Cell elasticity with altered cytoskeletal architectures across multiple cell types

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Abstract

The cytoskeleton is primarily responsible for providing structural support, localization and transport of organelles, and intracellular trafficking. The structural support is supplied by actin filaments, microtubules, and intermediate filaments, which contribute to overall cell elasticity to varying degrees. We evaluate cell elasticity in five different cell types with drug-induced cytoskeletal derangements to probe how actin filaments and microtubules contribute to cell elasticity and whether it is conserved across cell type. Specifically, we measure elastic stiffness in primary chondrocytes, fibroblasts, endothelial cells (HUVEC), hepatocellular carcinoma cells (HUH-7), and fibrosarcoma cells (HT 1080) subjected to two cytoskeletal destabilizers: cytochalasin D and nocodazole, which disrupt actin and microtubule polymerization, respectively. Elastic stiffness is measured by atomic force microscopy (AFM) and the disruption of the cytoskeleton is confirmed using fluorescence microscopy. The two cancer cell lines showed significantly reduced elastic moduli values (~ 0.5 kPa) when compared to the three healthy cell lines (~ 2 kPa). Non-cancer cells whose actin filaments were disrupted using cytochalasin D showed a decrease of 60-80% in moduli values compared to untreated cells of the same origin, whereas the nocodazole-treated cells showed no change in elasticity. Overall, we demonstrate actin filaments contribute more to elastic stiffness than microtubules but this result is cell type dependent. Cancer cells behaved differently, exhibiting increased stiffness as well as stiffness variability when subjected to nocodazole. We show that disruption of microtubule dynamics affects cancer cell elasticity, suggesting therapeutic drugs targeting microtubules be monitored for significant elastic changes.

Keywords

Atomic force microscopy; cell mechanics; elasticity; cytoskeleton; cancer

1. Introduction

The interdependence of cell elasticity and cytoskeletal components is a critical step toward understanding the mechanics of living tissue. Cellular responses and their microarchitecture react and adapt to their environment and disease state.¹⁻³ Changes in cell elasticity have been implicated in the pathogenesis of many human diseases⁴ including vascular disorders,⁵ malaria,⁶⁻⁷ sickle cell anemia,⁸ arthritis,⁹ asthma,¹⁰ and cancer.^{7, 11-16} Therefore, there is a practical need to measure cell mechanics quantitatively to understand how diseased cells differ from healthy ones. In particular, investigating the mechanical properties of cancer cells may help to better understand the physical mechanisms responsible for cancer metastasis.

Common techniques to measure the mechanics of cells, include the use of magnetic beads,^{2, 17} optical tweezers,¹⁸⁻¹⁹ micropipette aspiration^{9, 20-22} and atomic force microscopy (AFM).^{12, 23-26} Since the application of AFM to living cells, it has readily been adapted to characterize cell topography²⁷⁻²⁸ as well as mechanical properties^{12, 24, 29-31} with nanoscale precision. The AFM has been further adapted for microrheology,³²⁻³³ frequency modulation,³⁴⁻³⁵ and creep^{25, 36-37} experiments to study the viscoelastic properties of various cell lines. Of interest to this work, is the evaluation of elasticity of endothelial cells,³⁸⁻⁴¹ fibroblasts,^{28, 42-44} chondrocytes,^{22, 45-46} fibrosarcomas⁴⁷⁻⁴⁹ and hepatocellular carcinoma cells.⁵⁰ Kuznetsova *et al.* includes a table of expected moduli values for various cell lines.⁵¹ Based on elastic modulus measurements, many groups show that cancerous cells are softer than their non-transformed counterparts.^{12, 42} This stands to reason since increased deformability of cancer cells allows them to metastasize and infiltrate tissues.⁵² Such behavior is attributed to modification in the cytoskeletal organization of the cells.⁵³⁻⁵⁴

