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Characterizing cellular mechanical phenotypes with mechano-node-pore sensing

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The mechanical properties of cells change with their differentiation, chronological age, and malignant progression. Consequently, these properties may be useful label-free biomarkers of various functional or clinically relevant cell states. Here, we demonstrate mechano-node-pore sensing (mechano-NPS), a multi-parametric single-cell-analysis method that utilizes a four-terminal measurement of the current across a microfluidic channel to quantify simultaneously cell diameter, resistance to compressive deformation, transverse deformation under constant strain, and recovery time after deformation. We define a new parameter, the whole-cell deformability index (wCDI), which provides a quantitative mechanical metric of the resistance to compressive deformation that can be used to discriminate among different cell types. The wCDI and the transverse deformation under constant strain show malignant MCF-7 and A549 cell lines are mechanically distinct from non-malignant, MCF-10A and BEAS-2B cell lines, and distinguishes between cells treated or untreated with cytoskeleton-perturbing small molecules. We categorize cell recovery time, ΔTr, as instantaneous (ΔTr < 40 ms), transient (ΔTr < 40 ms), or prolonged (ΔTr > 40 ms), and show that the composition of recovery types, which is a consequence of changes in cytoskeletal organization, correlates with cellular transformation. Through the wCDI and cell-recovery time, mechano-NPS discriminates between sub-lineages of normal primary human mammary epithelial cells with accuracy comparable to flow cytometry, but without antibody labeling. Mechano-NPS identifies mechanical phenotypes that distinguishes lineage, chronological age, and stage of malignant progression in human epithelial cells.

Keywords: microfluidics; mechanical phenotyping; node-pore sensing; biosensors; label-free

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INTRODUCTION
Cells derive their mechanical properties from the structure and dynamics of their intracellular components, including the cytoskeleton, cell membrane, nucleus, and other organelles—all of which, in turn, emerge from cell type-specific genetic, epigenetic, and biochemical processes. The ability to identify differences within a population of one cell type or different cells among heterogeneous populations, or to detect changes due to disease or environmental interactions all based on cellular mechanical properties has potentially important implications for cell and tissue biology and clinical metrics. As examples, metastatic potential1–4, cell-cycle5–4, differentiation state5–10, the outcome of tissue self-organization11, and infection with intracellular pathogens12,13 have all been shown to correlate with changes in cellular. Even the process of aging has been shown to affect the ability of cells within the vascular system and musculoskeletal system to recover from mechanical deformation14. Thus, methods to measure multiple cellular mechanical properties rapidly and accurately have tremendous potential as label-free research tools and diagnostics.

Atomic-force microscopy (AFM)15–17 and micropipette aspiration18,19 are the gold standard for performing mechanical measurements on cells. These methods provide controlled loading conditions (for example, stress relaxation and creep indentation) and quantify such cellular properties as elastic modulus and cortical tension. They are, however, burdened by slow throughput, capable of analyzing only just a few cells per hour7,20, although recent adaptations of both methods have demonstrated higher throughput via more efficient analysis21,22. Likewise, optical tweezers23,24 and micropipette rheometry25—two other well-established methods to measure cellular mechanical properties—also suffer from low throughput. Given these drawbacks, a number of microfluidic platforms have consequently been developed, including hydrodynamic stretching cytometry26–28, suspended microchannel resonators (SMR)29, and real-time deformability cytometry (RT-DC)30, to name only a few. Each of these methods, through optical imaging or measuring changes in resonant frequencies, can analyze populations of cells in a relatively short time (for example, 2000–65 000 cells per for hydrodynamic stretching cytometry26–28, 30 cells per s for SMR29, and 100 cells per s for RT-DC30). To identify specific cell types, these methods most often focus on correlating cell size or mass with a specific mechanical property. For example, hydrodynamic stretching cytometry and RT-DC compare cellular deformability with cell size, and SMR determines the transit time of cells through a narrow channel with respect to cell mass. Populations of cells are...
complex with respect to the continua of cell states that are represented within, and as such, multiple biophysical parameters are necessary to deconvolve and identify complex cellular mixtures. Recently, Masaee et al.\textsuperscript{31,32} and Lin et al.\textsuperscript{31,32} have reported using deformability cytometry to measure multiple parameters, such as cell size, shape, and relaxation rate, while cells undergo deformation. In so doing, they were able to identify different cellular states associated with pluripotent and neural stem-cell differentiation, respectively. While this achievement emphasizes the need for measuring multiple biophysical parameters to identify specific cell types, Masaee et al.\textsuperscript{31} and Lin et al.\textsuperscript{31,32} focus on defining cellular phenotypes only while cells undergo deformation. Since overall recovery of a cell once released from deformation plays significant roles in cellular migration processes such as cancer metastasis\textsuperscript{34} and in providing a protective mechanism of cells against mechanical damage\textsuperscript{24-26} it is imperative for mechano-phenotyping platforms to have a temporal window sufficient enough to analyze the recovery that a cell undergoes after deformation.

Here, we describe a novel microfluidic platform called ‘mechano-Node-Pore Sensing’ (mechano-NPS). Mechano-NPS involves integrating a node-pore sensor\textsuperscript{37,38} with a contraction channel and performing a four-terminal measurement of the current across the integrated microfluidic channel to quantify four biophysical properties of a single cell, simultaneously: diameter, recovery from deformation. This electronic-based method of multi-dimensional mechanical phenotyping provides the means to use these biophysical parameters as label-free biomarkers for identification and differentiation among cell types and, uniquely, to determine the effects of chronological age and malignant progression on cell elasticity and recovery from deformation. Mechano-NPS distinguishes malignant from non-malignant immortal epithelial cells and measures deformability changes in the cytoskeleton. In addition, mechano-NPS can discriminate between sub-lineages and among chronological age groups of primary normal human mammary epithelial cells (HMECs) based solely on their mechanical properties. Mechano-NPS represents an efficient, simple, and direct means to quantify multiple mechanical properties of single cells in heterogeneous populations.

