Cells sense and respond to their environment. Mechanotransduction is the process by which mechanical forces, stress, and strains are converted into biochemical signals that control cell behavior. In recent decades it has been shown that appropriate mechanical signals are essential to tissue health, but the role of friction and direct contact shearing across cell surfaces has been essentially unexplored. This, despite the obvious existence of numerous biological tissues whose express function depends on sliding contacts. In our studies on frictional interactions of corneal cells we find that the friction coefficients are on the order of $\mu = 0.03–0.06$ for in vitro and in vivo experiments. Additionally, we observe cell death after single cycles of sliding at contact pressures estimated to be $\approx 12$ kPa. These experimental results suggest that frictional contact forces produce mechanical stresses and strains that are in the cellular mechanosensing ranges.

1. Introduction

The human body is an extremely complex moving mechanical assembly of living tissue, with a myriad of contacting interfaces. In tissues, the cell’s ability to sense and respond to static and dynamic mechanical cues is essential to physiological processes in development, health and disease; mechanical sensing by the cell is implicated in wound healing, angiogenesis, stem cell differentiation, cancer metastasis, and tissue homeostasis. Cells sense mechanical signals through mechanotransduction, the process by which physical strains are converted into intracellular biochemical signals, analogous to the conversion of mechanical strain into electrical current in piezoelectric transducers. Mechanotransduction elicits many types of active cellular responses. A cell senses the stiffness of its surroundings and generates increased contractile forces when adhered to increasingly stiff materials. The higher level of contractility results in higher levels of tensile stress within the cell, stiffening the cell itself. Moreover, at higher levels of contraction, the cell increases the strength of its adhesions to remain attached to its surroundings, which modulates cell shape, surface spreading, and migration rate. Thus, the material properties of a tissue feed back to cell mechanical behaviors through mechanotransduction to influence tissue function or malfunction.

Just as cells are sensitive to their static extracellular mechanical environment, dynamic forces and stresses will also elicit cellular responses. The complexity of tissues and organs within living organisms allow for many types of forces to be generated within tissues and between contacting tissues. Great progress has been made in understanding mechanotransduction in cell–ECM interactions and cell–cell interactions, in which forces are transmitted directly through adhesions. However, forces can be transmitted through sliding contacts, from cell to cell,
from ECM to cell, or from tissue to tissue. Although sliding contacts are tremendously numerous in living organisms, the role of friction forces in mechanotransduction has been almost entirely unexplored. Here we report on our in vitro and in vivo effort to measure friction coefficients under direct contact stimulation.

2. Mechanotransduction: a biochemical stress–strain sensor

Cells in tissues are anchored to an extracellular matrix (ECM) or to other cells by membrane-bound adhesion proteins. When a cell is strained, the membrane-bound proteins are physically forced to undergo conformational changes, exposing otherwise hidden domains to the intracellular cytoplasm, or to the extracellular space. These exposed domains bind signaling molecules, setting off a cascade of shifts in biochemical equilibria which, ultimately, results in changes in gene expression. By this mechano-chemical process, physical forces can mediate the expression levels and the activity of cytoskeletal filaments, motor proteins, adhesion proteins, and adenosine tri-phosphate (ATP). In turn, the expression levels and activities of these cytoskeletal components create a mechanical feedback, mediating cell-generated forces and the elasticity of the cell itself.

When a single cell probes its surroundings, mechanotransduction occurs by the action of the cell and the response of the environment. The cytoskeleton of a cell in isolation, anchored to a solid surface through membrane proteins, contracts to sense the stiffness of the substrate. If the substrate is very compliant, the elastic restoring forces are low, and the cell must generate high levels of strain to unfold the mechanotransductive proteins. By contrast, if the substrate is very rigid, the mechanotransductive proteins will unfold at low levels of average cell-generated strain. Through this mechanosensitive mechanism, the cell actively responds to the stiffness of its substrate. The cell responds to a stiffer substrate by exerting higher levels of stress, increasing its adhesion to the surface, and stiffening its cytoskeleton. Remarkably, the same correspondence between substrate stiffness, stress generation, and intracellular cell stiffness occurs in tissues; compliant neural tissue, less-compliant soft tissue, and rigid bone tissue follow this trend. Moreover, substrate stiffness and mechanosensing is essential for most cell types to proliferate in culture.