The influence of cytoskeletal drugs on cell elasticity has allowed the decoupling of cytoskeletal components and their contribution to overall cell elasticity. Theoretically, actin filaments in the cell periphery are the most rigid of the main components of the cytoskeleton but due to their tubular structure, microtubules have a larger bending stiffness.¹¹ The resulting contribution of each cytoskeletal component toward cell elasticity does not depend solely on the bending

stiffness of individual components, but will factor based on organization and concentration, which will be dependent on cell type. For example, Jackson *et al.*, indicates that changes in cytoskeletal cross-linking proteins in response to mechanical loading would be sufficient to increase whole cell stiffness.⁵⁵ AFM studies that investigate the influence of cytoskeletal drugs on cell elasticity have shown the elastic modulus to be highly affected by actin filament disruption,^{24, 28, 37, 56-58} but there are groups that find cell elasticity is relatively unaffected by microtubule-targeted drugs^{28, 57-58} and some that find microtubules contribute greatly.^{2, 44, 50} While many AFM studies examine drug responses of one cell type, or one healthy and one cancerous cell type, our goal is to present a comprehensive AFM study across multiple cell lines, healthy and cancerous, that examines the effect of cytoskeletal destabilizers on cell elasticity using the exact same method. We find destabilizing the microtubule network produces no effect in healthy cell lines, while producing a significant increase in elastic moduli in the fibrosarcoma line. We aim to elucidate the functional relationship between cellular mechanics and cytoskeletal stability and whether that relationship is conserved across different cell types.

2. Experimental Methods

The methods here are designed to probe the mechanical behavior across all cell lines providing a comprehensive head to head comparison across 15 conditions: control, actin destabilized and microtubule destabilized for each of 5 cell types. The same plating conditions (Section 2.1), cytoskeletal visualization technique (Section 2.2) and experimental test protocol (Sections 2.3, 2.4, 2.5) allow for direct comparison. Furthermore, the control of the AFM parameters, indentation depth and approach velocity, maintain the same probing frequency for each condition, which is critical for comparing the mechanical properties of materials that exhibit a frequency dependence.

2.1. Cell culture

Five cell lines were cultivated for this study: human dermal fibroblasts, bovine chondrocytes, HUVECs, HT 1080 (fibrosarcoma), and HUH-7 (hepatocellular carcinoma). Fibroblasts and HUVECs were cultured in Fibrolife (Lifeline Cell Technologies) and Vasculife (Lifeline Cell Technologies), respectively. HT 1080 fibrosarcoma cells, HUH-7 hepatocarcinoma cells, and chondrocytes were cultured in DMEM L-glutamine supplemented with 10% fetal bovine serum and 1% penicillin streptomycin. All cells were incubated at 37 °C in a humidified atmosphere

with 5% CO₂. Cells were grown to confluency and harvested by trypsinization. Coverslips were coated for 30–40 min with 5 µg/mL fibronectin (BD Biosciences) dissolved in PBS prior to plating. Cells were initially plated at 50-75k density on glass coverslips (22 x 40 mm) for all studies, and were incubated for 48 hrs prior to experiments. Three conditions were studied: control (recording buffer only), 2.5 µM Cytochalasin D (Sigma Aldrich) in recording buffer for 30 min, and 10 µM Nocodazole (Sigma Aldrich) in recording buffer for 30 min. Recording buffer solution was made as follows: 10% 10x HBSS (Gibco), 1% Heparin, 1% Glutamax, and 1% FBS in DI water. Solution was then pH-balanced to 7.4 using NaOH.

2.2. Tubulin and actin visualization

Cells were incubated in 250 nM TubulinTracker Green (Life Technologies) for 30 min at 37°C, then rinsed three times, and placed in recording buffer for fluorescence imaging with a standard FITC filter. For actin visualization, cells were fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA; diluted with HBSS from a 16% stock solution) for 15 min, then permeabilized for 3 min with 0.1% Triton, followed by 20 min of dye-loading with 100 nM Acti-stain 488 phalloidin (Cytoskeleton, Inc, Denver, CO) taken from a 14 µM stock prepared according to the manufacturer's instructions. Images were taken on an Olympus IX 51 with a 40x oil-immersion objective.

