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Single-cell mechanical analysis and tension quantification via electrodeformation-relaxation

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Abstract

The mechanical behavior and cortical tension of single cells are analyzed using electrodeformation-relaxation. Four types of cells, namely, MCF-10A, MCF-7, MDA-MB-231, and GBM were studied, with pulse durations ranging from 0.01 to 10 s. Mechanical response in the long-pulse regime is characterized by a power-law behavior, consistent with soft glassy rheology (SGR) resulting from unbinding events within the cortex network. In the sub-second, short-pulse regime, a single timescale well-describes the process, and indicates the "naïve" tensioned (prestressed) state of the cortex with minimal force-induced-alteration. A mathematical model is employed, and the simple ellipsoidal geometry allows for use of an analytical solution to extract the cortical tension. At the shortest pulse of 0.01 s, tensions for all four cell types are on the order of 10^{-2} N/m.

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Introduction

Cortical tension of cells plays ubiquitous roles in the organization of cell aggregates, tissues, and cell clusters, and in biological processes such as mechanotransduction, morphogenesis, cancer metastasis, and wound healing [1–8]. Co-active with adhesion and other mechanisms, they determine the tissue "surface tension" and cell sorting [9, 10], the "rigidity" transition in a confluent tissue [11], and the translocation behavior of a cluster of circulating tumor cells [12]. They are the key cellular-level properties affecting the collective behavior, especially in regimes where strong bonds between the cells and extracellular matrix are absent or not yet formed.

Quantifying cortical tension, however, is a challenging task, as it is a *state* variable, and subtly different although closely related to other properties such as the apparent moduli. In typical studies using AFM or pipetting methods, the force (indentation or suction pressure, respectively) is usually applied for 1-100 seconds, and the cortical tension is extracted via a cortical-shell-and-liquid-core or elastic shell model [13–18]. While these approaches indeed provide cues on the cell mechanical behavior, from a quantitative perspective, the properties already deviate from those in the "naïve", undisturbed state due to prolonged force application. For example, Trepat *et al.* demonstrated that a single transient biaxial stretch of 10% and 4 seconds can decrease cell stiffness by 50% [19]. This is not surprising - an extensive body of data exists and establishes the mechanically adaptive nature of cells, in part because the cell cortex is a "nonpermanent network" [20]. Prior studies commonly indicate different regimes of behavior as a function of force application time or frequency [18, 20–23]. In the lower-frequency regime, cell deformation follows a well-established power-law behavior with a typical exponent around $0.2 - 0.4$; in the high, the exponent may eventually approach $3/4$. From a theoretical perspective, the former is interpreted with soft glassy rheology (SGR) [24, 25], characterized by "yielding" events. In case of cells, these yielding events are presumably due to unbinding of crosslinked actin filaments [20, 22, 26–28]. On the other hand, the exponent of $3/4$ on the high-frequency side can be readily derived from a worm-like-chain (WLC) theory in the low-tension limit, and the measured properties better reflect the viscoelastic behavior of the actin filaments *per se* [29]. The demarcating frequency is typically around several hertz, corresponding to an unbinding timescale around a second [28, 30]. These prior works therefore allude to the possibility that cortical tension in its naïve state is best quantified in the moderate-to-high frequency (low force application time) regime where the structure-modifying unbinding events are not or minimally initiated.

This work tackles single-cell mechanical analysis and cortical tension quantification via electrodeformation. Electrodeformation is a contactless method where whole-cell deforma-

tion is induced via electrostatic forcing, through the application of an external direct- or alternate-current electric field [31–33]. Forces (known as the Maxwell stress) focus on the membrane/cortex, which is the primary conductance barrier separating the cytoplasm and the surroundings. Both stress and strain distributions are usually simple - an advantage that avoids difficulty in analysis due to complex geometry. Indeed, deformations are typically ellipsoidal which is the leading-order mode in spherical harmonics [34–36]. This technique is largely implemented in a microfluidic setting, and hence has the potential to achieve high throughput [37–42]. On the other hand, these studies all probe the cells in the low-frequency, SGR regime, with very long pulse durations ranging from 25 to 75 seconds. In contrast, the current work intends to achieve two objectives. In the first, we will systematically vary pulse duration by three orders of magnitudes, to quantify the frequency-dependent behavior of whole-cell deformation in this scheme, and to identify regimes of behavior. Second, with particular attention to the short-pulse (high frequency) regime, we will extract cortical tension based on the deformation-relaxation. We achieve this objective by using an analytical solution derived from a rigorous mathematical model describing the viscoelastic behavior of the cortex in a tensioned/prestressed state.