MATERIALS AND METHODS

Experimental design

The platform consists of a 30 μm-high microfluidic channel embedded in a polydimethylsiloxane (PDMS) mold bonded to a glass substrate with pre-defined platinum (Pt) electrodes and gold (Au) contact pads (Figure 1a). The central part of the channel, which we refer to as the ‘contraction channel’, is long (2055 μm) and narrow (10 or 12 μm-wide) and flanked on either side by a series of nodes and pores that are 85 and 25 μm wide, respectively.

Figure 1 Principle of mechanical phenotyping via mechano-NPS. (a) A photographic image of the microfluidic platform. The scale bar corresponds to 4 mm. Red-dashed box shows a close-up view of the entire microfluidic channel. The microfluidic channel (pore) is segmented by nodes and a contraction channel. Two electrodes at both ends of the channel apply a constant voltage (1 V), and two inner electrodes measure the change of current across the channel. The regions where free-cell diameter, deformed diameter, and cell recovery are measured are as indicated. (b) Expected current pulse generated by a cell transiting the microfluidic channel. $I$, $\Delta I_{np}$, $\Delta I_c$, and $\Delta I_r$ correspond to the baseline current and the current drop by a cell transiting a node-pore, a contraction channel, and a node-pore after the contraction channel, respectively. Numbers in parentheses (1–4) correspond to the same specific segments of the microchannel (pore, node, and contraction channel) in (a). $\Delta T_{node}$ corresponds to the time duration of a cell passing through the contraction channel, and $\Delta T_r$, indicates the time needed for $\Delta I_r$ to equal $\Delta I_{np}$ (see Supplementary Figure S6 for detailed information). (inset) An actual current pulse caused by a human mammary epithelial cell traversing the channel. (c) Time-snapshots of an MCF-7 cell (bordered by a white circle) in each of the different segments of the microfluidic channel (white dashed line; see Supplementary Videos 1 and 2 for detailed information). Numbers in parentheses (1–4) correspond to the same specific segments of the microchannel (pore, node, and contraction channel) in (a). (d) Cross-sectional diagram of the channel segments occupied by a cell. ‘AA’ and ‘BB’ indicate the corresponding cross-sections in (c). $W_{pore}$, $W_{node}$, $W_{c}$, and $h_{channel}$ correspond to the widths of the pore, node, and the contraction channel, and the height of the channel, respectively. $D_{cell}$ and $L_{deform}$ correspond to the free-cell diameter in the node-pore channel and the elongated length of the deformed cell in the contraction channel, respectively.
(Figure 1a, inset). The length of the contraction channel was chosen to provide sufficient time (~30 ms) over which a cell experiences constant applied strain. The node and pore dimensions were chosen for sufficient signal-to-noise ratios. Given the flexibility and ease of device design and fabrication, different contraction channel lengths and node and pore dimensions could be employed. Filters that are 25 μm in width (the width chosen based on the size range of cells measured in these studies, approximately 15–20 μm in diameter) are included at the entrance of the microfluidic channel in order to remove cellular clusters that may otherwise clog the device. Applying a constant DC voltage (1 V) across the channel, we employ a four-terminal measurement technique37–40 to measure the current pulses caused by cells transiting across the microfluidic channel when a non-pulsatile pressure of ~21 kPa (determined by a commercial pressure gauge, SSI Technologies) is utilized (Figure 1b and Supplementary Figure S1). After low-pass filtering all current versus time data, we employ custom-written software to extract both the magnitude and duration of each current sub-pulse (∆I, ∆I, ∆τ, and ∆T, in Figure 1b) (Supplementary Figure S2).

Power analysis was employed to ensure that our sample size for mechanical phenotyping offers adequate power (≥80) to detect differences between experimental groups within a 95% confidence interval41 from the measured data set. For all cases which have a P-value < 0.05, the analyzed sample size (N) provided sufficient power value to measure statistical differences (Supplementary Table S1). Statistical significance was determined by performing a paired t-test or χ²-test. To ensure repeatability of results, all data presented in this study were measured using multiple microfluidic devices. The wCDI of MCF-7 cells obtained with different device replicas showed no statistical difference (Supplementary Figure S3, P = 0.173).

Device fabrication
To make the PDMS molds of our microfluidic platform, we employ standard soft-lithography. Briefly, we fabricate negative-relief masters onto polished silicon wafers. After mixing and degassing, we pour a 9:1 pre-polymer: curing agent mixture of PDMS (Sylgard 184, Dow Corning) onto the masters and subsequently cure them at 80 °C for 60 min. A slab of PDMS with the embedded microfluidic channel is excised from the master, and entry and exit ports are cored with a 1 mm diameter biopsy punch. To complete the device, we first expose the PDMS mold and a glass substrate with pre-defined electrodes to an oxygen plasma (470 mTorr, 80 W, 1 min), then align and mate the two together, and finally place the device onto a hotplate set to 80 °C for 60 min. For the specific surface-treatment experiments described, we injected either poly-D-lysine (PDL, 1 μg mL⁻¹ in PBS) or bovine serum albumin (BSA, 2% w/v in PBS) into the completed device. After incubating for 2 h at 37 °C, we flushed the device with PBS and immediately began screening cells.

To fabricate the Pt electrodes and the Au contact pads onto the patterned substrates, we utilize standard photolithography for patterning. Using electron-gun evaporation, we deposit a 75-250 Å Titanium (Ti)/Pt/Au thin film onto the patterned substrates. We then use a gold wet etch (GOLD ETCHANT TFA, Transene Company) to expose the Pt electrodes.

Cell culture
MCF-10A cells (ATCC CRL-10317) were cultured in MEBM medium, supplemented with 0.1% insulin, 0.1% hEGF, 0.4% hydrocortisone, and 10% cholaoroxin. MCF-7 cells (ATCC HTB-22) were cultured in DMEM (Fisher Scientific, BW12719F), supplemented with 10% fetal bovine serum (FBS), 0.1 mM MEM Non-Essential Amino Acids (NEAA), 2 mM L-glutamine, and 1% Pen-Strep. BEAS-2B cells (ATCC CRL-9609) were cultured in BEGM BulletKit (Lonza, CC-3170). A549 cells (ATCC CRM-CCL-185) were cultured in F-12K medium (Fisher Scientific, MT10025CV), supplemented with 10% FBS and 1% of Pen-Strep. Jurkat cells (ATCC TIB-152) were cultured in RPMI 1640 medium (Thermo Fisher Scientific, Grand Island, NY USA), supplemented with 10% fetal bovine serum (FBS), and 1% Pen-Strep. All cell cultures were maintained at 37 °C in 5% CO₂ and routinely passaged, per published protocols42,43, once they reached 80% confluence.