2.3. Elastic stiffness measurement

Cell stiffness was measured using force spectroscopy via an atomic force microscope (MFP 3D, Asylum Research, Oxford Instruments) as shown schematically in Fig. 1 A. The technique includes indentation of an AFM tip into the cell and retraction of the tip resulting in the deflection of the AFM cantilever (Fig. 1 B). Proper calibration of the AFM cantilever stiffness relates the deflection of the cantilever to force. The force-indentation curve in addition to a nanomechanical model appropriate for the material and tip shape is used to extract an elastic modulus. For our work, the Hertz model best represents the tip-sample interaction. The application of the Hertz model to extract an elastic modulus for biological materials is common, but some specific requirements must be met. The Hertz model requires a small strain assumption, which is satisfied when the AFM tip radius is sufficiently large and depth of indentation is relatively small. We modify an AFM cantilever with a silica colloid with radii of

~ 4 μm (Fig. 1 C) and limit the depth of indentation (365 nm on average) thus satisfying this requirement. Another requirement is to consider substrate effects on the measurement due to the spread nature of attached cells. Because measurements are taken perinuclear where cell thickness is near its maximum, we limit AFM tip indentations to 10% of the expected cell height for each cell type. After calibrating the AFM cantilever using the thermal tuning method to obtain the cantilever stiffness (~ 0.05 N/m), subsequent measurements are set to trigger on cantilever deflection. Approach velocity of the AFM cantilever during indentation is set at 500 nm/s and kept constant. This velocity precludes any hydrodynamic effects experienced by cantilevers at high velocities.⁵⁹ Because cells can exhibit frequency dependence,³⁵ we limit the indentation depth and set the same approach velocity so that the same probing frequency is maintained for all cells regardless of stiffness. All measurements were conducted in recording buffer solution.

2.4. Force curve analysis

Stiffness measurements were acquired on multiple cultured substrates for a given test condition and were repeated. One perinuclear location on each cell was indented for elasticity measurements. Five successive measurements were taken and the moduli values from the Hertz model were averaged with good agreement (Fig 1B). The trigger point was set to cantilever deflection indicated in Fig 1B, which ranged 5-20 nm. The amount of resulting indentation within the cell will depend on the stiffness of the cell; therefore the cantilever deflection trigger set point was varied to maintain similar indentation depths for all cell lines. The average indentation depth across all measurements was 360 ± 200 nm. The number of cells tested in each condition ranged from 33-49, an overall average of 36 cells per condition, resulting in 542 total number of cells tested.

Elastic parameters are calculated by using the Hertz nanomechanical model for an infinitely stiff colloid tip and a soft, flat sample. Material properties are extracted by applying the model to force-indentation data obtained from AFM experiments. The relationship between force, F , cell indentation, δ , probe tip radius, R , and E^* , the complex elastic modulus, is described by the following,

$$F = \frac{4}{3} E^* \sqrt{R} \delta^{\frac{3}{2}}, \quad (1)$$

where E^* is given by,

$$\frac{1}{E^*} = \frac{1 - \nu_1^2}{E_1} + \frac{1 - \nu_2^2}{E_2}. \quad (2)$$

The material parameters E_1 (Young's modulus) and ν_1 (Poisson's ratio) of the colloid (silica glass) are 68 GPa and 0.19, respectively. The cell modulus, E_2 is an outcome of the model fit using 0.37 as ν_2 , the cellular Poisson's ratio.⁶⁰ Young's moduli were obtained by a least square analysis of the force-indentation curve using routines in the Igor Pro (WaveMetrics, Inc.) software package and the material properties listed above.

Sufficient cleaning of the AFM probe tip prior to testing with a methanol rinse and ozone treatment for 15 min resulted in little to no adhesion between the probe tip and cell surface. Adhesion between the tip and cell surface would result in a retraction curve that significantly deviates from the approach curve at the end of indentation. Significant adhesion would render the Hertz model invalid and a more appropriate model such as JKR or DMT should be applied.

