

# Nanomechanical Probes of Single Corneal Epithelial Cells: Shear Stress and Elastic Modulus

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**Abstract** Living human corneal epithelial cells have been probed in vitro via atomic force microscopy, revealing the frictional characteristics of *single* cells. Under cell media, measured shear stresses of 0.40 kPa demonstrate the high lubricity of epithelial cell surfaces in contact with a microsphere probe. The mechanical properties of individual epithelial cells have been further probed through nanometer scale indentation measurements. A simple elastic foundation model, based on experimentally verifiable parameters, is used to fit the indentation data, producing an effective elastic modulus of 16.5 kPa and highlighting the highly compliant nature of the cell surface. The elastic foundation model is found to more accurately fit the experimental data, to avoid unverifiable assumptions, and to produce a modulus significantly higher than that of the widely used Hertz–Sneddon model.

**Keywords** Atomic force microscopy · Elastic modulus · Shear stress · Friction · Epithelial cells

## 1 Introduction

The structure and function of living cells is strongly dependent on local environment, entailing factors related to both chemical and mechanical stimuli [1]. Cell structure and growth rely upon the transport of biochemical species across membranes, processes intimately related to chemical gradients and signals within the environment. Furthermore, it is now understood that mechanical forces acting on and within the cell can trigger specific cellular responses. As a result, the relationship between mechanical forces and the response and function of living cells is of growing interest. For example, prior work has explored the response of cell modulus to changes in the microenvironment as well as a function of the health of the cell [2]. In addition, a significant body of work has explored cellular properties as a function of shear environment, primarily documenting the influence of shear at cell–liquid interfaces. The focus of these studies has been to identify active mechanobiological mechanisms under physiological conditions [3].

In the emerging area of mechanobiology, effort is also needed in the development of techniques used to probe the mechanical properties of biological entities as well as quantitative models used to describe these properties. In particular, techniques capable of probing individual living cells in a manner that allows access to fundamental mechanical properties are needed. These techniques ultimately should provide quantitative measurement of forces needed for the construction of detailed models of cellular structure and environment in addition to biomolecular sensitivity. In turn, the availability of such techniques and biospecific models will allow the exploration of cellular function in terms of physiological environment, disease, and external drug stimuli.

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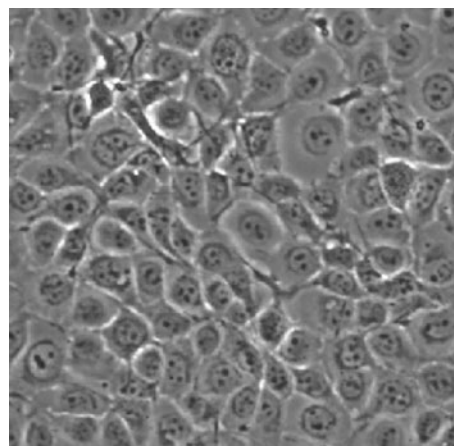
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A number of methods, with sensitivity in the pico- to nanoNewton range, have been developed to investigate forces associated with cellular structures and processes. These include atomic force microscopy (AFM), micropipette aspiration, stretching of underlying adhesive substrate, magnetic/optical tweezers, MEMs devices, and laminar fluid flow chambers. However, none of these systems are capable of quantifying tangential sliding forces, under an applied load, between a cell surface and a solid probe. A system capable of quantifying this category of surface interactions would enable new directions of investigation, applicable to diverse biological phenomenon such as the measurement of sliding forces between a cell and a synthetic solid surfaces (e.g., applicable to catheters, stents, contact lenses), between a cell and a biological substrate (e.g., applicable to cells migrating through porous 3D tissues), and between two cells (e.g., leukocyte extravasation from a blood vessel), as well as the measurement of protrusion forces of cellular extensions such as lamellopodia.