Cells were dissociated by treatment with 0.25% trypsin/EDTA for either 3 min (MCF-7 and A549 cells) or 5 min (MCF-10A and BEAS-2B cells) at 37 °C (Refs. 44–46), washed with the respective growth media, centrifuged at 0.2 RCF, and re-suspended at a concentration of ~20 000 cells per mL in PBS. To ensure cell viability, cells were injected into the prepared devices for screening immediately following re-suspension.

Primary human mammary epithelial cells
Primary HMEC strains were generated and maintained as described previously37,48. HMECs were grown in M87A medium containing cholaoroxin and oxytocin at 0.5 ng mL⁻¹ and 0.1 nM, respectively. Details on the derivation and culture of these HMEC can be found at Human Mammary Epithelial Cell (HMEC) Bank Website49. Research was conducted under Lawrence Berkeley National Laboratory Human Subjects Committee IRB protocols 305H002 and 108H004, which allows for the use of HMEC samples for future scientific research.

Pharmacological inhibition of cytoskeletal components
We disrupted actin polymerization with Latrunculin A and B (Enzo Life Sciences, Farmingdale, NY USA)50. Prior to deformability measurements, MCF-7 and MCF-10A cells were incubated with 2.5 or 5 μg mL⁻¹ LatA or LatB in each cell’s respective growth medium for one hour at 37 °C and 5% CO₂ (Refs. 29,51,52). Cells were then released from culture flasks with 0.25% trypsin/EDTA, rinsed once with PBS, centrifuged at 0.2 RCF, and re-suspended in PBS at a concentration of ~100 000 cells per mL. To confirm that actin polymerization was successfully inhibited after incubation, cells were fixed by 4% (w/v) paraformaldehyde in PBS for 15 min. They were then permeabilized with 0.1% Triton-X 100 (Sigma-Aldrich, St. Louis, MO, USA) in PBS for 5 min. Cell nuclei and F-actin were then counter-stained with 4’,6-diamidino-2-phenylindole (DAPI, Sigma-Aldrich, 10236276001) and rhodamine phalloidin (Thermo Fisher Scientific, R415), respectively, per manufacturer’s protocol, and then imaged with a Zeiss LSM710 confocal microscope.

Discriminating cell types based on the whole cell deformability index (wCDI)
We derived a dimensionless parameter, which we refer to as the whole cell deformability index (wCDI), to distinguish cell populations based on mechanical phenotype. We assume a functional relationship among the biophysical parameters of a cell and fluid flow as follows,

\[ F(E, D_{cell}, h_{channel}, U_{flow}, \Delta T_{cont}, \mu, L_c) = 0 \]  

where \( F \), \( D_{cell} \), \( h_{channel} \), \( U_{flow} \), \( \Delta T_{cont} \), \( \mu \), and \( L_c \) correspond to elastic modulus, free cell diameter, height of the microfluidic channel, flow velocity within the node segment leading to the contraction channel, the transit velocity of cells in the contraction channel, fluid viscosity, and the length of the contraction channel, respectively. Three fundamental dimensions (\( n = 3 \))—mass (M), length (L), and time (T)—are included in each of these six parameters (\( n = 7 \)) as follows,

\[ E = [ML^{-1}T^{-2}] \]  

\[ D_{cell} = [L] \]


\[ h_{\text{channel}} = |L| \]  

(2c)

\[ U_c = |LT^{-1}| \]  

(2d)

\[ U_{\text{flow}} = |LT^{-1}| \]  

(2e)

\[ \mu = [ML^{-1}T^{-1}] \]  

(2f)

\[ L_c = |L| \]  

(2g)

Following the Buckingham \( \pi \) theorem\textsuperscript{53}, the relationship among these parameters can be written in terms of a set of four dimensionless parameters \((n' - n = 4)\). To find these dimensionless parameters \((n_1; i = 1, 2, 3, \text{ and } 4)\), we select repeating variables \((h_{\text{channel}}, U_{\text{flow}}, \text{ and } \mu)\), where the number of required variables is equal to the number of fundamental dimensions \((n = 3)\). Multiplying one of the nonrepeating variables with the product of the repeating variables, we can define the following \( n \) terms,

\[ \eta_1 = \frac{h_{\text{channel}} E}{U_{\text{flow}} \mu} \]  

(3a)

\[ \eta_2 = \frac{h_{\text{channel}}}{\Delta T_{\text{cont}} U_{\text{flow}}} \]  

(3b)

\[ \eta_3 = \frac{D_{\text{cell}}}{h_{\text{channel}}} \]  

(3c)

\[ \eta_4 = \frac{L_c}{h_{\text{channel}}} \]  

(3d)

We define the dimensionless parameter, wCDI (Equation (6)), to be the product of \( n_1 \times n_2 \times n_3 \times n_4 \). The wCDI could also be defined as a function of \( n_1 \) in which \((n_1 = h(n_2, n_3, n_4))\), but the exact analytical expression can only be obtained by experiment\textsuperscript{59}. Comparing the wCDI with cellular cortical tension and the previously reported elastic modulus \( E \) of various cell lines (Supplementary Figure S4), we experimentally determined that the wCDI is inversely related to these traditional parameters.