Cells sense externally imposed forces through mechanotransduction. Spatially separated cells can sense one another by straining a shared substrate, and confluent layers of cells transmit forces over long distances through cell–cell junctions and cell–substrate adhesions, resulting in a macroscopic tug-of-war among hundreds of thousands of cells. Individual cells can sense shear flows; leukocyte activation is triggered by the mechanotransduction of fluid shear stresses at the blood vessel wall. Individual cells held between microcantilevers or held in optical cell stretchers exhibit active responses to externally applied forces. These responses suggest that tissue cells at interfaces sense contact and friction through the same mechanotransductive mechanisms.

3. Friction in living tissues

Exciting advances over the past decade have moved the traditional engineering field of tribology to the point of considering macroscopic interfaces in atomic and molecular terms. These developments have entailed ultra-low force measurements sensitive to the rupture of single chemical bonds and friction measurements spatially resolved to the level of individual atoms. The opportunity now exists to address the role of tribological action within biological systems, seeking to characterize, understand, and exploit, cellular interfaces and interactions on a molecular scale.

The most frequently discussed biological bearing surface is articular cartilage; unfortunately, cartilage’s remarkable ability to provide low friction and pain free motion is most obviously appreciated when it is lost (e.g. various forms of arthritis). The body also contains a number of other visible tribological systems such as the
eye, skin, and teeth (Fig. 1 and 2). In the case of eyes and knees, the lubrication mechanisms rely on the maintenance of the aqueous environment and the health of the cell surfaces.

**Cartilage lubrication: the role of fluid pressurization**

Articular cartilage is the tribological material in diarthrodial joints and consists of approximately 75% water and 25% cells and extracellular matrix (chondrocytes, collagen, and proteoglycans). Cartilage possesses a time-dependent mechanical response that serves critical roles during joint loading and sliding, controlled by the permeability and the bulk or aggregate modulus of the tissue.\textsuperscript{17,18} In vivo contact stresses in joints of humans, sheep, dogs, and cats lie in the range from 0.5–5 MPa,\textsuperscript{19} which is surprising considering the aggregate modulus of cartilage is in the same range (0.5–1 MPa).\textsuperscript{17,20,21} These joint contact pressures are in part supported by fluid pressure, where the interstitial fluid supports more than 90% of the normal stress. This fluid pressurization increases load capacity while reducing matrix stresses and friction.\textsuperscript{22} Interstitial fluid pressure is maintained between moving contacts, but is not maintained under the typical cartilage mechanics and stationary contact friction studies.\textsuperscript{23} Interstitial lubrication is sustained through motion in vivo\textsuperscript{24} and friction coefficients are maintained in the range from $\mu = 0.02$–0.03.\textsuperscript{23,25,26}

![Cartilage Lubrication Diagram](https://example.com/cartilage-lubrication.png)

**Fig. 1** The human body has a wide variety of contacting surfaces, and for most natural tribological systems from joints to eyes these contacts could be accurately described as soft. However, the pressure demands on these surfaces vary widely, from MPa in joints to single kPa in the eye. The cells that make up the intimate areas of contact have unique adaptations to enable low friction and provide durability. To date, upon failure of the systems engineering has provided materials systems to restore some degree of function (joint replacements, hernia repairs, bone fusion, crowns, stents, etc.). The future holds opportunities for regenerative medicine and tissue engineering to radically change the treatment strategies.
Interstitial pressurization effectively shields the matrix from the applied contact stress. While the apparent contact pressure in a joint can reach 5 MPa, it can be shown that the time constant for depressurization is on the order of 10 hours. Motion serves to continuously replenish interstitial pressure, and thus reduces matrix stresses, friction and wear by more than an order of magnitude.

**Cornea lubrication: boundary and hydrodynamic lubrication**

The cornea, which is the optical portal to the visual system, is also a tribological system. The cornea forms a dense, transparent connective tissue barrier that protects the eye. Most tribological studies of friction and lubricity in the eye focus on the hydrodynamic lubrication that occurs during the blink; however, recent work with contact lenses points to boundary lubrication being the key to providing comfort and maintaining lubricity during extended wear. Microtribological friction measurements on confluent layers of corneal epithelial cells gives friction coefficients on the order of $\mu = 0.03$. Maintenance of the proper cellular and extracellular matrix composition of the cornea is also essential to its function. The external surface of the cornea is lined with a thin epithelium composed of 5–6 layers of fibroblastic cells that form a protective layer over the corneal stroma. These cells rapidly regenerate the epithelium following injury. Trauma, inflammation or infection can have profound influences on the cells and extracellular matrix of the cornea, and in turn directly impact visual acuity. For example, damage to the corneal stroma can induce the local keratocytes to differentiate into mitotic fibroblastic cells that secrete altered extracellular matrix components, resulting in stromal scar formation and reduced transparency (cornea).