Materials and Methods

Cell Culture

MDA-MB-231, MCF-7, and MCF-10A cells were obtained from American Type Culture Collection (ATCC, Manassas, VA). Glioblastoma multiforme (GBM) cells were provided by one of our laboratories (RAF) and were previously isolated and characterized [43]. Cells were maintained in a cell culture incubator (5% CO2, 37 ï¿œC) in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum, 1% Penicillin-Streptomycin, and 1% L-Glutamine (Sigma-Aldrich). Cells were trypsinized for 5 minutes in the incubator using 0.5% trypsin/EDTA (Sigma-Aldrich) followed by centrifugation for 2 minutes at 2000 rpm (Allegra X-21, Beckman Coulter, Brea, CA) in culture media, and then twice in electrodeformation isotonic buffer containing 250 mM sucrose. The osmolarity and electrical conductivity are measured with osmometer (3D3 Osmometer, Advanced Instruments, Norwood, MA) and conductivity meter (CON 6, Oakton Instruments, Vernon Hills, IL), and adjusted to 310 mOsm/kg and 10 μS/cm, respectively. To ensure the cell viability and membrane integrity during the experiment 1% (v/v) 40 μg/ml Propidium Iodide (Molecular Probes, Inc., Eugene, OR) was added to the electrodeformation buffer.

Device fabrication and experimental Setup

Indium Tin Oxide (ITO, 140 nm) coated glass slide was purchased from Structure Probe, Inc. (SPI Supplies, West Chester, PA). The ITO coated glass slide was soaked in Acetone, Isopropanol, and DI water respectively for 10 minutes each, dehydrated in 200 ï¿œC for 30 minutes, and then a S1818 photoresist layer was deposited on top. The electrodes' gap pattern was developed by a photomask with a 35 μm-gap fabricated by CAD Art Services (CAD/Art Services, Inc. Bandon, OR). The photomask and general design followed prior work [44, 45]. The coated glass slide was exposed to UV light through a mask aligner and the parallel microelectrodes were developed onto the photoresist. Unprotected ITO regions were etched with 5% Hydrochloric acid for 15 to 20 minutes, and photoresist was removed with Acetone. An exemplary image of the chip near the electrode gap is shown in Fig. 1a.

The chip was placed on an inverted microscope (Olympus IX71, Center Valley, PA) with a 40× objective, and was connected via conductive tapes to a high-voltage, high-frequency amplifier (Trek Model PZD 350, Lockport, New York, NY), which in turn connected to a function generator (Tektronix AFG3022C, Melrose, MA, Fig. 1b). Pulses were delivered to the chip which resulted in electrodeformation. Images of the cells were recorded with a synchronized high speed-camera (pco.edge sCMOS, PCO AG, Kelheim, Germany) at 20-1,000 fps.