### Cortical tension measurement using micropipette aspiration

Cortical tension was measured by micropipette aspiration as described previously\textsuperscript{54,55}. Briefly, cells were trypsinized and resuspended in growth medium, and were transferred to the imaging chamber. Suction pressures in the range of 0.03–0.3 kPa were applied to the cells through an 8–10 \( \mu \)m glass micropipette. At each pressure, the cellular deformation inside the pipette was allowed to stabilize for 20–30 s before imaging. The average measurement from three images was used to calculate the length of deformation \( L_d \). Subsequently, applied pressure was increased in 0.03 kPa increments till the \( L_d \) exceeded the radius of the pipette \( (R_p) \). Any cell that blebbed was discarded. The critical pressure \( P_{\text{crit}} \) is defined as the pressure at which the deformation inside the pipette is hemispherical, that is, \( L_d = R_p \). The cortical tension \( T_{\text{crit}} \) was then calculated using the following equation, where \( R_c \) is:

\[ \Delta P_{\text{crit}} = 2T_{\text{crit}} \times \frac{1}{R_p} \times \frac{1}{R_c} \]  

(4)

The cortical tension measurements from Jurkat, NIH 3T3, and HeLa cells are plotted from Schifffauer et al.\textsuperscript{56}.

### RESULTS

Population characterization of mechanical phenotypes at single-cell resolution

The repeated expansion and contraction of the width of our overall microfluidic channel shown in Figure 1a produces a unique and symmetric current pulse, consisting of sub-pulses, for each cell that transits the channel. Upon entering the microfluidic channel, a cell partially blocks the flow of current, and consequently, the measured current immediately drops from a baseline value, \( I \) (Figure 1b). When the cell enters the first node, the current returns to baseline only to drop again once the cell exits that node. This is a hallmark of node-pore sensing (NPS)\textsuperscript{37,38}. The rise and fall of current repeats as the cell enters and exists the next two nodes. Upon entering the contraction channel where the width is narrower than the diameter of the cell, the cell deforms as shown in Figures 1c and d. Because the cell blocks nearly all of the current flow in this part of the channel, the current drop from baseline is far more dramatic than that resulting from the cell transiting the earlier pores (Supplementary Figure S5 and Supplementary Video 1). The cell subsequently enters and exits a series of node-pore pairs following the contraction channel, ultimately leading to the symmetrical shape of the overlap current pulse. This symmetry is intentional by design and critically allows the monitoring of a cell’s recovery from constant strain deformation (Supplementary Video 2).

The magnitude of the current sub-pulse produced in the node-pore sequence \( (\Delta n_{\text{np}}) \) and the contraction channel \( (\Delta L) \) corresponds to the free-cell diameter \( (D_{\text{cell}}) \) and cell elongation length \( (L_{\text{deform}}) \), respectively (Figure 1d). The relationship among the current drop \( (\Delta I) \), baseline current \( (I) \), particle diameter \( (d) \), the overall channel length \( (L) \), and the channel’s effective diameter \( (D_e) \) is defined as\textsuperscript{39,57,58}:

\[
\Delta I = I \left[ \frac{d^3}{D_e^2} \left( 1 - 0.8 (d/D_e) \right) \right]
\]

To determine \( D_e \), we measure polystyrene microspheres of known size with the microfluidic channel (Supplementary Table S2). Using the values of \( \Delta I/I \) arising from the microspheres, along with the known values of \( L \) and \( d \) (the size of the microspheres in this instance), we can numerically solve for \( D_e \) in Equation (5). Once \( D_e \) is known, we can subsequently determine \( D_{\text{cell}} \) of a screened cell by numerically solving for \( d \) in Equation (5) using the obtained values of \( \Delta n_{\text{np}}/I \). We can also determine the volume of the deformed cell, \( V_{\text{deform}} \), by the relationship\textsuperscript{39,57,58}, \( \Delta L/I = V_{\text{deform}}/V_{\text{contraction}} \), where \( V_{\text{contraction}} \) is the volume of the contraction channel. To calculate \( D_{\text{deform}} \), we assume the cell undergoes an isometric deformation in the direction of both the channel’s longitudinal axis and channel height, resulting in an oblate-spheroid shape. From the relationship between the volume and major radius of the oblate spheroid, \( V_{\text{deform}} = \pi w_c L_{\text{deform}}^2 / 6 \) where \( w_c \) is the contraction-channel width, we can determine \( D_{\text{deform}} \) from \( \Delta L/I \). We quantify the transverse deformation of the cell, \( L_{\text{deform}} = L_{\text{deform}}/L_{\text{cell}} \) as it transits the contraction channel.

As a cell traverses through each section of the channel, the duration of the resulting sub-pulse produced by a cell corresponds to the cell’s transit time \( (\Delta T) \) through that part of the channel. To quantify the resistance to compressive deformation, we utilize \( \Delta T_{\text{cont}} \). To determine the recovery time of a cell from compressive deformation \( (\Delta T_r) \), we note the time required for the sub-pulses produced by the cell after exiting the contraction channel to return to the same shape and magnitude as those produced by the cell prior to entering the contraction channel, that is, when the cell returns to its original size and shape (Figure 1b and Supplementary Figure S6). Given the number of node-pore pairs and the overall length of the node-pore sequence we employ after the contraction channel, our device’s temporal window for...
measuring cell recovery is 40 ms. The flexibility of our device design and ease of fabrication allow for the inclusion of many more node-pore pairs after the contraction channel, which in turn would lead to an increase in time over which to observe recovery (Methods: Experimental Design). Based on all the recovery times we recorded with our particular device, we discriminate among three different cell-recovery types—instant ($\Delta T_{\text{cont}} \sim 0$ ms), transient ($0 < \Delta T_{\text{cont}} \leq 40$ ms), and prolonged ($\Delta T_{\text{cont}} > 40$ ms) (Supplementary Figure S6).

Thus, from just a single current pulse produced by a cell transiting through the entire microfluidic channel, four biophysical properties of that cell—size ($D_{\text{cell}}$), resistance to compressive deformation ($\Delta U_{\text{cont}}$), transverse deformation ($\Delta U_{\text{trans}}$), and recovery from deformation ($\Delta U_{\text{rec}}$)—are extracted. These parameters are what we collectively use to mechanically phenotype a single cell, distinguish among cell types in a heterogeneous population, and determine subtle cellular changes.

