

Characterizing deformability and surface friction of cancer cells

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Metastasis requires the penetration of cancer cells through tight spaces, which is mediated by the physical properties of the cells as well as their interactions with the confined environment. Various microfluidic approaches have been devised to mimic traversal in vitro by measuring the time required for cells to pass through a constriction. Although a cell's passage time is expected to depend on its deformability, measurements from existing approaches are confounded by a cell's size and its frictional properties with the channel wall. Here, we introduce a device that enables the precise measurement of (i) the size of a single cell, given by its buoyant mass, (ii) the velocity of the cell entering a constricted microchannel (entry velocity), and (iii) the velocity of the cell as it transits through the constriction (transit velocity). Changing the deformability of the cell by perturbing its cytoskeleton primarily alters the entry velocity, whereas changing the surface friction by immobilizing positive charges on the constriction's walls primarily alters the transit velocity, indicating that these parameters can give insight into the factors affecting the passage of each cell. When accounting for cell buoyant mass, we find that cells possessing higher metastatic potential exhibit faster entry velocities than cells with lower metastatic potential. We additionally find that some cell types with higher metastatic potential exhibit greater than expected changes in transit velocities, suggesting that not only the increased deformability but reduced friction may be a factor in enabling invasive cancer cells to efficiently squeeze through tight spaces.

cell mechanics | cell stiffness | biophysics |
suspended microchannel resonator | biosensors

The ability to quantify the changes that take place during the metastatic process remains a major challenge and is necessary for elucidating the underlying mechanisms as well as for identifying new therapeutic targets. Cellular biomechanics play an important role in metastasis, as cancer cells must not only squeeze through the extracellular matrix and endothelial cell–cell junctions, but they also must travel through small capillaries to reach a distant site. To begin this journey, a cell may undergo an epithelial–mesenchymal transition involving numerous biochemical and cytoskeletal changes that enable it to maintain a motile and invasive state (1–3). As the cell travels and circulates in the bloodstream, its trajectory is influenced by viscoelastic and frictional properties, the latter of which is governed by cell surface interactions as well as normal forces exerted by the cell on the channel wall.

A wide range of approaches for measuring the viscoelastic properties of single cells has been developed and used for addressing this challenge. Techniques such as micropipette aspiration (4, 5), atomic force microscopy (6, 7), and microrheology (8–11) provide highly quantitative and detailed information but have a low throughput. Alternatively, microfluidic approaches (12–20) have led to dramatic increases in throughput but are generally only semiquantitative. Consequently, there has been a general lack of approaches to measure cellular biomechanical properties with both high throughput and high precision. Despite these limitations, two consistent findings have been observed

across multiple measurement platforms: (i) cancer cells are more deformable than normal cells, and (ii) cancer cell deformability correlates with increased metastatic potential (2, 21, 22).

Despite the progress that has been made toward understanding the viscoelastic and frictional properties of single cells in the context of metastasis, the relative significance between the two has been relatively underexplored. Perhaps one of the most straightforward approaches for studying this in vitro is to monitor the movement of a cell as it travels through a fluidic constriction. Such a strategy was initially used to study neutrophil activation (23) and was subsequently adopted for other cell types. In these experiments, deformability was assessed by measuring the total passage time required for the cell to deform into and pass through the constriction. However, there are two obstacles that have made it difficult to delineate the relative contribution of deformability and friction to the total passage time. First, passage time is known to have a power law dependence on cell size (24). Various strategies have been developed for decoupling this dependence. For example, bright-field microscopy has been used to measure cell diameter before entry into the constriction (17), although with limited resolution due to aberrations that can arise from the three-dimensionality of the cell. The Coulter principle has the advantage that it can be implemented without sensitivity to cell shape and orientation, but has the drawback of being difficult to calibrate due to the presence of the constriction (12, 13, 20). Second, in addition to depending on cell size, total passage time lumps together the time required for the cell to squeeze into the constriction, which is primarily dependent on deformability, and the time required to pass through the constriction, which depends on friction with the channel wall. These two regions can be delineated by microscopy (17) but have not yet been observed by the Coulter principle.

To address these obstacles, we have integrated a constriction near the apex of a suspended microchannel resonator (SMR) as shown in Fig. 1A. In brief, the SMR consists of a hollow microchannel embedded in a silicon cantilever, whose resonant frequency is detected by the deflection of a laser beam (25). In each experiment, a solution with cells that are denser than the surrounding fluid is flowed into the device. Once a cell enters the SMR, the resonant frequency is lowered by an amount that depends on its buoyant mass and position away from the resonator base (Fig. 1B) (25). By tracking the resonant frequency as a cell traverses through the SMR, the position of its center of mass along the channel and its buoyant mass can be measured with a precision near 100 nm and 1 pg, respectively, for a cell that weighs ~100 pg