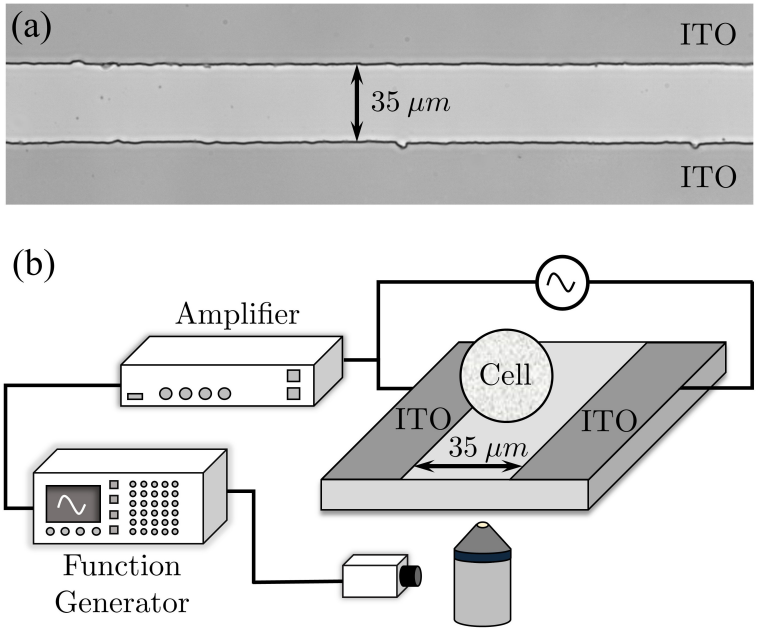

Figure 1: (a) An exemplary image of the etched ITO slide where the conductive coating is separated by a 35-μm gap. (b) A schematic of the experimental setup.

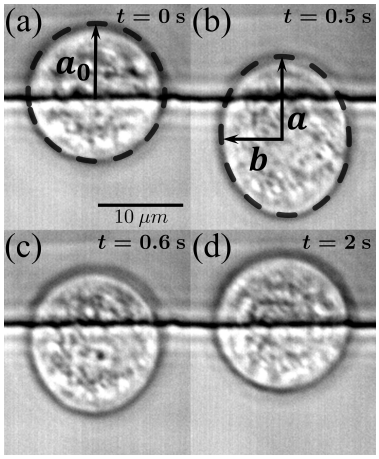

Figure 2: Exemplary images of the cell deformation-relaxation process. (a) An MDA-MB-231 cell at rest prior to the deformation pulse ($t = 0$ s, $\delta = 0$); the horizontal line is one of the electrode edges. (b) The same cell is deformed with a high-amplitude, high-frequency pulse ($t = 0.5$ s, $\delta = 0.12$, $V_{pp} = 40$ V, $f = 5$ MHz). Here a and b denote the long and short axis of the ellipse, respectively. (c) The cell begins to relax once the pulse ceases ($t = 0.6$ s, $\delta = 0.05$). (d) The cell eventually recovers its shape at the end of relaxation ($t = 2$ s, $\delta = 0$).

Electrodeformation protocol

Approximately 40 μl of cell solution (200 cells/μl) was dropped on the chip. A coverslip was gently placed on top to contain the drop, and to minimize motion due to flow. A small AC voltage ($V_{pp} = 4$ V, $f = 5$ MHz) was first applied to capture the cells near one of the electrode edges via dielectrophoresis. This minimized the translation of cells during deformation-relaxation. Subsequently, a high-amplitude, high-frequency AC pulse was applied to deform the cells (1-15 kV/cm, 5-8 MHz, 0.01-10 s). This pulse range was carefully designed to effectively deform the cells without electroporation - the high-frequency range led to small transmembrane potentials (0.06 to 0.62 V per calculation using a COMSOL simulation - see Appendix E). Upon pulse cessation, the deformed cell shape relaxed to its original shape. Before and after the electrodeformation-relaxation process, cell membrane integrity was assayed by a standard Propidium Iodide (PI) test - cases (around 5% of total) showing membrane permeabilization are not included in analysis due to the compromised structure. In addition, Joule heating is estimated to cause a temperature rise of 1-3 °C for typical pulse conditions, which we regard as negligible.

Exemplary images of cell deformation-relaxation are shown in Fig. 2. A custom-made image-processing code was developed in MATLAB (MathWorks, Natick, MA) to automatically detect the cell boundaries (dashed in Fig. 2), and also to automatically identify a and b with Fourier analysis, following one of our prior work [46].

Results

Data and Analysis

Figure 3 demonstrates exemplary deformation-relaxation process for pulse durations of $t_p =$ 0.01 and 0.5 s, respectively, for MDA-MB-231 cells. The applied voltage was 40 and 25 V (peak-to-peak, denoted by V_{pp}), respectively. Here, we use a shape factor, $\delta = a/a_0 - 1$ (see Fig. 2 for definitions of a and a_0) to quantify the degree of deformation, and data from the measurements are shown in green. We apply two different approaches of analysis to all data. The first one accords to a standard power-law model [22, 23, 41, 47–49],

$$\delta(t) = \frac{T_0}{\lambda\Gamma(\alpha+1)}[t^\alpha H(t) - (t-t_p)^\alpha H(t-t_p)], \tag{1}$$