In this study, we report the first account of solid–solid shear stress measurements on individual living cells as well as a revised analysis of cell elasticity as measured through related nanomechanical probes. While studies of cell surfaces under shear flow forces have been previously reported, heretofore, little has been accomplished in the exploration of a single cell's response to the shearing action of a solid body [4, 5]. Specifically, we have employed AFM to characterize both lateral shear and normal forces present at the contact of a colloidal probe tip and individual living human corneal epithelia cells. A quantitative analysis of the measured forces via an elastic foundation model highlights significant differences from results obtained with the widely employed Hertz–Sneddon contact mechanics model. In addition, information of contact area with the cell surface derived from the elastic foundation model allows calculation of the load-dependent shear stresses incurred at the living cell surface. As these stresses result from the interaction of proteins and membrane components across the interface, these studies demonstrate a clear path to directly probing the molecularly specific mechanobiological properties of cells found within endothelial and epithelia linings where mechanical action is known to play a significant role. Furthermore, the approach highlights the opportunity for cellular level investigations of interactions with external solid bodies.

## 2 Experimental

Human corneal epithelial (HCE) cells from an immortalized cell line (HCE-T) were cultured under Dulbecco's Modified Eagle Medium (DMEM)/F12 containing 100 U/mL



**Fig. 1** Phase contrast image ( $\times 20$ ) of a confluent layer of HCE cells seeded on fibronectin-coated glass cover slips. The positioning and imaging capabilities of AFM allow the interrogation of the mechanical properties of individual cells. All measurements were performed at the approximate center of the cell

penicillin and streptomycin, 5% fetal bovine serum, 0.1  $\mu\text{g/mL}$  cholera toxin, 5  $\mu\text{g/mL}$  insulin, 0.5% DMSO, and 10 ng/mL human epithelial growth factor. The cells were incubated at 37  $^{\circ}\text{C}$  and passaged twice weekly with 0.25% trypsin until a confluent monolayer was obtained (Fig. 1). The cells were then plated overnight onto fibronectin-coated glass cover slips, which were affixed to 60  $\times$  15 mm Petri dishes using small amounts of silicone grease. The cells were maintained under sterile cell media during experimentation and regularly checked for viability with respect to the duration of the experiment and applied loads.

AFM served to probe the biomechanical properties of the cells through load–displacement and friction-load measurements, the details of which have been previously described [6, 7]. The microscope (MFP-3D, Asylum Research, Santa Barbara, CA, USA) uses Igor Pro Software (WaveMetrics, Inc., Portland, OR, USA) to obtain and analyze the collected datasets. AFM sampling utilizes a set of piezoelectric motors to control the interaction of a cantilever probe with the surface of the sample. A laser focused on the backside of the cantilever provides optical measurements of cantilever deflections reflecting information about the sample surface. The  $z$ -axis motion of the probe is precisely regulated through a closed-loop feedback control of the  $z$ -axis piezoelectric motor. This feature allows for an accurately measured displacement of the tip holder during load–displacement measurements. The use of a modified scan head and custom software allowed for scanning in  $x$  and  $z$ -directions simultaneously, thus permitting the collection of friction data as a function of continuously varying load. All measurements were conducted at ambient temperatures (25  $^{\circ}\text{C}$ ). Given the recent study of temperature-dependent cell mechanics [8], some

variation in reported values may result at physiological conditions; the extent of such changes represents the focus of future studies. Friction measurements were conducted at scan speed of 1.4  $\mu\text{m/s}$ .

A silicon nitride cantilever modified through the attachment of a 5  $\mu\text{m}$  silica sphere (Novascan Technologies, Inc., Ames, IA, USA) and possessing an experimentally determined spring constant of 0.05 N/m was used in each experiment. The choice of the silica sphere as a contacting surface was motivated by the need to reduce contact pressures at the cell surface; the surface composition of the spheres used in this study is understood to be modified through the spontaneous adsorption of proteins from the solution medium as described below. The normal spring constant was calculated from the slope of the curve (the  $z$ -piezo voltage required per nanometer of displacement) and the resonant frequency of the thermal power scan. Lateral force values were referenced to prior studies of the poly(L-lysine)- $g$ -poly(ethylene glycol) (PLL- $g$ -PEG) system for which calibrated friction forces have been published [9–12].