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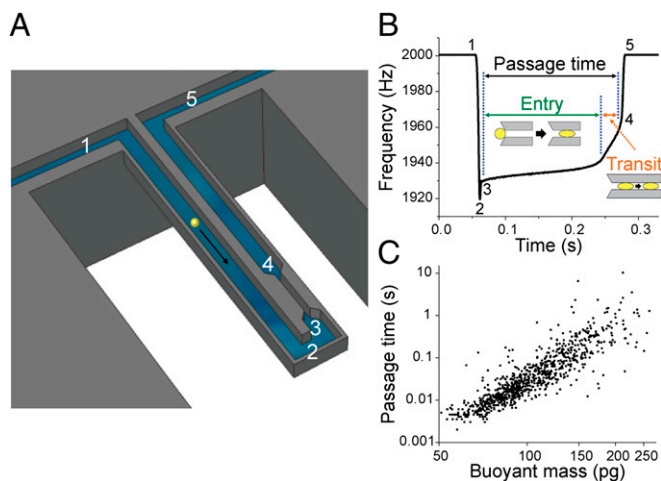


Fig. 1. Schematic diagram of the instrument and data extracted from the measurement. (A) Suspended microchannel resonator (SMR) with a constriction (6 μm wide, 15 μm deep, and 50 μm long) located at the apex. A cell passing through an embedded microfluidic channel is deformed as it flows into the constriction. Numbers 1–5 indicate different positions within the microchannel to demonstrate the trajectory of a cell flowing inside the channel. (B) The resonant frequency response of the SMR as the cell passes through the microfluidic channel. The numbers 1–5 correspond to the position of the cell in the cantilever, as marked in A. The height of the peak corresponds to the buoyant mass of the cell (1 \rightarrow 2). The cell slows down as it deforms to enter the constriction (transit), and then speeds up as it travels through the constriction (transit). The passage time corresponds to the sum of the entry and transit times (3 \rightarrow 4). (C) Power law dependence of passage time versus buoyant mass for the H1975 cell line ($n = 967$). Measurements were acquired with a PEG-coated channel surface and using a pressure drop of 1.8 psi.

and travels through the SMR in ~ 1 s. As a result, a cell's buoyant mass, passage time, velocity upon entering the constriction (entry velocity), and velocity as it transits through the constriction (transit velocity) can be extracted with a throughput of a few thousand cells per hour. Buoyant mass, which is the metric we use in this study for cell size, is defined by the product of the cell's volume and its density difference from the surrounding fluid.

Using the SMR with the integrated constriction, we demonstrate that precise, single-cell buoyant mass measurements in conjunction with passage time information enable the distinction between cell lines bearing different physical characteristics. More specifically, these combined measurements reveal differences between cell lines arising from blood and epithelial tissue, as well as between cell lines having varying metastatic potential. To assess factors affecting cell passage through the constriction, we further show that entry and transit velocity measurements enable us to identify the relative importance of deformability and surface friction, respectively. Changing the deformability of the cell by perturbing its cytoskeleton primarily alters the entry velocity, whereas changing the surface friction by immobilizing positive charges on the constriction's walls primarily alters the transit velocity. To demonstrate the insight that these parameters provide, we compare the properties of both mouse and human cancer cell lines having known metastatic potentials. When accounting for cell buoyant mass, we find that cells possessing higher metastatic potential exhibit faster entry velocities than cells with lower metastatic potential. However, in some cases, the increase in transit velocities associated with faster entry velocities was considerably greater than expected, suggesting that reduced friction may be a factor in enabling invasive cancer cells to efficiently squeeze through tight spaces. Finally, we demonstrate that combined buoyant mass and passage time measurements can identify tumor cells spiked into blood with a throughput of $\sim 10^5$ cells per h.

Results

Single-Cell Measurement of Buoyant Mass, Passage Time, and Comparison with a Biophysical Model. We first measured the buoyant mass and passage times of hundreds of single cells from a human lung adenocarcinoma cell line, H1975 (Fig. 1C), to validate our method. As expected, a cell's passage time through the constriction has a power law relationship to its buoyant mass (24). Interestingly, the range of passage times spans nearly four orders of magnitude as it changes with cell buoyant mass. In addition, the passage time of cells having the same buoyant mass varies by up to an order of magnitude, suggesting the influence of other effects. Compared with other approaches for measuring cell size and passage time through a constriction (12, 13, 17, 20), our results show a thousandfold larger range in passage time across the population and a nearly 10-fold larger variation in passage time for cells of similar buoyant mass. One possible explanation for this discrepancy is that, because the passage times in these previous studies were typically on the millisecond scale and below (12, 20), the relatively higher strain rates diminished the range in the mechanical properties. Although it seems surprising that cells of similar buoyant mass could have such a significant variation in passage time, time lapse microscopy of a cell passing through multiple constrictions in existing literature (26) suggests that, in addition to inherent biological variation, the orientation of the cell upon entry into the constriction could give rise to this spread.