**Bone, teeth, skin: cell generated structural materials**

Bone, teeth and skin are also important tribological materials, but unlike cartilage and the cornea, the contacting surfaces are not cellular. The cells that generate and maintain these structures are sensitive to mechanical extracellular stimulation. However, these cells are not themselves the primary interface in direct contact and tribology.

**4. Corneal tribology in vitro and in vivo**

The stratified epithelial cells of the cornea form the protective barrier for the eye, and work together with the eyelid and tear film to provide low friction and low stress.
lubrication during ocular activity. Similar stratified epithelial cells can be found in the lung and mouth, and interestingly as linings of blood vessels and pericardium.

**In vitro experiments with human corneal epithelial cells**

Tribological experiments on corneal epithelial cells were performed using a microtribometer, which consist of a flexure based biaxial load transducer on a series of piezo-positioning stages. This apparatus can simultaneously apply the normal force while measuring the friction force response. The displacement of the flexure was measured via capacitive sensors. These tribometers and the associated uncertainties have been previously reported in the literature. In these experiments with cells the goal is to perform direct contact friction measurements on living cells, which require very fine load control with low contact pressures and relatively low sliding speeds to eliminate hydrodynamic effects.

The cells used in this study were immortalized human corneal epithelial cells and the cell culture process is more completely described in prior publications; briefly, they 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\(^{-1}\) each of penicillin and streptomycin, 5% (v/v) fetal bovine serum (FBS), 0.1 μg cholera toxin ml\(^{-1}\), 0.5% (v/v) dimethyl sulfoxide, 5 μg insulin ml\(^{-1}\), and 10 ng human epidermal growth factor ml\(^{-1}\). When the single cell layers reached confluence they were rinsed in Hank’s Balanced Salt Solution and detached with 0.25% (w/v) trypsin-EDTA, and then subsequently seeded into the specialized cell holders at a density between 5 \times 10^4 and 1 \times 10^5 cells cm\(^{-2}\). The cells were then 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\(^{-2}\). Before and after testing, cells were submerged in 10% trypan blue staining in order to evaluate damage before and after tribological testing.

In order to perform friction experiments on a single layer of cells a special pin sample made from a hydrogel was prepared. The hydrogel material was taken from a commercially available contact lens, and had a bulk modulus of approximately 250 kPa. As a contacting probe, the hydrogel was bent around a spherical pin giving a radius of curvature of approximately 1 mm. Experiments were performed under a contacting load of 500 mN, and 2–20 cycles of reciprocation were performed (see schematic in Fig. 3a). The contact pressure was estimated to be on the order of 12 kPa (based on the imaging of cell disturbances and elastic contact models). Fig. 3b shows the surface of the cells after 5 cycles of sliding. There are clearly a number of dead cells (dark stained) within the contacting zone. The friction loop (friction coefficient vs. track position) for a representative cycle of testing is also shown. Based on all of our experiments the friction coefficient between a hydrated hydrogel and the living epithelial cells is \(\mu = 0.03\). After cell death and detachment the friction coefficients rise approaching \(\mu = 0.06\) (Fig. 3c), which is the value that we have seen for hydrogels in aqueous environments containing proteins when run against highly polished glass surfaces.

**In vivo experiments with murine corneas**

A portable microtribometer was designed and constructed with the express purpose of performing friction experiments on animals. In this configuration, the flexures and the probe move on a multi-axis piezoelectric stage and the animal is held stationary under the frictional probe. The same experimental uncertainties regarding the measurements of forces apply (~20 μN), and the dynamic effects are negligible at the 250 μm s\(^{-1}\) sliding speeds.