2.5. Statistical analysis

Moduli values exhibited a log-normal distribution as indicated by high r-squared values in quantile-quantile plots discussed below. Since a non-normative distribution exists, an arithmetic average and standard deviation would be insufficient parameters to perform tests for statistical significance. Instead, a Wilcoxon rank sum test was performed to determine statistical significance using commercially available software (MATLAB, MathWorks).

3. Results

3.1. Visual confirmation of cytoarchitectural effects of cytochalasin D and nocodazole

Imaging of the cells indicated a well spread morphology as expected two days after plating, the same time point as elastic stiffness measurements. Cancer cells tended to form colonies, especially in the case of hepatocellular carcinoma. Fluorescent staining of tubulin (Fig. 2, 3, left column) and actin (Fig. 2, 3, right column) was performed on each of the five cell lines (Fig. 2: HUVECs, chondrocytes, fibroblasts, Fig. 3: fibrosarcoma, hepatocellular carcinoma) including control cells and drug treated cells. Actin filaments in control cells showed some qualitative degree of orientation or alignment as well as clearly visible fibers across the length of the cell (tens of microns). Fluorescence was highly visible in the actin-rich cortex region of control cells. Actin staining of cytochalasin D-treated cells demonstrated the effect of the drug by the

lack of long stress fibers. Residual patches of actin left behind by depolymerized stress fibers were located with high concentration at the cell perimeter, likely indicating focal adhesion sites. Tubulin staining in control cells revealed a dense microtubule network across all cell lines. The chondrocyte cell line had the least efficient uptake of the tubulin dye, which we attribute to cell line differences. Tubulin staining in nocodazole-treated cells resulted in a much less dense network of microtubules, where often the microtubule spindle is visible. For example, white arrows in Fig 3 (Hepatocellular carcinoma) indicate a spindle-like formation of tubulin after nocodazole treatment. The concentrations were chosen such that though the molarity differs, both cytoskeletal components are destabilized by the prescribed molar concentration of each drug.

3.2. Elastic stiffness measurements exhibit a log-normal distribution

The values for elastic stiffness were compared to a standard normal distribution and a log-normal distribution. Of those two, the log-normal distribution compared more favourably with our discrete measurements. To quantify the appropriateness of a log-normal distribution to describe our elastic stiffness measurements, a quantile-quantile plot (q-q plot) was produced with a corresponding r-squared value to assess linearity. A q-q plot is used to compare two distributions for similarity; if the resulting plot is highly linear, the distributions agree, whereas the converse is true: if the resulting plot is not linear, the two distributions are likely dissimilar. Histograms and q-q plots for the HUVEC cell line are included in Fig. 4. The highly linear q-q plots demonstrate that cellular elastic moduli follow a log-normal distribution. The r-squared value reported is between a line generated between the first and third quantiles and the measurement quantiles. R-squared values generated from q-q plots comparing $\log(\text{modulus})$ values to a standard normal curve ranged from 0.87 to 0.98, thereby indicating strong agreement with a log-normal distribution. Others^{13, 25} have found modulus measurements of living cells to be log-normally distributed but dependent on cell type. We measure a strong correlation with a log-normal distribution across all five cell types. Histograms and q-q plots for the other four cell lines are included in SI.

3.3. Actin filaments contribute more than microtubules to elastic stiffness in normal cells

All elastic stiffness measurements for the five cell lines are included in Fig. 5, which also includes an overlaid standard box plot. Statistical significance is indicated by stars over the compared data sets. Since a non-normative distribution exists, a Wilcoxon rank sum test was performed to determine statistical significance; corresponding p-values are given in Table 1. There is a dramatic decrease in cell elasticity when actin filaments are targeted via cytochalasin D for HUVECs, chondrocytes, and fibroblasts. The median values drop to between 18-38% of the control value when cytochalasin D treated cells are compared to control cells for these three cell lines. Specifically, the median value of HUVEC control cells, 1.37 kPa, drops to 0.24 kPa upon cytochalasin D treatment ($p = 5e-11$), the median value of chondrocyte moduli at 2.07 kPa for control cells drops to 0.60 kPa ($p = 8e-11$), and the median value of fibroblast moduli at 2.19 kPa for control cells drops to 0.82 kPa ($p = 1e-7$). Considering the wide distribution of values for nocodazole treatment differed only slightly from controls: HUVEC median moduli value increased to 1.75 kPa ($p = 0.08$), chondrocyte median moduli value decreased to 1.98 kPa ($p = 0.47$), and fibroblast median moduli value decreased to 1.60 kPa ($p = 0.19$). Thus, destabilizing the microtubule network had little effect on the modulus for HUVECs, chondrocytes and fibroblasts; whereas destabilizing the actin filament network, decreased the measured modulus by half or more.