Distinguishing malignant and non-malignant epithelial cell lines based on mechanical phenotyping

We investigated whether mechano-NPS could distinguish between immortal malignant and non-malignant states in two different epithelial tissue types based on their mechanical properties alone. We compared the mechanical properties of malignant MCF-7 with non-malignant MCF-10A breast epithelial cells and malignant A549 with non-malignant BEAS-2B lung epithelial cells when individual cells were subjected to a constant applied strain along the length of the contraction channel they traversed. Because strain, $\varepsilon$, is a function of both cell size and contraction channel width ($w_{\text{ch}}$), $\varepsilon = (D_{\text{cell}} - w_{\text{ch}})/D_{\text{cell}}$ and prior independent measurement of $D_{\text{cell}}$ showed that malignant MCF-7 and A549 cells are, on average, larger than non-malignant MCF-10A and BEAS-2B cells (Supplementary Table S3), we utilized a 12 μm-wide contraction channel to measure MCF-7 and A549 cells and a 10 μm-wide contraction channel to measure MCF-10A and BEAS-2B in order to achieve the same average $\varepsilon$ (~0.3) for all cell types (Supplementary Table S3). As shown in the four dimensional (4D) graphs in Figure 2a, $D_{\text{cell}}$ and $U_{\text{defom}}$ of MCF-10A and BEAS-2B cells are significantly different from those of MCF-7 and A549 cells, respectively. Moreover, MCF-10A and BEAS-2B cells transit the contraction channel more slowly as compared to MCF-7 and A549 cells, respectively. When comparing transverse deformation ($\Delta U_{\text{trans}}$), we find that while A549 deform significantly less than BEAS-2B cells, MCF-7 and MCF-10A cells have similar deformation (Figure 2b).

Although our results clearly show that the transit time through the contraction channel ($\Delta T_{\text{cont}}$) is dependent on cell type (that is, malignant vs. non-malignant), so too could cell diameter affect transit time (Figure 2a)48–61. Because this could lead to difficulties in distinguishing cells within a heterogeneous population (Supplementary Figure S7), we employ the Buckingham $\pi$-technique$^{53}$ to define a new dimensionless parameter, the whole-cell deformability index (wCDI), which relates $D_{\text{cell}}$ and $\Delta T_{\text{cont}}$ by the following:

$$wCDI = \frac{L_c}{U_{\text{flow}} h_{\text{channel}}} \frac{D_{\text{cell}}}{\Delta T_{\text{cont}}}$$

(6)

where $U_{\text{flow}}$ is the fluid velocity in the node section leading into the contraction channel, $L_c$ is the length of contraction channel, and $h_{\text{channel}}$ is the contraction-channel height (see detailed information in Methods: discriminating cell types based on the wCDI). $U_{\text{flow}}$, $L_c$ and $h_{\text{channel}}$ are fixed values for any given experiment, and consequently, $D_{\text{cell}}$ and $\Delta T_{\text{cont}}$ become the key parameters. Physically, the wCDI describes the deformability of the cell as a whole, including the cytoskeleton, nucleus, and organelles. Cells that are more deformable (that is, less stiff) transit through the contraction channel more easily, and subsequently at higher velocities, than those that are less deformable (that is, more stiff). Correspondingly, these cells will have a higher wCDI as compared to the latter, in accordance with Equation (6). Moreover, cells which are larger (smaller) will transit the contraction channel more slowly (quickly), and Equation (6) effectively negates this cell-size effect. While the Buckingham $\pi$-technique relates the wCDI to the cell’s elastic modulus, $E$, (see Methods, Equation (3a)), it does not define the explicit relationship between the two. We, therefore, performed side-by-side measurements of different cell lines (Jurkat, MCF-7, and MCF-10A) with the gold standard, micropipette aspiration, and also compared our measurements of MCF-7, MCF-10A, A549, and BEAS-2B cell lines with those obtained by AFM in the published literature$^{15,37,62–67}$.

Our results and subsequent analysis (Supplementary Figure S4) show that the wCDI is inversely proportional to both cortical tension and $E$, confirming our original physical description of the wCDI. While future studies are necessary to determine the exact analytical expression between the wCDI and $E$, mechano-NPS’s ability to mechanically phenotype cells successfully for cell-type discrimination is clearly demonstrated.

Figure 2c shows the wCDI distribution of non-malignant vs. malignant cells. The solid lines correspond to the fitted normal distribution of each population and the red-shaded region is the overlap area of the two distributions. As shown in Figure 2c, the wCDI of MCF-7 cells is significantly greater than that of MCF-10A cells with a 2.6% overlap. Similarly, A549 cells have a greater numerical wCDI than BEAS-2B cells, but with only a 1.6% overlap. Given the sensitivity demonstrated using the wCDI vs. $\Delta T_{\text{cont}}$ or cell size, alone (Figure 2c vs. Supplementary Figure S7), mechano-NPS and correspondingly the wCDI could potentially be utilized as a method for detecting subtle heterogeneities within cell populations such as those found in primary tissue$^{68,69}$, heterogeneous cell lines and strains$^{70}$, and biopsied tissue samples$^{71,72}$.

Clear differences were observed in the recovery time after mechanical strain between breast and lung epithelial cell lines and, in the case of the latter, between malignant and non-malignant cell lines. Figure 2d shows that there was no statistical difference (using a Chi-square Analysis) regarding instantaneous recovery from mechanical deformation among breast epithelial cells (38.3% malignant MCF-7 cells vs. 50% MCF10-A cells, $P = 0.101$). This is in striking contrast to lung epithelial cells in which there was a strong statistical difference ($P < 0.0001$) between malignant and non-malignant cell lines: 37.0% of malignant A549 cells recovered instantaneously vs. 82.0% of non-malignant BEAS-2B cells screened (Figure 2d). Even though both are malignant cell lines, MCF-7 and A549 cell populations show surprising differences in their composition of transient and prolonged cell-recovery types. Whereas the majority of screened A549 cells transiently recovered (53.0%), MCF-7 cells were nearly evenly divided between transient and prolonged recovery (38.3% and 47.5%, respectively).

