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Short communication

Elastic modulus and hydraulic permeability of MDCK monolayers

K.D. Schulze [a], S.M. Zehnder [a], J.M. Urueña [a], T. Bhattacharjee [a], W.G. Sawyer [a,b], T.E. Angelini [a,c,d,*]

[a] Department of Mechanical and Aerospace Engineering, University of Florida, Gainesville, FL, United States
[b] Department of Material Science and Engineering, University of Florida, Gainesville, FL, United States
[c] J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville, FL, United States
[d] Institute for Cell Engineering and Regenerative Medicine, University of Florida, Gainesville, FL, United States

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ABSTRACT

The critical role of cell mechanics in tissue health has led to the development of many *in vitro* methods that measure the elasticity of the cytoskeleton and whole cells, yet the connection between these local cell properties and bulk measurements of tissue mechanics remains unclear. To help bridge this gap, we have developed a monolayer indentation technique for measuring multi-cellular mechanics *in vitro*. Here, we measure the elasticity of cell monolayers and uncover the role of fluid permeability in these multi-cellular systems, finding that the resistance of fluid transport through cells controls their force–response at long times.

1. Introduction

The material properties of the cytoskeleton and the cell as a whole correlate with cell behavior and tissue-level physiology (Discher et al., 2005). Numerous experimental methods for measuring the material properties of cells at local spatial scales exist. Measurements at sub-cellular length-scales have been performed by attaching super-paramagnetic beads to the cytoskeleton and applying a torque with a magnetic field (Bursac et al., 2005; Wang et al., 2002). Indentations have been performed on the actin cortex using atomic-force microscopes, also at the sub-cellular length-scale (Mahaffy et al., 2000; Sen et al., 2005). Whole cells have been stretched between micro-cantilevers (Fernández et al., 2006) and within optical traps (Guck et al., 2005). Some of these single-cell methods have been applied to cells in monolayers to gain insight into tissue-level multi-cellular mechanics (Trepat et al., 2006). Within tissues, cell groups are often under compression, and may exhibit collective mechanical responses different from those previously determined from local shear and tensile testing methods. Thus, *in vitro* measurements of cells under compression at the multicellular scale may reveal unexplored forces potentially at play within tissues.

Here we apply gentle, direct contact forces to Madin Darby Canine Kidney (MDCK) cell monolayers, compressing cell groups with steady forces and with no apparent cell damage. At short times, we determine an elastic modulus of 33.0 kPa, which drops to 15.6 kPa when the cytoskeleton is relaxed with blebbistatin. Over long times, the cells under the indenter compress slowly without translating, indicating that fluid driven out from under the contact at a rate limited by the monolayer's permeability. We combine Darcy's law with a contact mechanics model for thin layers to determine the monolayer permeability. These results show that while cell elasticity may dominate force–response in tissues at short times, dissipative resistance to fluid flow controls tissue response at long time-scales.

2. Methods and materials

2.1. Cell culture protocols

MDCK cells are cultured in Dublecco's modified Eagle's media supplemented with 10% fetal bovine serum and 1% penicillin streptomycin at 37 °C in a 5% CO_2 atmosphere. Monolayer islands, 3–5 mm in diameter, are spotted onto fibronectin coated, glass-bottomed culture dishes and fluorescently dyed with 5-chloromethyl-fluorescein diacetate (CMFDA). Detailed protocols for creating monolayer islands, fluorescent labelling, and several different pharmaceutical treatments employed in this study can be found in the Supplementary information.

2.2. In situ monolayer indentation

To perform tests on monolayers, we designed a micro-indentation system delicate enough to deform cells without damage, using a maximum force of 50 μN (Fig. 1). At this load, the contact width is about 250 μm, so the average pressure

* Corresponding author at: Department of Mechanical and Aerospace Engineering, University of Florida, 338 MAE-B, Gainesville, FL 32611, United States. Fax: +1 352 392 6565.
E-mail address: t.e.angelini@ufl.edu (T.E. Angelini).

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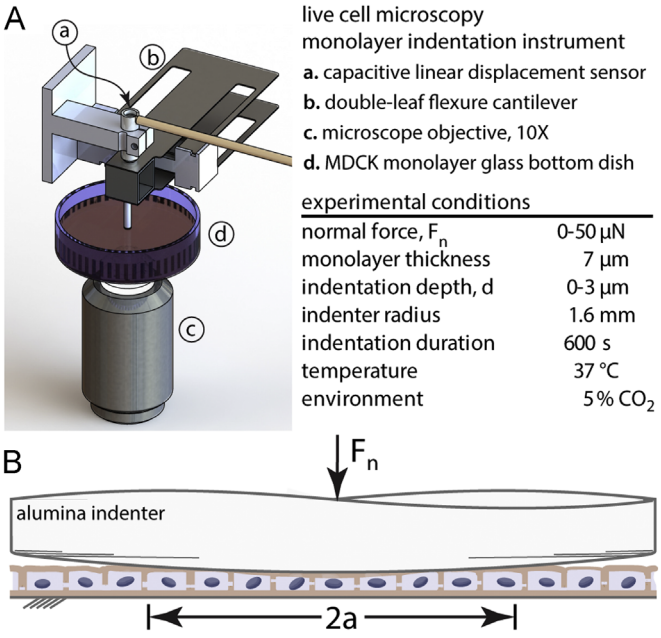

Fig. 1. (A) A micro-indenter is positioned over an inverted microscope and translated vertically with a piezo driven stage. Deflection of the double-leaf flexure is measured with a capacitive linear displacement sensor (3 nm resolution). A constant load is held with closed-loop feedback. (B) The 1.6 mm radius indenter presses into the 7 μm thick cell layer, applying a normal force, F_n, making a contact diameter 2*a*. The true disparity between the indenter radius and the monolayer thickness is much greater than illustrated here.

under contact is about 1 kPa, or roughly 1/10 the typical modulus of epithelial tissue (Fig. 2). We are able to repeatedly apply direct pressure to monolayers, hold a normal load, and retract without observing any cell damage (Fig. 2B–D). To eliminate adhesion to the cells, the indenter tip is coated in f-127 pluronic before each experiment. Each of the tests described below were performed on three different monolayers.