3.4. Destabilizing microtubule dynamics has a greater stiffness effect in cancer cell lines than normal cell lines

Moduli values for the cancer cells were less than half that of the three other cell lines. Median moduli values for fibrosarcoma and hepatocellular carcinoma cells were 0.48 kPa and 0.30 kPa, respectively. The response to cytochalasin D resulted in a decrease in the median modulus value for both the fibrosarcoma and hepatocellular carcinoma cells. The median modulus value decreased to 0.39 kPa for fibrosarcoma cells ($p = 0.70$, not significant) and to 0.21 kPa for the hepatocellular carcinoma cells ($p = 9e-4$). After nocodazole treatment, the median modulus value of both cancer cell lines *increased* to 0.97 kPa (fibrosarcoma, $p = 3e-3$) and 0.38 kPa (hepatocellular carcinoma, $p = 0.13$, not significant), respectively. While the hepatocellular carcinoma line did not show a statistically significant increase after nocodazole treatment, the data are trending in that direction. The median value for control HCC is 0.30 kPa and for HCC after nocodazole treatment it increases to 0.38 kPa. Additionally, the geometric mean is 33%

higher after nocodazole treatment. Both median and geometric mean indicate increased stiffness. While use of Student's t-test to compare HCC and HCC after nocodazole treatment results in a statistically significant metric at $p < 0.05$, we have chosen the Wilcoxon rank sum test as a very robust and distribution-independent measure of significance, which may as a result not highlight all trends. Thus, it is evident that nocodazole treatment altered cell stiffness within the two cancer lines more than in the three normal cell lines for which measurements were made.

4. Discussion

Multiple different methodologies have been used to assess and compare mechanical properties of live cells. Each type of measurement has technical advantages as well as methodological disadvantages in terms of both performance of the measurement and robustness of the measured value as a true and representative reflection of cell elasticity. There being no standardized technique, this imposes significant challenges in interpreting and comparing values of mechanical properties of live cells obtained using different methods. For example, some techniques deform the entire cell (micropipette aspiration) as opposed to relatively small, localized indentations as in AFM. Even within AFM, depending on tip shape, values for elastic moduli can be severely overestimated without a model correction.⁶¹ Our work advances previous studies by providing a head to head comparison across 15 conditions: 5 cell lines under control, actin destabilization, and microtubule destabilization, using the same AFM method, plating protocol, and nanomechanical analysis. Our report also includes a relatively large number of experiments, for which we demonstrate the lognormal distribution of values utilizing quantile-quantile plots. We compare our modulus results from each cell line to those of the literature attempting to compare with AFM experiments where extant.

4.1. Endothelial cells

Endothelial cells have a reported elastic moduli of 1-5 kPa measured by various AFM experiments.³⁸⁻⁴¹ Ingber *et al.*² showed a slight decrease in endothelial cell elasticity using magnetic twisting cytometry when nocodazole was applied though their results differed minimally from control values. Moduli measurements of our HUVECs agree with the expected range of endothelial cell elasticity, which was affected by cytochalasin D, the actin destabilizer,

while nocodazole, the microtubule destabilizer, did not have a statistically significant effect on cell stiffness.