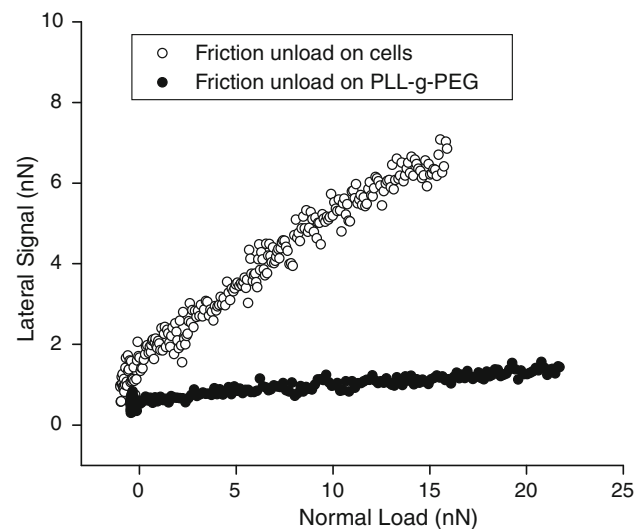
Prior to each experiment, the cantilever was rinsed in alcohol and exposed for 15 s to an oxygen plasma (Harrick Plasma, Ithaca, NY, USA) to remove any contaminants. All AFM experiments were performed under liquid media. A self-mated polymer brush sample was interrogated with the same tip-cantilever assembly for comparison purposes; prior studies have considered this system as an analogue to the highly hydrated glycocalyx of the epithelial cell surface. Specifically, a PLL- $g$ -PEG copolymer, consisting of 5 kDa PEG side chains grafted onto a 20-kDa PLL backbone at a ratio of 1 PEG unit per 3.5 PLL units [9–12], was coated onto a silica substrate and the AFM microsphere. For these samples, friction measurements were conducted under a 10-mM HEPES buffer solution (4-[2-hydroxyethyl]piperazine-1-[2-ethanesulfonic acid]) (pH 7.4).

### 3 Results

#### 3.1 Friction Measurements

Navigation across the sample surface, guided by both optical microscopy and AFM imaging, permitted the lateral placement of the probe tip at the approximate center of individual cells. All friction measurements entailed 500 nm lateral displacements at a scan rate of 6.1  $\mu\text{m/s}$ . Normal loads were varied between 0 (no contact) and a maximum of 20 nN in an effort to avoid cell damage due to excessive contact pressures. Minimal adhesion was detected under the aqueous media. The frictional properties of the endothelial cells were evaluated by monitoring lateral

deflections of the cantilever as it was rastered across the cell surface. The reciprocating motion of the cantilever produced a friction loop, from which a kinetic friction force, averaged over sample position and scan direction, could be deduced for each specific load [13]. Control experiments demonstrated that the HCE-T cells did not experience permanent deformation during the course of these load-unload cycles, yet sufficient pressure was imparted in order to collect friction data. Associated contact pressures deduced from a contact mechanics analysis are described below. In the assessment of frictional properties, data were acquired at least five times from each of three characteristic cells. Representative friction-load data, measured as a function of decreasing load, are presented in Fig. 2 for HCE-T cells and the self-mated polymer brush sample. Similar data were obtained for increasing loads, indicating that little plastic deformation occurred during the course of the friction measurements. The friction-load behavior of the self-mated PLL- $g$ -PEG interface has been previously characterized through similar approaches [9–12]. For the polymer system, the exceptionally small increase in friction with increasing load is understood to result from the highly hydrated, low shear interface of the densely packed polymer brush. The comparative results presented here depict a significantly higher friction response for the biological interface, comprised of HCE-T cells in contact with a protein-coated microsphere [9–12].