To render our measurement more readily comparable to those obtained via other assays of cell deformability such as micropipette aspiration, we compared the cell trajectories observed in our SMR device to a classical biophysical model of cell entry into a constriction (Fig. 2). We modeled the cell as a homogeneous liquid drop with a viscosity μ_0 that decreases with shear rate according to a power law coefficient b (*SI Materials and Methods*). Our goal in applying it here is to highlight the conceptual similarity between our technique and the classic techniques, not to attempt to derive absolute measures of cell deformability. This “shear-thinning” model has previously been applied to model the aspiration of human neutrophils and other cells into a micropipette (4, 27, 28).

We used the shear-thinning model to predict both the entry times (the time it takes a cell to deform into the constriction; Fig. 1B) as well as the trajectories of H1975 cells ($n = 343$;

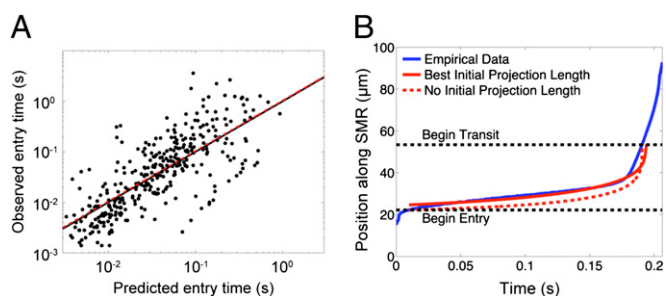


Fig. 2. Trajectories observed in the SMR can be predicted with high accuracy from a power law viscosity model, similar to Tsai et al. (28). (A) Prediction of entry times into the constriction for H1975 cells (test set, $n = 343$). Cells are modeled from a training set ($n = 388$) as having a shear rate-dependent viscosity $\mu = \mu_0(\dot{\gamma})^{-b}$, where a single best-fit model is chosen for all of the cells. The black line shows equality between predicted and observed entry times, the black dots represent individual cells, and the red dashed line is the best fit line for the test set. Predicted entry times and observed entry times demonstrate a high correlation of $r = 0.76$ in log space. (B) Prediction of the detailed trajectory through the constriction using the power law viscosity model, shown for a typical cell with its own best-fit model. The blue line represents the observed trajectory of the cell through the constriction, the dotted red line represents the model prediction, and the solid red line shows the model prediction when an initial projection is incorporated into the model.

SI Materials and Methods). The model's predicted entry times show a strong correlation with the observed entry times (Fig. 2A, $r = 0.76$ on a log-log scale). Similarly, strong correlations were obtained for HCC827 (Fig. S1), human lung cancer cell line, which is known to be less invasive than H1975 (29, 30). The shear-thinning model captures the dynamics of entry (Fig. 2B) comparably to results obtained on previous platforms. Similar to prior applications of the model, incorporating a small initial projection of the cell into the constriction (28) improves the fit (Fig. S2) (*SI Results*) but does not otherwise significantly alter model parameters. These results suggest that our measurement characterizes the deformation behavior of single cells in a manner akin to those previously achieved by micropipette aspiration.

Then, to determine whether the large span and variance in passage time is exhibited by other types of mammalian cells, we measured seven additional adherent cell lines including mouse embryonic fibroblasts (MEFs), mouse lung cancer cell lines (T_{Met} , T_{nonMet} , and $T_{Met-Nkx2-1}$) (31), human lung cancer cell lines (H1650, H1975, and HCC827), as well as a mouse lymphoblastic leukemia cell line (L1210) that was grown in suspension. Measurements were all acquired with a poly(ethylene glycol) (PEG)-coated channel surface and under a constant applied pressure drop across the microchannel (0.9 psi). All of the measured cell lines exhibited a similar power law dependence between passage time and cell buoyant mass (Fig. 3). However, it is important to note that cell buoyant mass was not the only determining factor of the passage time. As demonstrated by the comparison of the T_{Met} and L1210 cell lines in Fig. 3C (Fig. S3), the epithelial lung cancer cells require more time to pass through the constriction than blood cells of similar buoyant mass. From these data, it is clear that neither cell buoyant mass nor passage time alone would be sufficient to distinguish between these two populations of cells. Rather, the combination of the two metrics allows for a clear distinction.