Evaluating the contribution of cell-surface interactions and the cytoskeletal component, F-actin, to the mechanical phenotypes measured

To determine whether cell-surface interactions greatly affect the passage of a cell within the contraction channel, and in turn contribute significantly to its wCDI, we screened MCF-7 cells in channels coated with either PDL or BSA and compared the resulting wCDI with that obtained by screening with a bare-PDMS channel (Figure 3a). PDL increases cell-surface interactions by adding positive charges on the PDMS channel walls$^{73,74}$ and would therefore lead to a lower wCDI. In contrast, BSA inhibits cellular adhesion to the PDMS surface$^{75}$ and would result in a higher wCDI. Figure 3b compares the wCDI obtained when MCF-7 cells were measured with bare-PDMS and PDL- and BSA-coated channels at different inlet pressures, that is, flow speeds. At low
their respective controls (Figure 3d), with MCF-7 cells generally treated with LatA and LatB was significantly reduced compared to the bare-PDMS control channel. At magnitudes, (Figure 3c), and subsequently screened them under a strain perturbations. Thus, we treated MCF-7 and MCF-10A cells with the based on mechanical differences, we should detect cytoskeletal surface-interactions do contribute to the wCDI, they are not the epithelial cell types (Figure 2b). We, therefore, conclude that while difference in wCDI among the different surface treatments vs. the that measured with the bare-PDMS control channel. Moreover, the either the PDL- or BSA-coated channel is not a dramatic shift from As shown in Figure 3b, the obtained wCDI at this inlet pressure for surface interactions are minimized within the contraction channel. experiments, cells

Because we propose that mechano-NPS distinguishes cells Because we propose that mechano-NPS distinguishes cells between untreated and treated cells regarding instantaneous recovery ($P \leq 0.0001$) and transient recovery ($P \leq 0.0001$).

pressures ($P_{\text{inlet}} = 7$ kPa and 14 kPa), the average wCDI is appreciably lower in the PDL-coated channel and higher in the BSA-channel as compared to the bare-PDMS control channel. At $P_{\text{inlet}} = 21$ kPa, the inlet pressure at which we performed all our experiments, cells flow at a sufficiently high enough rate that cell-surface interactions are minimized within the contraction channel. As shown in Figure 3b, the obtained wCDI at this inlet pressure for either the PDL- or BSA-coated channel is not a dramatic shift from that measured with the bare-PDMS control channel. Moreover, the difference in wCDI among the different surface treatments vs. the bare-PDMS control channel at 21 kPa inlet pressure is significantly less than that measured between malignant and non-malignant epithelial cell types (Figure 2b). We, therefore, conclude that while surface-interactions do contribute to the wCDI, they are not the dominant factor at the higher inlet pressures or flow rates used for these studies.

Because we propose that mechano-NPS distinguishes cells based on mechanical differences, we should detect cytoskeletal perturbations. Thus, we treated MCF-7 and MCF-10A cells with the actin polymerization inhibitors, Latrunculin A (LatA) or B (LatB) (Figure 3c), and subsequently screened them under a strain magnitude, $\varepsilon_{\text{avg}} \approx 0.3$. We found that the cellular deformation in the transverse direction ($\Delta_{\text{deform}}$) of both MCF-7 and MCF-10A cells treated with LatA and LatB was significantly reduced compared to their respective controls (Figure 3d), with MCF-7 cells generally more so than MCF-10A cells. Furthermore, we found that the wCDI increased for both LatA- and LatB-treated MCF-7 cells, and for LatA-treated MCF-10A cells as compared to the untreated control cells (Figure 3e). In subsequent experiments, we observed that the change in wCDI caused by LatA treatment correspondingly increased with concentration for both MCF-7 and MCF-10A cells, with the latter more sensitive to the treatment (Supplementary Figure S8). This is in contrast, however, to no detectable change in wCDI of MCF-10A cells no matter the LatB concentration. Overall, the different response of MCF-7 and MCF-10A cells to LatA and LatB may be due to differences in F-actin content, but further experiments are warranted here. As we confirmed with staining and confocal microscopy that the F-actin filaments were indeed inhibited in the Lat A- and B-treated cells (more so with Lat-A than with Lat-B as shown in Figure 3c), we conclude that mechano-NPS successfully detects cytoskeletal perturbations induced by exogenous chemicals.

While differences between the wCDI of LatA-treated cells are more pronounced with MCF-10A cells than MCF-7 cells, the differences in recovery time for Lat A- and LatB-treated cells in both cell types vs. the control are far more significant. Figure 3f shows that Latrunculin treatment results in the slow recovery of both MCF-7 and MCF-10A cells from the sudden relief of deformation. Moreover, there is a statistically significant difference between untreated and treated cells regarding
recovery. In the case of MCF-7, only 8.1% of LatA-treated and 24.2% of LatB-treated cells instantaneously recover vs. 38.3% of untreated cells. For MCF-10A, the majority of LatA- and LatB-treated cells (66.7% and 41.4%, respectively) do not recover within the 40 ms time window our device offers (vs. 9.7% of untreated control cells). As we also found, the changes in cellular recovery are generally more pronounced at higher concentrations of Latrunculin treatment (Supplementary Figure S8). These results support the notion that actin filaments contribute to the ability of cells to retain their original shape. Moreover, mechanochip detects differences in recovery from deformation, either transiently or not at all, between LatA- and LatB-treatment that are consistent with LatA being the more avid inhibitor of actin polymerization.

**Mechanical phenotyping of human mammary epithelial cells**

To determine whether our platform could discriminate different lineages within a population of primary epithelial cells, we screened the mechanical phenotypes of HMECs, which broadly consist of two lineages: myoepithelial (MEP) cells and luminal epithelial (LEP) cells (Figure 4a). MEP and LEP cells have distinct roles in breast tissue. MEP cells play active roles in ductal contraction and in tumor suppression, and LEP cells produce milk and may represent a target-cell-type for carcinogenesis. Previous studies of mammary epithelia have implicated profound roles of cytoskeletal components in morphogenesis. We measured the mechanical characteristics of these two lineages of cells. Since both MEP and LEP cells have a similar size range (Supplementary Figure S7), we employed a 10 μm-wide contraction channel, which allowed us to discriminate between the two lineages.