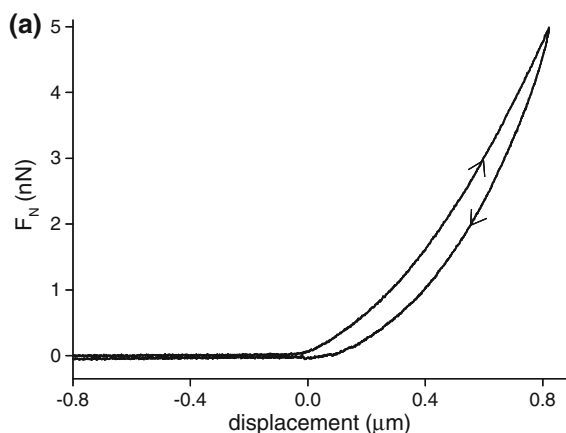


**Fig. 2** The frictional properties of a materials interface can be represented via the unitless slope of a plot of lateral signal and normal force, as shown above. In order to gain perspective on the friction properties of a single HCE-T cell, control samples were analyzed under analogous experimental conditions with the same AFM probe, with the results indicated on the graph

### 3.2 Elastic Modulus

In order to quantify the deformation properties of HCE-T cells under these conditions, force curves were analyzed and fit to a mathematical model. To determine the Young's modulus, an indentation plot was first created, in which the normal force is monitored as a function of displacement along the probe-substrate separation axis. Here, the force–displacement curves obtained on cell surfaces were referenced to the data measured from a glass slide modeling an incompressible substrate in order to obtain the response of the cells to indentation forces.

These indentation curves have been fit to an elastic foundation model [14]. In this model, a one-dimensional differential element of an elastic solid is taken to behave like a linear spring. Thus, the elastic foundation model is often described as a bed of springs. In reality, the material modulus, Poisson's ratio, and the lateral confinement of the material being compressed are all used to derive the effective spring constants. This is discussed in greater detail in Johnson's *Contact Mechanics* [14]. In this study, the silica probe is assumed to be incompressible such that the entirety of the deformation is due to compliance on the part of the cell in contact with the probe tip. As such, calculated pressures are taken as an upper bound and related contact areas as a lower bound. Furthermore, it is assumed that the underlying fibronectin layer does not contribute to the measured modulus, given the relative depth of penetration and separation from that layer. Based on the structure and reproducibility of the force curves over the load range 0–20 nN, the cells are presumed to respond elastically, without any plastic deformation taking place.



**Fig. 3 a** A force–displacement plot measured at the epithelial cell surface that deforms upon loading and recovers upon unloading of the normal force. The contribution of the underlying glass substrate has been subtracted from these data. Additional measurements systematically explored higher force ranges (up to 20 nN) with no

observable change in behavior. **b** Schematic representation of the elastic foundation model in which a rigid, spherical indenter contacts a cellular substrate modeled by a bed of springs. The parameters indicated in this figure are used in Eqs. 1–3

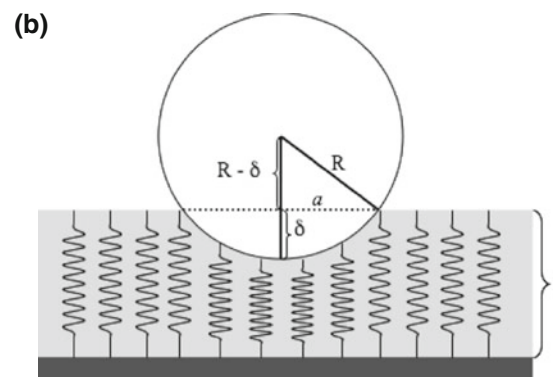
$$P = E' \frac{\delta}{t} \quad (1)$$

$$F_n = \int_0^{2\pi} \int_0^a P \cdot r \cdot dr \cdot d\theta = \int_0^{2\pi} \int_0^a \frac{E' \delta}{t} \cdot r \cdot dr \cdot d\theta \cong \frac{\pi E' a^4}{4tR} \quad (2)$$

Equation 2 results from the integration of the pressure distribution in Eq. 1 and defines normal force ( $F_n$ ) in terms of the half-width of the indentation ( $a$ ), the height of the cell ( $t$ ), the effective elastic modulus ( $E'$ ), and the radius of the probe ( $R$ ). The only dynamic variable in Eq. 2 is  $a$ , which is related to the indentation value  $\delta$ . Figure 3b shows the geometric relationship among the tip radius, indentation value, and the half-width of the indentation ( $a$ ). This relationship is quantified in Eq. 3.