In a similar manner, we found that cell lines with higher metastatic potential exhibit shorter passage times compared with

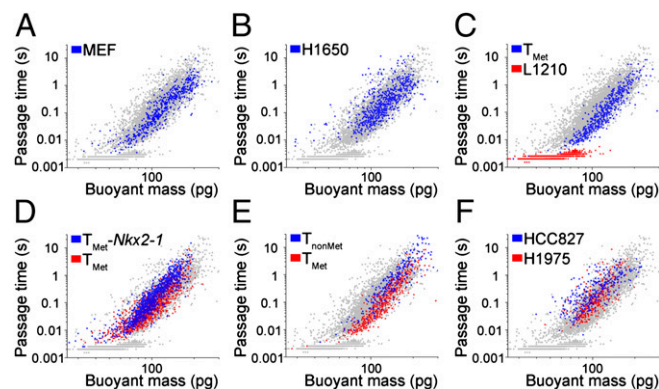


Fig. 3. Power law relationship between passage time and cell buoyant mass is demonstrated by measurements of various cell lines, including (A) mouse embryonic fibroblast (MEF) ($n = 511$), (B) H1650 ($n = 639$), (C) T_{Met} (blue, $n = 512$), L1210 (red, $n = 1401$), (D) $T_{Met-Nkx2-1}$ (blue, $n = 1065$), T_{Met} (red, $n = 1028$), (E) T_{nonMet} (blue, $n = 252$), T_{Met} (red, same dataset as in C), (F) HCC827 (blue, $n = 278$), and H1975 (red, $n = 307$). Measurements were made in a PEG-coated channel under a constant pressure drop of 0.9 psi. The gray dots shown as a background correspond to the collection of all measured cell lines. Notably, as shown in C, adherent mouse lung cancer cells in suspension require a longer time to pass through the constriction than mouse blood cells of similar buoyant mass. Three pairs of cancer cell lines having different known metastatic potentials are compared in D–F. In each pair, the cell line with the higher metastatic potential (red dots) exhibits shorter passage times than those with the lower metastatic potential (blue dots). The difference in passage time was statistically significant for all three pairs in D–F (Fig. S4).

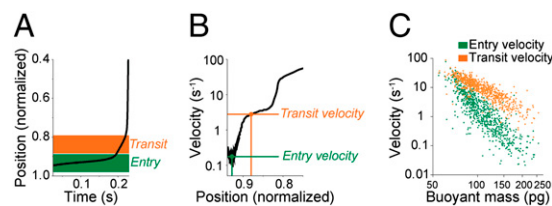


Fig. 4. Extracting entry and transit velocities from single cell measurement. (A) The resonant frequency response (positions 3–5 in Fig. 1B) is converted to the normalized position of the cell in the cantilever and plotted versus time. The length of the cantilever was normalized to 1 to represent the cell's position, where 1 and 0 correspond to the tip and base, respectively. (B) Cell velocity is obtained by taking the time derivative of the normalized position. Entry and transit velocities are extracted at specific locations that correspond to the entrance and the inside of the constriction, respectively. (C) Entry (green) and transit (orange) velocities versus buoyant mass for the data set from Fig. 1C.

cell lines with lower metastatic potential (Fig. 3 D–F). Three pairs of cancer cell lines were compared, and in each pair, one cell line was known to be more metastatic than the other. HCC827 and H1975 are both human lung adenocarcinoma cell lines, but H1975 is more metastatic than HCC827. T_{nonMet} and T_{Met} cell lines were derived from nonmetastatic and metastatic primary mouse lung adenocarcinomas, respectively, with the T_{Met} cell line having greater metastatic ability (31). Down-regulation of the *Nkx2-1* transcription factor is a key event in lung cancer metastasis; therefore, T_{Met} cells reexpressing this transcription factor ($T_{Met-Nkx2-1}$) have restricted metastatic potential compared with T_{Met} (31). Hence, the distinction between each pair of cell lines bearing different metastatic potentials is evident when both passage time and buoyant mass information are used together. The difference in passage times was statistically significant for all three pairs (Fig. S4).

Entry and Transit Velocities Reveal the Relative Significance Between Deformability and Surface Friction.

To assess the components that govern passage time, the entry and transit velocities were extracted from the SMR measurements. The passage of each cell through the constricted microchannel comprises the initial deformation of the cell to enter the constriction as well as the subsequent transit of the cell through the constriction. The velocity of each cell during its entry and transit can be obtained by monitoring the position of its center of mass within the microchannel (Fig. 4A and B), as given by the resonant frequency shift of the cantilever (32). Both entry and transit velocities have a power law dependence on the buoyant mass, similar to the passage time, but the velocities decrease with increasing buoyant mass (Fig. 4C). Also, for a given cell, the entry velocity is slower than the transit velocity. However, the difference between the entry and transit velocities is less pronounced for smaller cells, because smaller cells require less time to deform into the constriction in comparison with larger cells. Hence, in complement with passage time information, the entry and transit velocities can provide more insight into cellular properties governing the passage through the constriction.