**Figure 3** Contribution of cell-surface interaction and cytoskeletal component, F-actin, to the mechanical phenotypes of epithelial cells. (a) Schematic of experimental conditions used to measure the effects of cell-surface interaction on the wCDI. While poly-D-lysine (PDL) increases the positive charges on the channel wall for increased cell-surface interaction, bovine serum albumin (BSA) minimizes cellular adhesion to the channel wall. The control for all experiments was bare PDMS. (b) The difference in wCDI measured when MCF-7 cells transit a bare PDMS contraction channel (control) and a PDL-coated channel or a BSA-coated channel (n=99 for all cases) under various fluidic conditions. The difference in wCDI becomes smaller with greater Prel. Within each box, the central red line corresponds to the median, and the edges of the box to 25% and 75% of the population. (c) Fluorescence images of MCF-7 and MCF-10A cells after treatment with Latrunculin A (LatA, 5 μg mL⁻¹, 1 h) or Latrunculin B (LatB, 5 μg mL⁻¹, 1 h). Cell nuclei and F-actin are stained with 4,6-diamidino-2-phenylindole (DAPI, blue) and rhodamine Phalloidin (red), respectively. Scale bar corresponds to 20 μm. (d) Transverse deformation (Δdeform) of untreated, LatA-, and LatB-treated MCF7 and MCF10A cells (n=99). Statistical differences were determined by a paired t-test. (e) wCDI distribution of MCF-7 (n=99, Ctrl vs LatA: P=0.0074, Ctrl vs LatB: P=0.0253) and MCF-10A cells (n=99, Ctrl vs LatA: P=0.99, Ctrl vs LatB: P=0.9758), in which cells were either untreated or treated with LatA or LatB. Statistical differences were determined by a paired t-test. Within each box, the central red line corresponds to the median, and the edges of the box to 25% and 75% of the population. (f) The proportion of untreated and treated MCF-7 and MCF-10A cells screened that recovered instantaneously (ΔT ≥ 0), required 40 ms or less (0 < ΔT ≤ 40 ms), or did not recover within window time measured (ΔT > 40 ms). The statistical differences between the proportions of recovery types of untreated and treated cells were evaluated by a Chi-square test. For all graphs, *, **, ***, and **** indicate P ≤ 0.05, P ≤ 0.01, P ≤ 0.001, and P ≤ 0.0001, respectively.
Outliers over 3 standard deviation of the mean were removed. The black dashed line corresponds to the cellular diameter, there are clear differences between the and transit time of both lineages are dependent on the free width direction. Furthermore, while the deformed diameter that they are more deformable to an applied strain in the channel-

Although LEP cells, on average, had a similar transverse were FACS-enriched ahead of mechano-NPS characterization.

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Figure 4  Mechanical phenotyping of HMECs. (a) Cellular structure of the human mammary gland. The mammary duct consists of an outer layer of myoepithelial cells (red) that surround an inner layer of luminal epithelial cells (blue). (b) 4D plot of the cell diameter \(D_{\text{cell}}\), elongated length \(L_{\text{deform}}\), transit time through the contraction channel \(\Delta T_{\text{cont}}\), and recovery time \(\Delta T_r\) of myoepithelial (MEP, \(n = 99\)) and luminal epithelial (LEP, \(n = 104\)) breast cells. Dotted ovals group each sub-lineage (MEP: red and LEP: blue). Pre-sorted MEP and LEP cells were screened with an applied strain magnitude \(\varepsilon \sim 0.4\). (c) wCDI distributions of MEP and LEP lineages \((P = 1.2047 \times 25)\). Statistical differences were determined by a paired \(t\)-test. The red and blue lines correspond to the fitted normal distribution of MEP \((\text{wCDI} = 0.865 \pm 0.107)\) and LEP \((\text{wCDI} = 1.133 \pm 0.144)\) cells, respectively. The wCDI overlap between the two lineages is 29.3%. (d) Distribution of pre-sorted MEP and LEP cells that have instant \((\Delta T_r \sim 0)\), transient \((0 < \Delta T_r < 40 \text{ ms})\), or prolonged \((\Delta T_r > 40 \text{ ms})\) recovery. (e and g) wCDI distribution of HMECs derived from young \((e, y = \text{age}, 240L; n = 54, 59L; n = 53, 51L; n = 50, 124; n = 54)\) and old women \((g, 112R; n = 62, 237; n = 59, 122L; n = 54, 29; n = 60)\). Outliers over 3 standard deviation of the mean were removed. The black dashed line corresponds to the fitted normal distribution of HMEC cells (MEP+LEP). The red and blue solid lines represent the normal distribution of MEP and LEP cells, respectively, with the ratio \((a)\) of each lineage in the HMEC population as determined by the Expectation-Maximization algorithm\(^7\). (f and h) The proportion of HMECs from young \((e)\) and old \((g)\) women that have instant, transient, or prolonged recovery from applied strain.

corresponding to an \(\varepsilon_{\text{avg}} \sim 0.4\) for all measurements. Figure 4b shows the relationship among the measured parameters of MEP and LEP cells (derived from a 66-year-old woman, strain 237) that were FACS-enriched ahead of mechano-NPS characterization. Although LEP cells, on average, had a similar transverse deformation as that of MEP cells, they required less time to pass through the contraction channel (Figure 4b), thus suggesting that they are more deformable to an applied strain in the channel-width direction. Furthermore, while the deformed diameter and transit time of both lineages are dependent on the free cellular diameter, there are clear differences between the wCDI distribution of MEP \((\text{wCDI} = 0.865 \pm 0.107)\) and LEP \((\text{wCDI} = 1.133 \pm 0.144)\) cells (Figure 4c). In terms of cell recovery, MEP and LEP cells show a similar distribution of recovery types (Figure 4d).