4.2. Fibroblasts

Many groups have used fibroblasts from mouse models and primary human cell lines to study cellular mechanics due to their mechanical function within the body. Published AFM analysis of the mechanics of fibroblasts (*e.g.*, 3T3, NRK, Human dermal) include a very large range of elastic moduli values 1-100 kPa.⁴³ The higher values within this range may be caused by overestimation of the elastic modulus measured by AFM techniques with small radii tips. Large stress concentrations can occur around the sharper tip and there is added error associated with prescribing an accurate contact radius, leading often to large indentations. There are new adaptations of the Hertz model available to correct for small radii AFM tips.^{44, 61} The qualitative shift in elastic moduli due to drug treatment was shown by Rotsch *et al.*, who demonstrated a 3-fold decrease in elastic moduli with cytochalasin D treatment by mapping the elastic modulus over the surface of fibroblasts resulting in moduli on the order of 10-100 kPa.²⁸ However, drug treatments in Rotsch's study affecting microtubules, colchicine and colcemide, which disrupt the network, and taxol, which stabilizes the network, had no effect on the elasticity maps of their fibroblast cells. Our results agree with literature where cytochalasin D treatment decreases elastic moduli, but not quantitatively. We provide a lower moduli value ~ 2 kPa as compared to techniques where a sharp tip or large indentations may be causing an overestimation of the elastic modulus. We also employ the same AFM technique across our drug conditions and across four other cell lines providing a more comprehensive view of cell elasticity.

4.3. Chondrocytes

Using various experimental methods including uniaxial compression,⁴⁵ creep indentation,⁴⁶ and micropipette aspiration,^{9, 22} the elastic moduli of chondrocytes have been measured in the range 0.5-10 kPa. Chahine *et al.*⁶² measured elastic moduli of bovine chondrocytes at varying indentation depths and found the effects of 5 μ M cytochalasin B (cytochalasin B and D are both inhibitors of actin polymerization) to decrease average elastic moduli from approximately 1 kPa to less than 0.5 kPa for indentations of 500 nm. Our AFM indentations were slightly more conservative, but the application of cytochalasin D resulted in a similar decrease (76%) in

average elastic modulus similar to the differences between control and cytochalasin B application found in Chahine's study.

4.4. Cancer lines

There are a myriad of reports on the relatively lower elastic moduli of cancer cells when compared to their healthy counterparts. Park *et al.*⁴² measured the elastic modulus of transformed (0.42 ± 0.35 kPa) and normal mouse fibroblasts (1.01 ± 0.40 kPa). Efremov *et al.*⁴⁹ used AFM to characterize the mechanical properties of various fibroblastic cancers including the HT 1080 line, which resulted in an elastic modulus of 310 ± 210 Pa. Wu *et al.*⁵⁰ uses micropipette aspiration to study the mechanics of hepatocellular carcinoma subjected to various cytoskeletal drugs. Similar concentrations of cytochalasin D resulted in a reduction of elastic modulus to about one-third that of untreated HCC. Interestingly, Wu *et al.* report the elasticity of HCC to be affected by microtubule-targeted drugs: vinblastine and colchicine. At increasing concentration, the microtubule destabilizers decreased the elasticity of HCCs. The micropipette aspiration technique used measures the mechanical properties of suspended cells, where a fibrillous contractile actin cytoskeleton is not developed,²¹ whereas the AFM technique used in the present study probes attached cells, more appropriate for examining a developed actin cytoskeleton. Of the two destabilizers, nocodazole treatment did not affect stiffness values in HUVECs, chondrocytes, and fibroblasts, but had a curious stiffening effect on fibrosarcoma and hepatocellular carcinoma lines. One possible explanation is that the application of nocodazole is linked to destabilizing spindle microtubule dynamics, which is a target in many chemotherapeutic drugs to combat cancer.⁶³ By disrupting the microtubule and therefore any microtubule docking/linking processes, we effectively alter the stiffness response of fibrosarcoma and hepatocellular carcinoma.