Next, the effects of deformability and surface friction on the passage of cells through the constriction were assessed by measuring H1975 cells under two separate conditions—having perturbed its cytoskeleton with latrunculin B (LatB), and having coated the microchannel surface with positively charged poly-L-lysine (PLL) (Fig. 5). First, as expected, the treatment with LatB decreased the passage time of the cells (Fig. S5A), corresponding to the LatB-induced increase in cell deformability by the disruption of actin filaments (33). Furthermore, the LatB treatment increased both entry and transit velocities, with the relative increase in entry velocity being greater than that of transit velocity

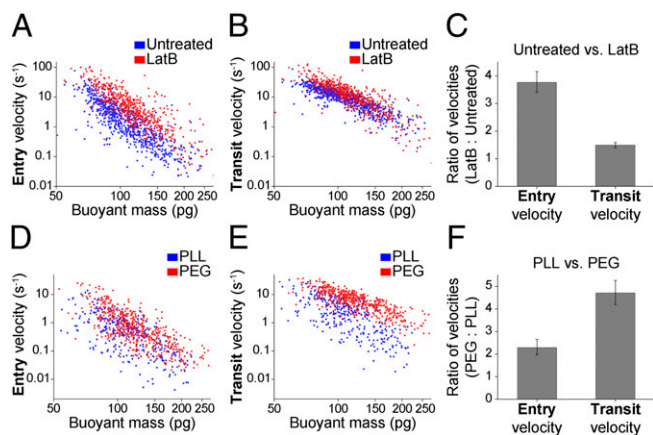


Fig. 5. Changes in the entry and transit velocities of H1975 cells after perturbing either deformability or microchannel surface charge. (A) Entry velocity and (B) transit velocity versus buoyant mass for H1975 untreated (blue, $n = 843$) and treated with LatB (red, $n = 907$, $5 \mu\text{g/mL}$ for 30 min) measured in a PEG-coated channel. Treatment with LatB decreases the passage time of H1975 (Fig. S5) and induces a larger shift in entry velocity than transit velocity. (C) A ratio of velocities from the two conditions was calculated as in Fig. S12. Changing the deformability of the cell by perturbing its actin cytoskeleton induces a 3.8-fold increase in the entry velocity, and only a 1.5-fold increase in the transit velocity. (D) Entry velocity and (E) transit velocity versus buoyant mass for H1975 cells passing through a microchannel whose surface is coated with positively charged PLL (blue, $n = 345$) or neutral PEG (red, $n = 649$). PLL increases the passage time (Fig. S5) and results in a greater shift in transit velocity than entry velocity. (F) Changing the surface friction from PEG to PLL caused entry velocity to decrease 2.3-fold, and transit velocity to decrease 4.7-fold. Error bars represent 95% confidence intervals. All measurements were acquired using a pressure drop of 1.8 psi.

(Fig. 5 A–C). Although the small change in transit velocity indicates that a change in cell stiffness can influence the friction because the normal force of the cell against the channel wall is altered (34), the greater change in entry velocity is consistent with the fact that perturbing the viscoelastic properties of the cell affects the passage most when the deformation of the cell is critical, i.e., at the entry rather than during transit. A similar behavior was observed when MEF cells were treated with nocodazole (Fig. S6), further demonstrating that a cytoskeletal deformability change in the cell corresponds to a larger shift in entry velocity than transit velocity.

Coating the microchannel constriction with PLL increased the passage time of the cells (Fig. S5B), corresponding to the increased interaction of the negatively charged cell surface with the positively charged PLL-coated channel surface than with the neutral PLL-graft-PEG-coated surface. The PLL coating decreased both the entry and transit velocities, but with a larger change in transit velocity (Fig. 5 D–F). Although the surface interaction during the deformation at the constriction entry was nontrivial, the effect was greatest when the contact area between the cell and the channel wall was maximum, i.e., during the cell's transit through the constriction. The transit velocity thus represents a friction measurement that encapsulates effects contributing to the normal force exerted by the cell on the channel wall, as well as interactions between the surface of the cell and the channel. The experiment involving a PLL surface coating demonstrates that enhancing surface interactions, such as those governed by charge, alters the transit velocity more than entry velocity. Similar results were obtained for various other cell lines, such as HCC827, T_{Met} , and T_{nonMet} (Fig. S7). Hence, even though deformability and friction are not completely orthogonal in our measurements, the relative differences in entry and transit velocities provide a metric for the relative importance of

deformability and friction during the passage of a cell through a constriction.