where T_0 denotes applied stress, λ is a modulus, Γ is the Gamma function, α is the exponent, and H is the Heaviside step function. (For details see Appendix B). The second is in the form of a single-timescale deformation-relaxation,

$$\delta(t) = \frac{F_0}{4\gamma_s a_0}\sqrt{\frac{5}{4\pi}}\left[(1-e^{-\frac{t}{t_r}})H(t) - (1-e^{-\frac{t-t_p}{t_r}})H(t-t_p)\right], \tag{2}$$

where the coefficients F_0 and γ_s are force and surface viscosity, respectively, derived from a viscoelastic surface model introduced later, and t_r is the single timescale. (For details see Appendix A). In both panels, power-law fitting per (1) is shown in black-dashed, and single-exponential fitting per (2) is shown in red-dashed. The coefficients of determination, R^2, are also provided. Implications of these two different approaches of analysis are deferred to later. Note that in Fig. 3 and in general, δ may not begin and/or end in 0 in the process, denoting a deviation from sphericity in the cell's relaxed shape. This arises due to the combined effects of the cell shape and numerical errors in our imaging and edge detection algorithm. To account for this deviation, we have used an offset value ($<$0.004) in both the power-law and the exponential-fittings above.

We investigate a total of 4 cell types, namely, MCF-10A, MCF-7, MDA-MB-231, and GBM, following the protocol established above. For each case, we vary the pulse duration, t_p, from 0.01 to 10 s, spanning 4 orders of magnitude. For MCF-10A, MCF-7, MDA-MB-231 and GBM, the total number of measurements are 42, 47, 52 and 42 respectively. The details on the number of repeats for each pulse duration is tabulated in Table F.1. For all cases we also vary the amplitude of the applied voltage, and found a consistent correlation between the maximum degree of deformations and the electric stress (Appendix D, Fig. D.1). However, key quantity, such as the exponent α from the power-law model, depends primarily on the

pulse duration t_p, which we demonstrate in Fig. 4a. Evidently, α assumes the highest value for the shortest pulse duration, ranging from 0.48-0.58 for all cell types. As t_p increases, α decreases to the range of 0.27-0.4 at t_p =0.1 s, but no obvious trend is observed beyond this duration.