$$R^2 = a^2 + (R - \delta)^2 \therefore a^4 = (2R\delta - \delta^2)^2 \quad (3)$$

The calculated average value for the effective Young's modulus of the HCE-T cells, based on the elastic foundation model, was 16.5 kPa for a sample size of 18 measurements, with a standard deviation of  $\pm 8.83$  kPa. These values have been determined using a cell thickness of  $10.9 \pm 0.7$   $\mu\text{m}$  determined from the evaluation of eight different cells. This thickness value has been derived by comparing AFM force–distance curves of individual cells and those acquired on the glass substrate. By mechanically



observable change in behavior. **b** Schematic representation of the elastic foundation model in which a rigid, spherical indenter contacts a cellular substrate modeled by a bed of springs. The parameters indicated in this figure are used in Eqs. 1–3

removing a swath of cells, an area of the glass substrate was exposed in close proximity to the characteristic monolayer of cells that was used as a reference for cell height. By overlaying the glass and cell force curves, the difference between the points of contact on the  $z$ -displacement axis reflect cell thickness. Previous studies have reported a range of data for single-cell elastic moduli, spanning from 1 to 100 kPa [17, 18].

#### 4 Discussion

When testing the properties of living cells, it is important to verify that the experimental conditions do not cause cell death. During these experiments, the cells were maintained outside of the controlled environment of the incubator for up to 4 h. Additionally, when implementing normal forces upon single cells, high forces disrupt the integrity of the cell membrane and cause rupture. However, there is a threshold below which resolution suffers, thus an appropriate range of forces must be defined that is specific to the equipment and cell line used.

In order to verify that the experimental conditions of temperature and atmosphere did not induce cell death, a control experiment was performed in which a fibronectin-coated glass cover slip containing a confluent monolayer of HCE-T cells was treated with 7-amino-actinomycin D (7-AAD) and allowed to remain at experimental conditions for 18 h. 7-AAD causes the nucleus of cells to be stained red if the cell membrane becomes permeable (characteristic of cell death). The cells were assayed hourly for the first 5 h and again after 18 h to determine viability. After 5 h, all cells remained viable, confirming that the length of experiment was not detrimental to the cells.

To determine whether the force range used in experimentation (0–20 nN) was appropriate, a specific region of cells was tested at the maximum normal force achievable using the 0.05 N/m AFM probe ( $\sim 60$  nN). Shortly after the experiment, the cells were treated with 7-AAD and the area of interest was assayed. The cells in this area did not stain red, thus verifying that a range of force from 0 to 20 nN was appropriate for HCE-T cells.

##### 4.1 Single Cell Friction Measurements

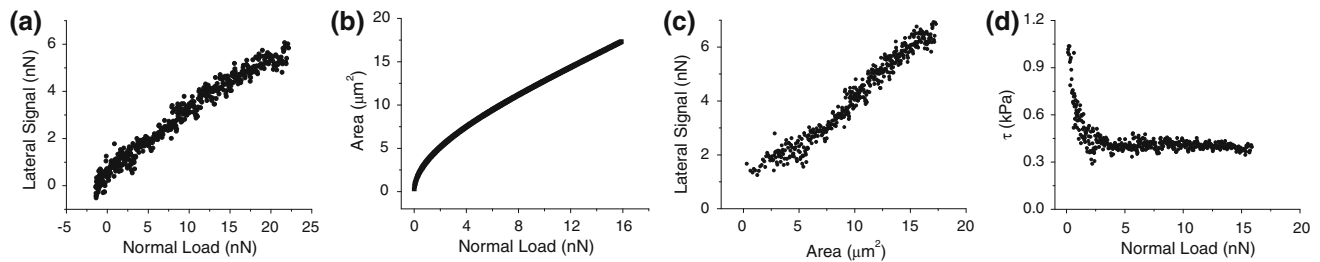
To the best of our knowledge, the data in this article mark the first quantified friction measurement of a single living cell and highlight the opportunity for biomechanical studies at the molecular level. In the past, AFM has been utilized to determine the friction coefficient of articular cartilage; however, the study was not conducted on the level of individual cells [19]. Here, measurements have been restricted to a single cell in contact with a colloidal