Characterizing Entry and Transit Velocities of Cancer Cells Reveals That Deformability and Friction Govern Cell Passage Time. We also found that the relative importance of the cell's deformability and surface friction to its passage through the constriction could be a unique indicator of cell state. The deformation and friction properties of cancer cells having different known metastatic potentials were characterized by entry and transit velocities on a PEG-coated channel (Fig. 6). In addition to having shorter passage times (Fig. 3 D–F), all three cell lines with higher metastatic potential exhibit faster entry and transit velocities. Interestingly, the relative differences in entry and transit velocities from each pair showed distinct patterns. Expressing the $Nkx2-1$ transcription factor in T_{Met} led to a change primarily in entry velocity, which is similar to what we observed in altering the cytoskeletal deformability of the cells with LatB (Fig. 5C). In contrast, T_{Met} versus T_{nonMet} showed significant differences in both entry and transit velocities, suggesting that changes in friction account for more of the difference between this pair of cells than between the pair with a single genetic alteration (T_{Met} versus $T_{\text{Met-Nkx2-1}}$). H1975 versus HCC827 also demonstrated that a considerable change in the transit velocity is associated with the entry velocity. These examples suggest that reduced friction may play a role in enabling invasive cancer cells to effectively squeeze through tight spaces. It is possible that such changes in friction may be related to differences in factors contributing to the normal force of the cell on the channel wall, such as cell relaxation times. Additionally, the observed changes may be caused by glycocalyx characteristics, such as the expression of particular cell surface molecules like sialic acid, because it is already known that increased expression of sialic acid may be accompanied with a higher metastatic potential in cancer cells (35).

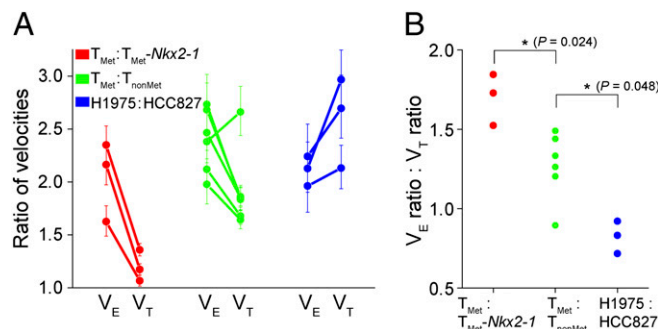


Fig. 6. Three pairs of cancer cell lines having different metastatic potentials (T_{Met} versus $T_{\text{Met-Nkx2-1}}$, red; T_{Met} versus T_{nonMet} , green; and H1975 versus HCC827, blue) were compared by measuring the changes in entry velocity (V_E) and ratio of V_T for three pairs of cancer cell lines. V_E and V_T ratios connected by a line represent one replicate. In contrast to T_{Met} versus $T_{\text{Met-Nkx2-1}}$, T_{Met} versus T_{nonMet} and H1975 versus HCC827 show that a significant change in transit velocity is associated with a change in entry velocity, suggesting that the role of friction is more significant in those pairs. T_{Met} versus $T_{\text{Met-Nkx2-1}}$, T_{Met} versus T_{nonMet} , and H1975 versus HCC827 were repeated from different cultures three, six, and three times, respectively. Error bars represent 95% confidence intervals. (B) For each measurement, the ratio of V_E divided by the ratio of V_T is shown, which confirms that the proportional change in V_E relative to V_T was significantly different among the three pairs ($*P < 0.05$, Mann-Whitney-Wilcoxon test). Measurements were acquired using a pressure drop of 0.9 psi for the mouse cell lines (T_{Met} , $T_{\text{Met-Nkx2-1}}$, T_{nonMet}) and a higher drop of 1.8 psi for the human cell lines (H1975, HCC827) to account for their larger size.

Effects of Cell Density on SMR Measurements. As a cell's buoyant mass depends on its volume and difference in its density with the surrounding fluid, we sought to identify effects of cell density on the trends found in the deformability and friction measurements made by the SMR. We measured the average density of each cell line (*SI Materials and Methods*) and thus converted the buoyant mass to volume (Fig. S8) (*SI Results*) (36). Passage times, entry velocities, and transit velocities of cancer cells with varying metastatic potentials (Figs. 3 D–F and 6) were compared again based on cell volume (Figs. S8 and S9). Interestingly, we found that the difference in density between human lung cancer cell lines was more significant than that between mouse lung cancer cell lines. Because HCC827 cells had a significantly lower density than H1975 cells, passage time properties for these two cell lines, when plotted versus cell volume, were similar. In contrast, the density of mouse lung cancer cell lines (i.e., T_{Met} versus $T_{Met-Nkx2-1}$ and T_{Met} versus T_{nonMet}) was only slightly different, and therefore the passage time properties of those cell lines remained similar when plotted versus the volume. Thus, differences between passage time properties for all three cell line pairs were consistent with expected deformability changes based on metastatic potential when accounting for buoyant mass, but not when accounting for volume. Furthermore, the differences in cell density did not change the findings from characterizing entry and transit velocities shown in Fig. 6 (Fig. S9). Density measurements in combination with deformability and friction measurements may shed light on further interesting characteristics of cancer cells, which can be studied more in depth in future work.