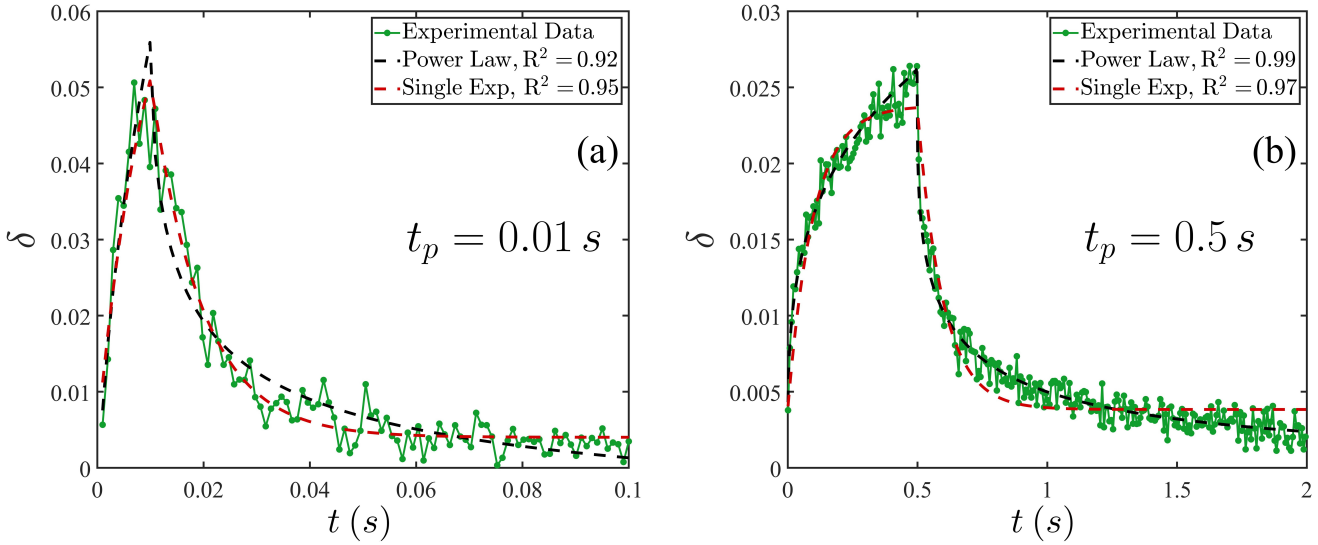

Figure 3: Evolution of the shape factor for two different pulse durations for a single MDA-MB-231 cell. Here $\delta = a/a_0 - 1$ (see Fig. 2). (a) $t_p = 0.01$ s, $V_{pp} = 40$ V, $f = 5$ MHz. (b) $t_p = 0.5$ s, $V_{pp} = 25$ V, $f = 7$ MHz. For both cases, two analytical strategies are attempted: power-law (black) and single-exponential (red). The coefficients of determination, R^2, are provided for both cases.

We also quantify the error of fitting for both models. Fig. 4b shows the RMS error differences between the data and the fitting (denoted RMSE) for MCF-7. In general, The power-law model performs better for longer pulse durations, whereas the single-timescale model demonstrates more accuracy for t_p shorter than 0.1 s. This trend is consistently corroborated in both MDA-MB-231 and GBM cells, shown in Fig. F.1. For MCF-10A, the two approaches provide comparable results for $t_p \leq 1$ s, whereas the superiority of the power-law fitting becomes only evident for the longer pulse durations of 5 and 10 s (see Appendix F).

These results corroborate with prior work [20–23, 28, 50] that distinguishable regimes exist in the response of cells to external mechanical forcing. In the limit of long timescales, the soft glassy rheology (SGR) theory is commonly accepted, which predicts the power-law behavior [24, 25]. For this regime ($t_p \geq 0.1$ s), our power-law exponents are consistent with those reported previously [49, 51–53]. On the other hand, for the shorter timescales, the material properties of the cell cytoskeleton are thought to be mediated by the elastic

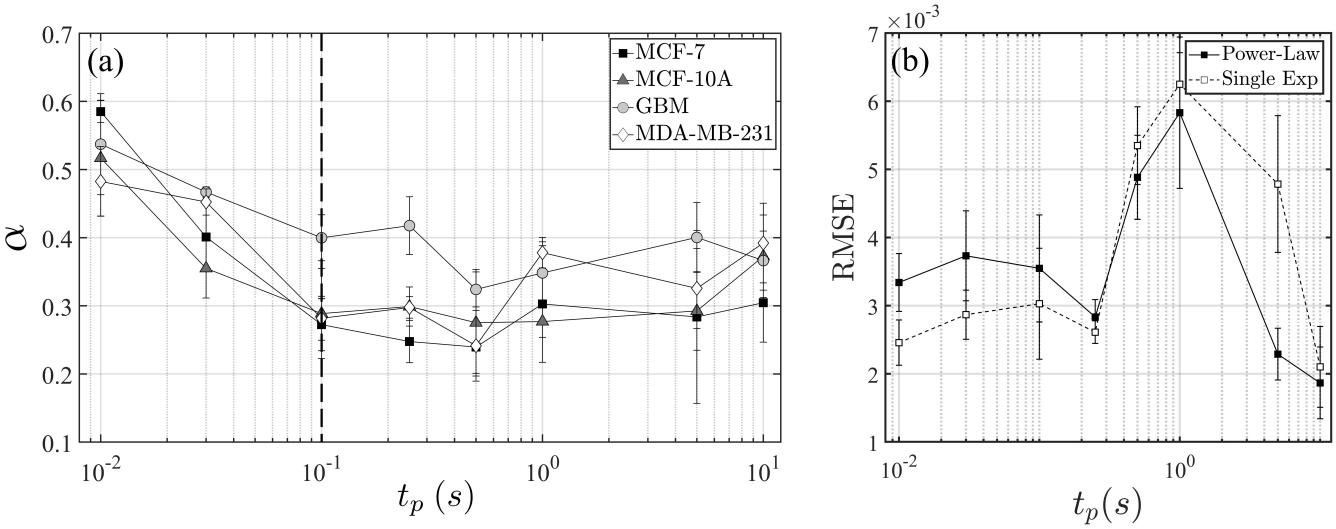

Figure 4: (a) The power-law exponent, α, versus pulse duration, t_p. Error bars indicate standard deviation. The number of cells examined in each data point is tabulated in Table F.1. (b) Error quantification (RMS error, or RMSE) for MCF-7. Results for other cell types are found in Fig. F.1.