probe. As all friction properties are dependent on the identity of both contacting surfaces as well as the sliding environment, it is of importance to acknowledge the propensity for protein adsorption on glass surfaces held in cell media. Prior studies have documented the degree of uptake of specific proteins from similar solutions onto clean glass surfaces [20]. With this in mind, the appropriate mental picture for the sliding interface reported here is that of a compliant living epithelial cell surface in contact with a rigid, yet protein-coated, microsphere. The measured friction response reflects the presence of this protein coating, yet the protein coating has been neglected in the evaluation of elastic modulus due to the limited thickness.

Two approaches have been adopted here to quantify the frictional response of individual HCE-T cells. First, using the results of the elastic foundation model, the load-dependent shear stress of the cell–probe interface has been evaluated. This has been accomplished by plotting the measured friction force as a function of the corresponding contact area, deduced geometrically from the values of  $R$  obtained through Eq. 3 (Fig. 4c). With shear stress ( $\tau$ ) defined as the ratio of the friction force to the real area of contact, a plot of these ratios from Fig. 4c reflects the load dependence of the shear stress of the interface (Fig. 4d). As apparent from the plot, these measurements depict a load-independent shear stress of  $\sim 0.40$  kPa at loads above  $\sim 2$  nN. The load independence over the higher load range is consistent with viability of the cell surface in this load range (no cell rupture) and portrays a characteristic shear stress for these cells and the specific interfacial conditions employed here. At the lowest loads, a sharp rise in shear stress is detected. Prior evaluation of shear stress with AFM have likewise detected increases in shear stress near the point of probe surface detachment and have argued that such increases arise from primarily from adhesive interactions. While such interactions are present at all loads, the increase in shear stress at low loads may arise from neglecting the contribution of adhesion to the calculated area of contact. Nonetheless, both load regimes provide sensitivity to cell surface composition and functionality and represent the path for future studies, entailing purposeful modifications of the cell surface and fluid environment.

Second, the friction results of the HCE-T cells can be compared to those of the self-mated PLL-*g*-PEG interface used as a control model. The PLL-*g*-PEG system has been previously characterized through a similar approach employing colloidal probe AFM [9–12]. The copolymer is understood to self-assemble under appropriate pH conditions onto oxide surfaces, forming a dense layer in which the PLL chains are electrostatically adsorbed on the surface, leaving the PEG chains to extend into solution in a brush-like conformation. In this configuration, extremely





**Fig. 4** **a** Friction–load data measured as a function of decreasing load for the epithelial cell–probe contact under cell medium. **b** Based upon the elastic foundation model, geometric relationships, and fits to indentation data, the calculated area of contact is plotted versus normal load. **c** The shear stress of the interface is derived from the

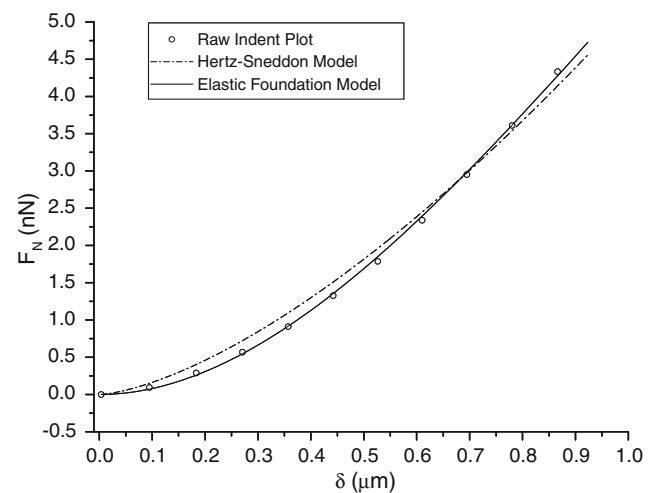
ratio of the friction force to the real area of contact. **d** A plot of shear stress versus normal load highlights a load-independent shear stress of 0.40 kPa above  $\sim 2$  nN. The large increase in shear stress at low loads is thought to arise from adhesive interactions and uncertainties in the molecular scale area of contact