Using SMR Measurements to Discriminate Between Human Epithelial and Blood Cells. As an application of using passage time measurements of single cells in the SMR, we demonstrate the ability of the SMR to discriminate between cells arising from human blood versus epithelium. A human buffy coat sample was spiked with H1650 cells, and the buoyant mass and passage time of each cell was recorded. Fig. 7 portrays that blood cells transit through the constriction several orders of magnitude faster than spiked H1650 cells, and that the SMR signals generated by the two types of cells are quite distinct. Hence, in general, H1650 cells can be distinguished from blood cells based on the passage time and buoyant mass measurements. Among the total of $\sim 10^4$ cells counted in the spiked sample, the number of cells having a long passage time (greater than 8 ms) was 103, which closely corresponds to the expected value from the spiked concentration of

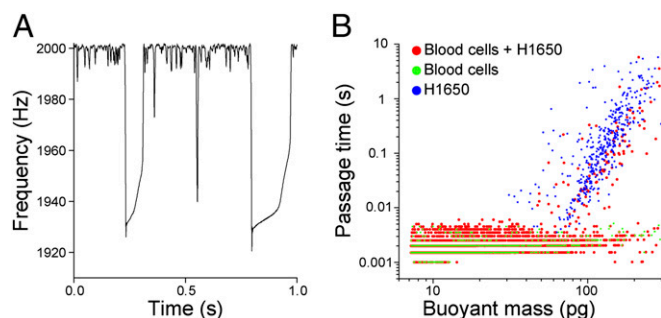


Fig. 7. Passage time versus buoyant mass measurements delineate H1650 cells from human blood cells. (A) Peaks detected from the blood cells spiked with H1650 cells, where each peak represents the transit of a cell through the SMR. (B) Passage time versus buoyant mass for H1650 and blood cells. Blood cells (green, $n = 2832$) traveled through the constriction several orders of magnitude faster than H1650 cells (blue, $n = 404$), suggesting that H1650 cells could be distinguished from blood cell populations by passage time. Indeed, the spiked sample (red, $n = 10810$) shows a subpopulation whose passage time and buoyant mass match that of the H1650 cells. Measurements were acquired with a PEG-coated channel surface and using a pressure drop of 1.5 psi.

H1650 cells ($\sim 1:90$). Thus, the SMR platform may provide a tool to distinguish circulating tumor cells (CTCs) from the surrounding blood cells, irrespective of molecular surface expression (37, 38). Moreover, the throughput of the SMR is $\sim 10^3$ cells per h ($\sim 10^4$ cells detected for 6 min), which is sufficient for interrogating patient samples provided upstream enrichment strategies are used (e.g., CD45 depletion) (39, 40). Therefore, in the future it may be feasible to use the SMR with constriction to identify CTCs in patient blood samples based on biophysical properties, which may reveal populations of CTCs that have gone undetected by common methods involving specific molecular probes. In addition, parsing out transit and entry velocity information could increase our understanding of the biophysical characteristics of CTCs. As the cells remain viable and proliferate well after measurement in the SMR (Fig. S10), we could later perform downstream molecular and functional analyses on identified CTCs.

Conclusion

The SMR can precisely weigh individual cells and measure their position along the channel with high spatial resolution. We have used this capability along with an integrated constriction in the microchannel, first to decouple cell size from passage time information, as a measure of holistic cellular deformability. More excitingly, the SMR further enables us to track a cell's velocity as it flows through the constriction and infer the relative significance between deformability and surface friction of cancer cells. Indeed, we have shown that deformability and friction characteristics can be linked to even a single genetic alteration known to govern metastatic potential in cancer cells. Our results suggest that there may be genetic alterations that preferentially modulate friction and that these could be possible drivers for metastatic potential. In addition, we have demonstrated a preliminary study that establishes the feasibility of using the SMR system to identify and study CTCs. This approach has the particular advantage of not relying upon specific molecular markers, which may bias the selection of CTCs. Thus, measurements of single-cell buoyant mass, deformability, and friction may help to elucidate biophysical properties of cancer cells in circulation and in relation to processes involved in metastasis.

Materials and Methods

Experimental System. As described by Burg et al. (25), cells suspended in solution flow through the SMR, and the resulting frequency shift depends on the buoyant mass and position of the cell. SMR devices with a constriction were fabricated by creating buried channels in silicon-on-insulator wafers, followed by wafer thinning and dry etching to form suspended microchannels with 2- to 3- μm thin walls and a 15- μm channel depth. Two hundred devices are fabricated and vacuum-packaged on a 6-inch wafer with yields exceeding 80%. A getter layer prevents slow degradation of the on-chip vacuum due to outgassing. Integrated under each cantilever is an electrostatic drive electrode and the cantilever vibration is detected by the optical lever. A gain controlled oscillator circuit is used to continuously track the resonant frequency of the SMR device. Although we can observe each cell as it enters the SMR, the constraints of our device make it impractical to obtain a precise measure of cell volume with our microscope. It is also important to note that to track the position of the cell as it travels through the constriction, we monitor the resonant frequency of the cantilever, which can achieve a precision near 100 nm for cell velocities of ~ 0.5 mm/s. The position of the cell cannot be tracked by microscopy because the walls of the suspended channel are not transparent. The length of the cantilever is 316 μm with the rectangular microchannel of 20 μm wide and 15 μm deep. The constriction located near the apex of the microchannel is 50 μm long and the width of cross-section is 6 μm . Cells enter and exit the SMR through two on-chip bypass channels that flank the SMR (Fig. S11). Fluid flow through the bypass channels is controlled by two electronic pressure regulators and three solenoid valves. Cells are introduced through the inlet of the bypass channel, whereas the other three outlets are filled with cell culture medium. A constant pressure drop is maintained across the fluidic channels by the pressure regulators that drive the cells through the SMR (Fig. S11).