We also measured the mechanical properties of primary HMEC cultures that consisted of mixtures of MEP and LEP cells from eight women of different chronological age (four pre-menopausal women aged < 30 years and four post-menopausal women aged > 55 years). Using the Expectation-Maximization algorithm\(^7\), in which the wCDI distribution function of sorted MEP and LEP cells obtained in our earlier experiments (Figure 4c) were used as initial
values, we determined the ratio (a) of MEP and LEP cells within each primary HMEC strain (Figures 4e and g) and subsequently compared this ratio to FACS analysis of CD10+/CD227− MEP and CD10−/CD227+ LEP (Supplementary Figure S9). The component ratios of MEP and LEP cells, as determined by the wCDI distributions, match exceptionally well with those obtained from FACS, as confirmed by a Chi-square test with a P-value = 0.05 (Supplementary Table S4). Indeed, the two methods are statistically indistinguishable. Although age-dependent differences in wCDI were not detected, age-dependent differences were readily apparent in recovery. Figures 4f and h show the composition of cell-recovery type for MEP and LEP cells of the young and old HMEC strains. Younger HMEC strains strikingly have a higher proportion of cells that recover instantaneously (an average of 47.8%) as compared to older strains (an average of 19.9%), suggesting that the cytoskeleton in younger cells is more resilient or more active, and in turn more responsive, to mechanical deformation.

We next determined whether HMEC traversing the stages of malignant progression have distinctive mechanical signatures that could be used to track these stages. We previously reported a method for producing post-stasis and immortal HMEC cell lines in the absence of gross, and confounding, genomic errors81. In this experiment, expression of p16 shRNA or cyclin D1 was used to bypass the stress-induced stasis barrier, and expression of c-myc was used to bypass the replicative senescence barrier and generate immortal non-malignant cell lines (Figure 5a). We used mechano-NPS to generate wCDI profiles and the recovery-type distribution of primary normal HMEC strains (240L and 122L), post-stasis finite strains (240L-p16sh, 240L-D1, 122L-p16sh, 122L-D1), and immortal non-malignant cell lines (240Lp16sMY, 240LD1MY, 122Lp16sMY, 122LD1MY). Each stage of malignant progression had a unique wCDI distribution. 240LD1MY, 122LD1MY, and 122Lp16sMY are known to have molecular and biochemical signatures of the luminal cancer subtype82. Their wCDI profiles show a mean that is greater than those of their normal isogenic HMEC predecessors, which also is consistent with a more LEP phenotype (Figure 5b). In contrast, 240p16sMY have a molecular and biochemical phenotype of basal breast cancers, which bear more similarity to MEP than to LEP lineage, and the wCDI distribution was more consistent with that of MEP (Figure 5b).

The post-stasis finite strains exhibited wCDI distributions that were intermediate phenotypes between normal HMEC and the isogenic immortal malignant cell lines, in a manner consistent with the eventual intrinsic luminal- or basal-like subtype of the immortal lines (Figure 5b). Interestingly, all immortal non-malignant cell lines screened have a greater fraction of cells that exhibit instant or transient recovery as compared to those of post-stasis finite strains (Figure 5c). When comparing the older pre-stasis strain, 122L to the isogenic immortal cell lines, there was a particularly stark decrease in recovery time (Figure 5c). Thus, we observed two different types of mechanical signatures: wCDI differed between the MEP and LEP lineages, whereas recovery from deformation was a distinguishing characteristic of chronological age. Moreover, these data provide functional evidence to suggest that the process of immortalization is associated with fundamental changes in the ability of cytoskeletons to respond to deformation.

**DISCUSSION**

Mechano-NPS is a versatile technique that can analyze populations of single cells for a number of biophysical properties,
mechano-NPS reveals and quantifies emergent functional properties of the cytoskeleton of cells. Consequently, mechano-NPS can evaluate cytoskeleton-targeted drugs (for example, estramustine, colchicine, and paclitaxel), which are often employed in cancer therapies, and may provide a new window into drug resistance of cancer cells, which could be caused in part by their cytoskeletal components. The ability of our platform to rapidly characterize mechanical properties in populations of cells lends itself to numerous applications in cell biology and basic research. For example, mechano-NPS could be used to assay rapidly common laboratory cell lines cultured under different conditions and confluences, and to determine whether cells coming out of culture are in a similar state from day-to-day. Clinically, mechano-NPS may yield a new approach to early detection of breast and other types of cancer genesis through analyzing epithelial cells and their composition ratio. Indeed, we have already demonstrated mechano-NPS’s ability to distinguish between LEP and MEP lineages in mixed populations, between epithelial cells from pre- or post-menopausal women, and between normal and immortal transformed epithelial cells from the same individual. The proportions of MEP and LEP subpopulations in mammary epithelium is highly associated with age of women, and when combined with distinct deformation recovery phenotypes in normal and transformed cells, mechano-NPS may yield valuable information regarding risk or diagnosis of breast cancer. We previously reported that the intrinsic subtype of immortal transformed HMEC was observable at the earliest stage of progression, bypass of stress-induced stasis, using molecular and biochemical markers of lineage. Here, we show that the stage of progression and the intrinsic subtypes are associated with distinctive mechanical phenotypes, opening up the possibility that wCDI could be used in a diagnostic setting as well.

CONCLUSION
Mechano-NPS is a multi-parametric, electronic-based, single-cell analysis method that can quantify cell diameter, resistance to compressive deformation, transverse deformation under constant strain, and recovery time after deformation, simultaneously. As demonstrated, the newly defined index wCDI, transverse deformation, and recovery time provide a quantitative mechanical metric for discriminating among different cell types, identifying sub-lineages of primary human mammary epithelial cells, and analyzing phenotypes that correlate with chronological age and malignant progression of human mammary epithelial cells. Mechano-NPS thus has great potential to be utilized as an efficient, label-free mechanical phenotyping tool for basic and clinical applications requiring characterization of cellular mechanics at the single-cell level.

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COMPETING INTERESTS
LLS is a founder of, and holds equity in, Nodexus Inc. The remaining authors declare no conflict of interest.
Screening cell mechanical phenotypes electronically

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Supplementary Information for this article can be found on the Microsystems & Nanoengineering website (http://www.nature.com/micronano).

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