5. Conclusion

We confirm across multiple cell lines that actin filaments, and not microtubules, contribute most to elastic stiffness in healthy cell lines. The contribution of microtubules toward overall cell elasticity is highly contested, however, we have no evidence that suggests destabilized microtubules leads to a decrease in stiffness, to the contrary, that it may in fact *increase* cell

stiffness in cells with disorganized cytoskeletal architectures such as cancer cells. Thus microtubule dynamics contributes more to elasticity in cancer cells than normal healthy cell lines. Our results provide a comprehensive platform upon which to build experimentally toward an understanding of the mechanisms by which microtubules or modifications thereof contribute to cell mechanical state. This, in turn, yields a foundation for linking mechanical and functional conditions, including cell bioenergetics in disease, susceptibility to toxins or therapeutic molecules in treatment regimens, or even metastatic potential for cancer phenotyping.

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Graphical Abstract

Figures

Fig. 1 (A) Side view schematic of an AFM cantilever over a cell, highlighting the substrate effects from measurements taken in the cell periphery. (B) Five successive force curve measurements taken on a fibroblast cell with Hertz model fit in black, approach curve in red, retraction curve in blue. Using Eq (2), average modulus value for the five curves is 1.97 ± 0.07 kPa. (C) Image from the inverted optical microscope (Nikon oil-immersion 100x objective) of a human dermal fibroblast cell underneath the AFM cantilever with colloid positioned perinuclear (outlines of cell and nucleus, and colloid for visualization).

Fig. 2 Effect of cytoskeletal destabilizers, 10 μ M Nocodazole (Noco) or 2.5 μ M Cytochalasin D (Cyto D), on HUVECs, chondrocytes, and fibroblasts. Tubulin TrackerTM was used to label microtubules. Phalloidin was used to label actin filaments. Images taken at 40 x magnification. Scale bars are 50 μ m.

Fig. 3 Effect of cytoskeletal destabilizers, 10 μ M Nocodazole (Noco) or 2.5 μ M Cytochalasin D (Cyto D), on fibrosarcoma cells and hepatocellular carcinoma. Tubulin TrackerTM was used to label microtubules. Phalloidin was used to label actin filaments. White arrows in Hepatocellular carcinoma with nocodazole treatment indicate a spindle-like formation of tubulin after treatment. Images taken at 40 x magnification. Scale bars are 50 μ m.

Fig. 4 Histogram of measured modulus values for the HUVECs (A), HUVECs with 2.5 μ M Cytochalasin D (B), and HUVECs with 10 μ M Nocodazole (C). Inset: Quantile-quantile plots (q-q plot), which are a graphical method of comparing the distribution of measured modulus values to a log-normal distribution. A highly linear q-q plot indicates similarity in the two distributions.

Fig. 5 Scatter plot of modulus values for HUVECs, chondrocytes, fibroblasts, fibrosarcoma cells, and hepatocellular carcinoma cells under control conditions (blue), 2.5 μ M Cytochalasin D treatment (red), or 10 μ M Nocodazole treatment (green), with standard box plots of 10, 25, 50, 75, and 90 percentiles. Cancer cell measurements are highlighted in the shaded region. Wilcoxon rank sum test conducted for significance (**p-value < 0.01, ***p-value < 0.0001). The following outliers are not shown: chondrocyte control 7.6 kPa, chondrocyte nocodazole 9.5 kPa, and fibrosarcoma nocodazole 5.6 kPa but are included in all tests for significance.

Table 1. P-values comparing measured elastic stiffness for each cell type and drug condition: control, cytochalasin D treatment (cytoD), and nocodazole treatment (noco) using the Wilcoxon rank-sum test. A red box is statistically significant, $p < 0.01$.

	HUVEC			Chondrocyte			Fibroblast			Fibrosarcoma			Hepatocellular carcinoma		
Drug Condition	Ctrl	CytoD	Noco	Ctrl	CytoD	Noco	Ctrl	CytoD	Noco	Ctrl	CytoD	Noco	Ctrl	CytoD	Noco
Ctrl	x	5e-11	0.08	x	8e-11	0.47	x	1e-7	0.19	x	0.70	3e-3	x	9e-4	0.13
CytoD	x	x	3e-11	x	x	6e-7	x	x	4e-6	x	x	4e-3	x	x	6e-5
Noco	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Number of Cells	33	33	33	49	35	35	38	34	37	36	33	36	41	36	33