Channel Wall Coating. The fluidic channel of the device was coated with PEG [1 mg/mL; PLL(20)-g[3.5]-PEG(2); Surface Technology] or PLL (0.1 mg/mL; R&D Systems) before all experiments. All results from Figs. 1–4, 5 A–C, 6, and 7 were measured with PEG-coated surface. In Fig. 5 D–F, the results from PLL-coated surface was compared with those from PEG-coated surface. Before the coating, the channel was cleaned with piranha solution (3:1 mixture of H₂SO₄ and H₂O₂) for 15 min at room temperature, followed by a thorough rinse with deionized H₂O (dH₂O). The channel was briefly dried with air before adding PEG or PLL solution. For a PEG coating, a slow flow of PEG was kept in the SMR for 1 h. The channels were then dried with air and rinsed with dH₂O. For a PLL coating, PLL was maintained in the SMR for 2 h, followed by drying with air and rinsing with PBS and dH₂O.

Cell Preparation. All cell lines, including MEF (33), T_{Met}, T_{Met}-Nkx2-1, T_{nonMet} (31), H1650, H1975, HCC827 (29, 30), and L1210 (41), were cultured under standard conditions as described elsewhere. Materials used for cell culture are listed in Table S1 for each cell line. The T_{nonMet} cell line (368T1) and T_{Met} cell line (393T5) were generated from tumors that developed in *Kras*^{LSL-G12D}; *p53*^{fllox/lox} mice after intratracheal lentiviral-Cre infection (31). T_{Met}-Nkx2-1 cells were generated through retroviral expression of Nkx2-1 in a T_{Met} cell line (389T2) (31). T_{nonMet} cells (368T1) were compared with T_{Met} cells (393T5). T_{Met}-Nkx2-1 cells were compared with its control T_{Met} cells (389T2). Cells were passaged 1 or 2 d before the experiment. To harvest the cells, the culture medium was aspirated, cells were rinsed with PBS, and trypsin was used to detach the adherent cell types from the flask. Cells were then aspirated and resuspended in culture media to a concentration of 0.5–1 × 10⁶ cells per mL. The cell solution was filtered through a 30-μm mesh (Miltenyi Biotec) before measurement in the SMR. To treat the cells with LatB, the cells were dissociated from the surface, resuspended in the medium with 5 μg/mL LatB, and kept in the 37 °C incubator for 30 min before loading the cells in the SMR device. To maintain the LatB-treated condition during the experiment, the medium used in the SMR device was also supplemented with LatB. Treating

the cells with LatB in a suspended state for up to 1 h did not alter the viability and proliferation rate when they were replated on a flask after replacing the medium with normal growth medium.

Spiked Cells Experiment. To test whether the SMR can identify tumor cells among a heterogeneous population of cells, human blood cells were spiked with a known concentration of human lung cancer cells, H1650. Human blood cells were prepared from buffy coat (Research Blood Components). First, red blood cells in the buffy coat were removed by Histopaque-1077 (Sigma-Aldrich). Briefly, 3 mL of buffy coat was overlaid onto an equal volume of Histopaque solution. It was centrifuged at 821 × *g* for 15 min at room temperature. The center layer containing nucleated cells was then carefully extracted using a pipette and resuspended in PBS (821 × *g*, 10 min). This was diluted to a final concentration of ~8 × 10⁶ cells per mL, which was measured by a Coulter counter (Multisizer 4; Beckman Coulter). Then, ~9 × 10⁴ cells per mL H1650 cells was spiked into the blood cells. Although CTC cells are much rarer in patient samples, the higher concentration of spiked cells enabled us to acquire enough data in a given time to demonstrate that tumor cells can be distinguished from blood cells using our instrument. The passage time for blood cells, H1650, and a mixture of blood cells and H1650 cells were separately measured under the same conditions, using a pressure drop of 1.5 psi. The resulting flow rate was 38 μL/h as measured with blank media.

Data Processing. Buoyant mass, passage time, entry velocity, and transit velocity information were obtained by processing the resonant frequency data from the SMR. Details are included in *SI Materials and Methods*.

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