The mouse used in this study was a C57 black 6, widely used for models of human disease. The mouse was first anesthetized using isoflurane 2–5% mixed with air, then the head was immobilized in a three-point stereotactic restrainer that provided
continuous inhalational anesthetic during all friction testing and imaging. This fixture was mounted onto a coarse vertical positioning stage that was located directly below the microtribometer. Before and after friction testing, the mouse left eye was rinsed for 2 min in a fluorescein saline solution, saline rinsed, and imaged under blue light to reveal any scratches or physical damage that was on the cornea. The same process was performed after tribological experiments. No measurable damage was observed on any of the corneas that were tested using this protocol. Post-testing, the mouse was removed from anesthesia and observed until normal activity resumed. The animal was housed in specific pathogen-free conditions in a microisolator cage and was treated in accordance with the guidelines provided in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

For these *in vivo* studies motion was provided by a 250 μm piezoelectric stage. At a sliding speed of 250 μm s⁻¹ the reciprocating frequency is 0.5 Hz. The pin was made by melting the end of a capillary tube to form a very smooth 0.5 mm radius spherical probe on the end of an 8 mm standoff. The entire probe assembly was adhesively mounted onto the flexure assembly. Fig. 4a shows a schematic of the mouse eye and probe assembly.

The experiments were performed by gently (but quickly) loading the 1 mm diameter glass probe into contact with the exposed cornea. The measured loads were between 3–5 mN and varied from experiment to experiment and spatially varied during an experiment due to the relatively small curvature of the mouse eye.
Fig. 4c shows a friction loop that was measured during an experiment. The average friction coefficient was found to be $\mu = 0.06$. Post experimental analysis of the cornea showed no measureable damage after fluorescein staining (Fig. 4d).

5. Closing discussion

The transduction of sliding contact forces into biochemical signals has not been extensively studied in vitro. However, the great depth of knowledge in the areas of cartilage tribology and cornea tribology can be used to make baseline predictions about the cell’s potential response to friction forces both in the laboratory and in the body. Oscillatory rheological measurements on a wide range of cell types in culture have been employed to explore the cell’s response to mechanical forces. In frequency response measurements in vitro, cells exhibit active and passive stress stiffening, as well as dramatic cytoskeletal re-organization, when strained at frequencies within the approximate range of 0.1 to 1 Hz.\textsuperscript{16,39,40} In the body, reciprocation frequencies of sliding contacts are within this range; the average time between blinks in the eye is on the order of 5 s, and the average joint reciprocation period is approximately 1 s.

In stress response measurements in vitro, cells also exhibit a wide range of sensitivity; shear stresses as low as 1 Pa can activate leukocytes and, by contrast, shear stresses as high as 20 kPa are required to elicit a mechanosensitive response of chondrocytes in cartilage.\textsuperscript{15,41} Threshold stresses required for mechanosensitive responses in most fibroblast-type cells lie in the middle of this range at approximately 0.01–1 kPa. In the body, shear stresses at reciprocating contacts can be estimated...
in multiple ways. Assuming hydrodynamic lubrication with sliding speeds of 1–100 mm s\(^{-1}\) and film thicknesses from 0.1–1 mm, the shear stresses would be on the order of tens to hundreds of Pascals. Alternatively, assuming boundary lubrication conditions, the shear stress at the tissue surfaces is given by \(\sigma_s = \mu N\), where \(\mu\) is the friction coefficient and \(N\) is the normal stress. In Fig. 3 and Fig. 4 we show that typical friction coefficients lie within the range of 0.03 to 0.06. Normal stresses vary from 6 kPa in the eye to 1 MPa in cartilage; we therefore estimate that shear stresses in these sliding contacts are within the range of 0.18 to 60 kPa, directly within the mechanosensitive range found in vitro.

Taken together, these comparisons suggest that typical sliding contact forces within the body occur within the range of shear stresses and frequencies necessary to generate a mechanotransductive response of interfacial cells. Recently it has become clear that mechanical signals have a major impact on cell fate and function, and we propose that frictional contact forces can generate these mechanical signals at the molecular level within interfacial cells. We have found in our studies of epithelial monolayers in vitro, and of stratified epithelial cells in vivo, that the friction coefficients in protein containing aqueous environments is \(\mu = 0.03–0.06\). Based on models and estimates of contact area, this corresponds to shear stresses on the order of 0.3–0.5 kPa. Such low values of shear stresses are consistent with brush type aqueous lubrication, and we suggest that the cell surfaces are maintaining low friction interfaces through similar mechanisms. To control the cellular mechanotransductive response in new engineering strategies for replacing biological tissues with synthetic materials like metal, ceramics, and plastics, these lubricating properties at the surfaces of living tissues must be employed. In general, the mechanical microcellular environment significantly impacts many kinds of cell behavior, including contractility, migration, proliferation, apoptosis, and stem cell differentiation; cell friction may be a key contributor to these mechanosensitive behaviors at interfaces.

References

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