low frictional forces are measured in an aqueous environment as result of a combination of the repulsive osmotic forces between opposing polymer layers and the low shear forces of the highly hydrated PEG chains. Although the shear stress of the polymer brush interface is not quantified here due to uncertainties in the contact area, a comparison of friction–load behavior of the two systems (Fig. 2) highlights the sensitivity of the approach presented here as well as the potential importance of frictional interactions at the cellular level. Specifically, the comparison indicates that the cell–probe interface, entailing the molecular level contact of membrane components and transmembrane proteins with proteins adsorbed from media on the rigid probe, exhibits measurably higher friction than a model aqueous based low-friction system. This observation highlights the role of biomolecular entities in determining the frictional response of HCE-T cells and the potential for following changes in cell surface responses through this approach.

#### 4.2 Modeling Contact Mechanics

To quantify the elastic modulus of an individual cell, multiple contact mechanics models were considered. The most widely utilized model for single cell biomechanics is the modified Hertz model [21] (also known as the Hertz–Sneddon model), which has been used to assess the character of many types of living cells, including human epithelial cells [22]. However, this contact theory acts under numerous assumptions which cannot be validated for the case of living cells. Namely, these include a flat contact surface, infinitesimal deformation, non-frictional contact, infinite sample thickness and dimensions, and homogeneous, isotropic properties. Alternative theories have been proposed in order to better model the contact mechanics of a cell (for a full discussion, see analysis from Costa [2]), yet each increasingly complex model remains founded on assumptions which cannot be corroborated. In the interest

of limiting unrealistic assumptions while maintaining accuracy of fit, the elastic foundation model was implemented to quantify the elastic modulus of living HCE-T cells. Although the response of a living cell to pressure is undoubtedly more complex than that of a bed of springs, the elastic foundation model does fit the force-indentation plot with accuracy, as seen in Fig. 5. Unlike the modified Hertz model, the elastic foundation model does not regard the cell as an infinitely thick substrate, but rather takes cell height into consideration (see Eq. 2). Although the elastic foundation does assume linear elastic behavior, the use of a spherical probe (as was used in these experiments) has been shown to alleviate the local strains



**Fig. 5** Comparative indentation curves simulated using the Hertz–Sneddon model and the elastic foundation models displayed with HCE-T cell data (the number of displayed data points has been reduced for clarity). The elastic foundation model constitutes a more accurate fit to the raw data than the Hertz–Sneddon model and provides a significant difference in determined modulus. It is relevant to note that the average elastic modulus obtained using the Hertz–Sneddon model (3.1 kPa) was significantly lower than the value produced with the elastic foundation model

associated with sharp tips that exceed the linear regime [23].

