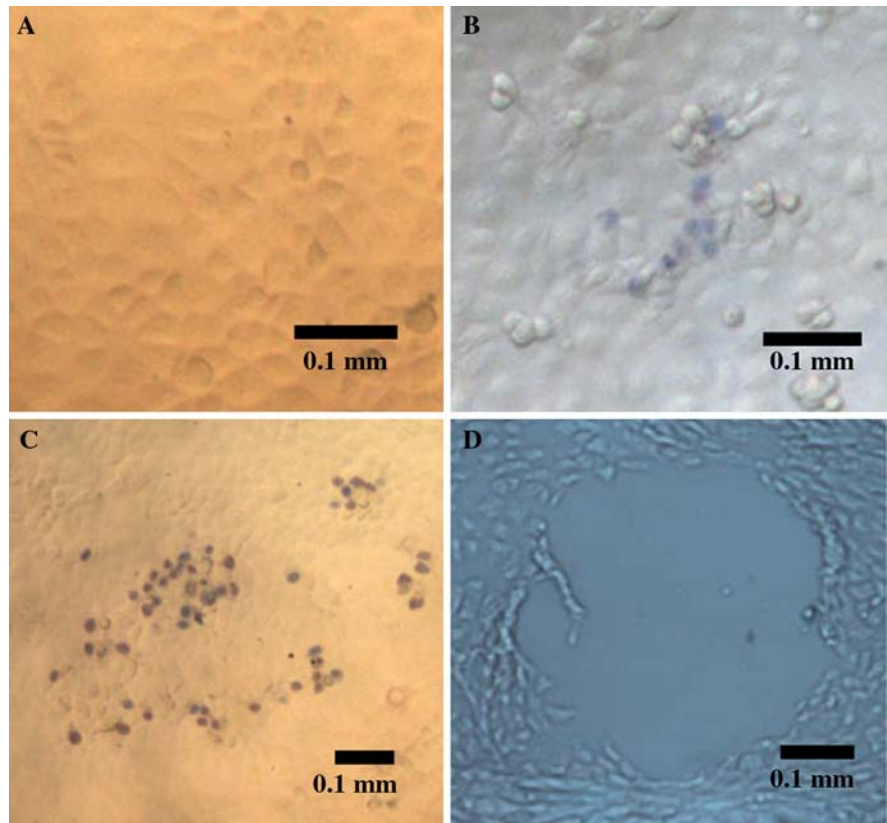
Applied normal loads of 0.5, 2, and 6 mN were used in the testing. Figure 4 shows the effects of friction in terms of the applied normal load force on the cell monolayer. The lowest applied normal force of 0.5 mN produced minimal cell death, as shown by Trypan Blue staining (Fig. 4b). Increasing the force to 2 mN increased the number of nonviable cells, as measured by Trypan Blue staining, but the cell monolayer remained intact (Fig. 4c). Applying 6 mN of normal force produced a large hole in the HCE-T monolayer (Fig. 4d).

Cell damage was also directly related to the number of cycles performed. When the applied load was kept constant, increasing the number of cycles increased the level of cell damage, as indicated by the increase in hole size for 5, 10, and 20 cycles (Fig. 5).

## Conclusion

A low load tribology technique was developed to allow direct contact friction testing on living cell monolayers. Additionally, protocols were developed to assess friction-induced damage at the cellular level. The methods described here can be used on a wide variety of adherent cell types. In addition, various materials can be tested by replacing the glass pin with a different material of interest. This would

**Fig. 4** HCE-T monolayer stained with Trypan Blue: (a) control, (b) 0.5 mN load for 5 cycles, (c) 2 mN load for 5 cycles, (d) 6 mN load for 5 cycles. Scale bars are 0.1 mm



**Fig. 5** HCE-T monolayer subjected to a 2 mN normal load for 5, 10, and 20 cycles. Holes within cell monolayer are outlined. Scale bar is 0.5 mm

be a useful tool for evaluating the clinical applicability of various materials for use in medical devices. In addition, it should be noted that cell damage can occur when a loading force as low as 0.5 mN is applied, even though the cells appear to be intact.

Ongoing work is being done to develop more sensitive assays for assessing cell damage. For instance, immunostaining of cytoskeletal components will show cellular response prior to death.

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