2.3. Indentation sequence for monolayer studies

To measure the monolayer response to contact forces, we rapidly ramp the applied load from 0 to 50 μN over a 10 s period, hold for 600–700 s, and remove the load by retracting the indenter. We see the indentation depth rise rapidly without any apparent lag as the force is ramped to 50 μN, then continue to rise slowly as the force is held – a behavior reminiscent of poroelastic materials under applied step-loads. Previously, we observed water passing between MDCK cells under low pressures over long times (Zehnder et al., 2015a, 2015c), further leading us to treat the monolayers as poroelastic in the analysis described here. To characterize poroelastic materials, experiments are typically split into three different regimes of mechanical response: short times, where no fluid can flow and the material is incompressible, having a Poisson's ratio of $\nu = ½$; long times where flow has stopped and equilibrium levels of compression are achieved; intermediate times where permeability limits the rate of indentation (Hu et al., 2010). At short times, since ν is known, $E^*=E/(1-\nu^2)=4/3E$, where E^* is the contact modulus and E is Young's modulus. This modulus is typically used again for analysis at long times to determine ν at equilibrium after flow has stopped. Here we follow the same protocol, but we do not attempt to determine ν because the internal cell architecture may evolve over very long times. Indentation tests with simultaneous imaging of the cytoskeleton are required to study the long time limit of a poroelastic interpretation of mechanical data.

3. Results

3.1. Thin slab contact mechanics of monolayers

At the start of the indentation protocol, the contact width, *a*, grows rapidly with indentation depth, *d*, due to the large disparity between the indenter radius (1.6 mm) and the monolayer thickness (7 μm). For example, *a* exceeds the layer thickness when *d* is just 15 nm. Thus, the 3D elasticity problem studied here falls within a thin slab limit that can be described by the Winkler elastic foundation model (Johnson, 1985). In this limit, for a spherical indenter pressing on a thin sheet of thickness *h* and contact modulus E^*, the relationship between normal force, F_n and indentation depth is given by $F_n = \pi E^* R h^{-1} d^2$. This relationship arises from the lateral confinement of local stresses by the rigid substrate; stresses are approximately uniform along the indentation direction and propagate laterally by the slab thickness, *h*. Consequently, this model will not apply to tests performed on soft substrates having an elastic modulus comparable to the cell layer.

To test whether this simple elastic slab model describes monolayer mechanics at short times, we examine the scaling between the *F* and *d* data-points during the first 10 s of indentation. We find that d^2 is proportional to *F*, and that data from different experiments can be collapsed onto a universal scaling curve when normalized by the constant system parameters (Fig. 3). For each measurement, E^* is determined by fitting the Winkler model to the data, with all other parameters fixed to their known quantities. Averaging across measurements performed on different monolayers, we find $E^* = 33.0 \pm 3.0$ kPa (mean ± standard deviation). Cell stiffness is linked to cytoskeletal pre-stress, driven by Myosin II, so to check whether the modulus measured here is driven by the same underlying mechanics, we repeat the experiments on cells drugged with 100 μM blebbistatin, a Myosin II inhibitor. We find $E^* = 15.6 \pm 5.5$ kPa for blebbistatin treated cells, about half that of untreated cells. At short times, when no fluid can flow, the effective Poisson's ratio is ½, allowing the Young's modulus, *E*, to be determined from E^*. We find $E =$ 24.8 kPa and 11.7 kPa for untreated and blebbistatin treated monolayers, respectively.

3.2. MDCK monolayer permeability

In poroelastic materials, fluid permeability controls the response to applied pressure over intermediate time-scales. Our measurements reveal that this time-scale for cell monolayers is on the order of hundreds of seconds for pressures in the kilopascal

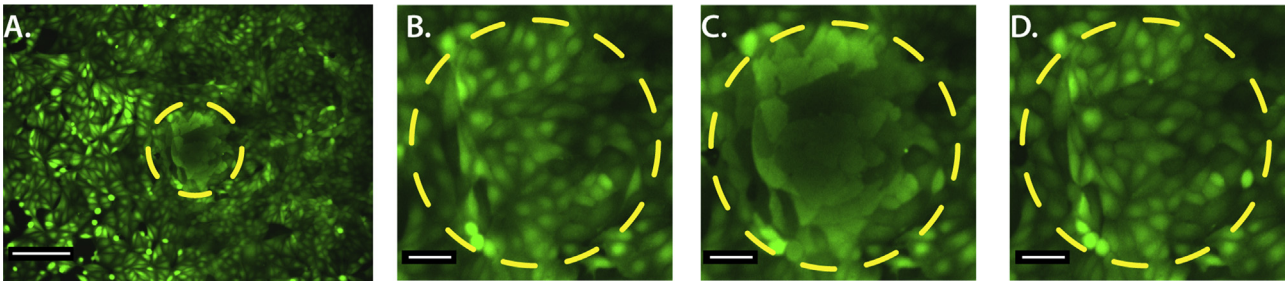

Fig. 2. (A) MDCK monolayer under persistent 50 μN load. Scale bar: 200 μm. Monolayer indentation viewed at higher magnification, before (B), during (C), and after indentation (D) is performed without apparent cell damage. Scale bar: 50 μm.