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## References

- Alcaraz, J., Nelson, C.M., Bissell, M.J.: Biomechanical approaches for studying integration of tissue structure and function in mammary epithelia. *J. Mammary Gland Biol. Neoplasia* **9**, 361–374 (2004)
- Costa, K.D.: Single-cell elastography: probing for disease with the atomic force microscope. *Dis. Markers* **19**, 139–154 (2003–2004)
- Wang, Y., Shyy, J.Y., Chien, S.: Fluorescence proteins, live-cell imaging, and mechanobiology: seeing is believing. *Annu. Rev. Biomed. Eng.* **10**, 1–38 (2008)
- Bhattacharyya, K., Guha, T., Bhar, R., Ganesan, V., Khan, M., Brahmachary, R.L.: Atomic force microscopic studies on erythrocytes from an evolutionary perspective. *Anat. Rec.* **279A**, 671–675 (2004)
- Kandori, T., Hayase, T., Inoue, K., Funamoto, K., Takeno, T., Ohta, M., Takeda, M., Shirai, A.: Frictional characteristics of erythrocytes on coated glass plates subject to inclined centrifugal forces. *J. Biomech. Eng.* **130**, 051007-1 (2008)
- Perry, S.S.: Scanning probe microscopy measurements of interfacial friction. *MRS Bull.* **29**, 478 (2004)
- Perry, S.S., Somorjai, G.A., Mate, C.M., White, R.L.: Friction and adhesion properties of hard carbon surfaces measured by atomic force microscopy. *Tribol. Lett.* **1**, 233 (1995)
- Sunyer, R., Trepatt, X., Fredberg, J.J., Farré, R., Navajas, D.: The temperature dependence of cell mechanics measured by atomic force microscopy. *Phys. Biol.* **6**, 025009 (2009)
- Yan, X., et al.: Reduction of friction at oxide interfaces upon polymer adsorption from aqueous solutions. *Langmuir* **20**, 423–428 (2004)
- Müller, M.T., Yan, X., Lee, S., Perry, S.S., Spencer, N.D.: Preferential solvation and its effect on the lubrication properties of a surface-bound, brush-like polymer. *Macromolecules* **38**, 3861 (2005)
- Müller, M.T., Yan, X., Lee, S., Perry, S.S., Spencer, N.D.: Lubrication Properties of a Brush-like Copolymer as a Function of the Amount of Solvent Absorbed within the Brush. *Macromolecules* **38**, 5706 (2005)
- Perry, S.S., Yan, X., Limpoco, F.T., Lee, S., Müller, M.T., Spencer, N.D.: Tribological properties of poly(l-lysine)-graft-poly(ethylene glycol) films: influence of polymer architecture and adsorbed conformation. *ACS Appl. Mater. Interfaces* **1**(6), 1224–1230 (2009)
- Ogletree, D.F., Carpick, R.W., Salmeron, M.: Calibration of frictional forces in atomic force microscopy. *Rev. Sci. Instrum.* **67**, 3298–3306 (1996)
- Johnson, K.L.: *Contact Mechanics*. Cambridge University Press, Cambridge, MA (1987)
- Dunn, A.C., Cobb, J.A., Kantzios, A.N., Lee, S.J., Sartinoranont, M., Tran-Son-Tay, R., Sawyer, W.G.: Friction coefficient measurement of hydrogel materials on living epithelial cells. *Tribol. Lett.* **30**, 13–19 (2008)
- Rennie, A.C., Dickrell, P.L., Sawyer, W.G.: Friction coefficient of soft contact lenses: measurements and modeling. *Tribol. Lett.* **18**, 499–504 (2005)
- Radmacher, M., Fritz, M., Kacher, C.M., Cleveland, J.P., Hansma, P.K.: Measuring the viscoelastic properties of human platelets with the atomic force microscope. *Biophys. J.* **70**, 556–567 (1996)
- Weisenhorn, A.L., Khorsandi, M., Kasas, S., Gotzos, V., Butt, H.-J.: Deformation and height anomaly of soft surfaces studied with an AFM. *Nanotechnology* **4**, 106–113 (1993)
- Park, S., Costa, K.D., Ateshian, G.A.: Microscale frictional response of bovine articular cartilage from atomic force microscopy. *J. Biomech.* **37**, 1679–1687 (2004)
- Bausch, A.R., Ziemann, F., Boulbitch, A.A., Jacobson, K., Sackmann, E.: Local measurements of viscoelastic parameters of adherent cell surfaces by magnetic bead microrheometry. *Biophys. J.* **75**, 2038–2049 (1998)
- Sneddon, I.: The relation between load and penetration in the axisymmetric boussinesq problem for a punch of arbitrary profile. *Int. J. Eng. Sci.* **3**, 47–57 (1965)
- Berdyeva, T.K., Woodworth, C.D., Sokolov, I.: Human epithelial cells increase their rigidity with ageing in vitro: direct measurements. *Phys. Med. Biol.* **50**, 81–92 (2005)
- Dimitriadis, E.K., Horkay, F., Maresca, J., Kachar, B., Chadwick, R.S.: Determination of elastic moduli of thin layers of soft material using the atomic force microscope. *Biophys. J.* **82**, 2798–2810 (2002)