response of the actin filaments in conjunction with thermal fluctuation [21, 29, 54]. In particular, our data indicate that in general, a single-timescale fitting outperforms power-law fitting. This timescale reflects the mechanical state of the actin filaments themselves without the structure-modifying unbinding events, and is consistent with the behavior predicted by Broedersz *et al.* [20] for intermediate frequency ranges (further discussed in the Discussion section). The two regimes are demarcated by a value of t_p around a fraction of a second, in agreement with those reported in literature, namely, around 0.1-1 s [27, 28, 30, 50, 55–58]. Note, however, transition between the regimes is gradual, and selection of the threshold value is approximate.

Model interpretation

We further elaborate on the two model analyses based on the observation above. Both evidences from data and prior theory indicate that a single-timescale model is more appropriate for the shorter pulse times. We present a spatially resolved analysis assuming that the cell cortex is a viscoelastic "membrane" (in the sense of a mechanical entity) with surface tension (Fig. A.1). This model allows us to extract cortical tension in a regime better capturing properties in the "naïve" state without structural modification. For the longer timescales, we will use a standard power-law model, in which the power-law behavior derives from the structure-modifying "yielding" events per standard SGR model. Details are presented below.

The surface tension model. In the first, we focus on the cell cortex, which is assumed to be an infinitesimally thin shell with a surface tension, γ_s , and a surface viscosity η_s (Fig. A.1). In the regime of small-to-moderate deformation, the problem can be solved analytically for the dominant, ellipsoidal mode as the leading order term in a spherical harmonic expansion [34, 59]. The full governing equations are reduced to a single ordinary differential equation,

$$\frac{4}{3}\eta_s\dot{X}_2(t) + 4\gamma_s X_2(t) = F_0 H(t_p - t), \tag{3}$$

where X_2 is the coefficient of spherical harmonic model $Y_{2,0}$, and is related to δ via

$$X_2 = \sqrt{4\pi/5}a_0\delta. \tag{4}$$

On the right-hand side, F_0 corresponds to electrostatic forcing in the $Y_{2,0}$ mode, which is calculated using a COMSOL simulation capturing the electrode geometry and pulsing conditions realistically (Appendix E). The Heaviside function takes into account that the pulse has a finite duration of t_p. Details on the model and derivation are found in Appendix A as well as our recent work [59]. Solving (3) and considering (4) reveals (2) as the final solution used for fitting, and the timescale is

$$t_r = \frac{\eta_s}{3\gamma_s}. \tag{5}$$

Figure 5 summarizes results analyzed with this model, in which we temporarily focus on the 3 shorter pulse durations, namely, $t_p = 0.01$, 0.03, and 0.1 s. More complete results are shown in Fig. A.2 in Appendix A and Fig. 7 below. In Fig. 5a, the timescale t_r is extracted. The values for the first two pulse durations are comparable, and does not depend on the applied voltages/electric fields. Appreciable increases are demonstrated at $t_p = 0.1$ s, which we speculate correlates with the onset of actin crosslink unbinding and transition to the power-law (SGR) regime.

The availability of t_r and F_0 allows us to extract the mechanical properties γ_s and η_s, which are shown in Fig. 5b and c. Using this analysis, cortical tension demonstrates values in the range of 10^{-2}-10^{-1} N/m, whereas surface viscosity is on the order of 10^{-3} Pa $\cdot$ s $\cdot$ m.

The power-law model. We now turn to the power-law model, in which we used a lumped stress-strain relation but with fractional derivative:

$$\lambda D_t^\alpha(\delta(t)) = T_0 H(t_p - t),$$

where $D_t^\alpha(\cdot)$ is Riemann's fractional derivative, T_0 is constant applied stress, δ is strain, α

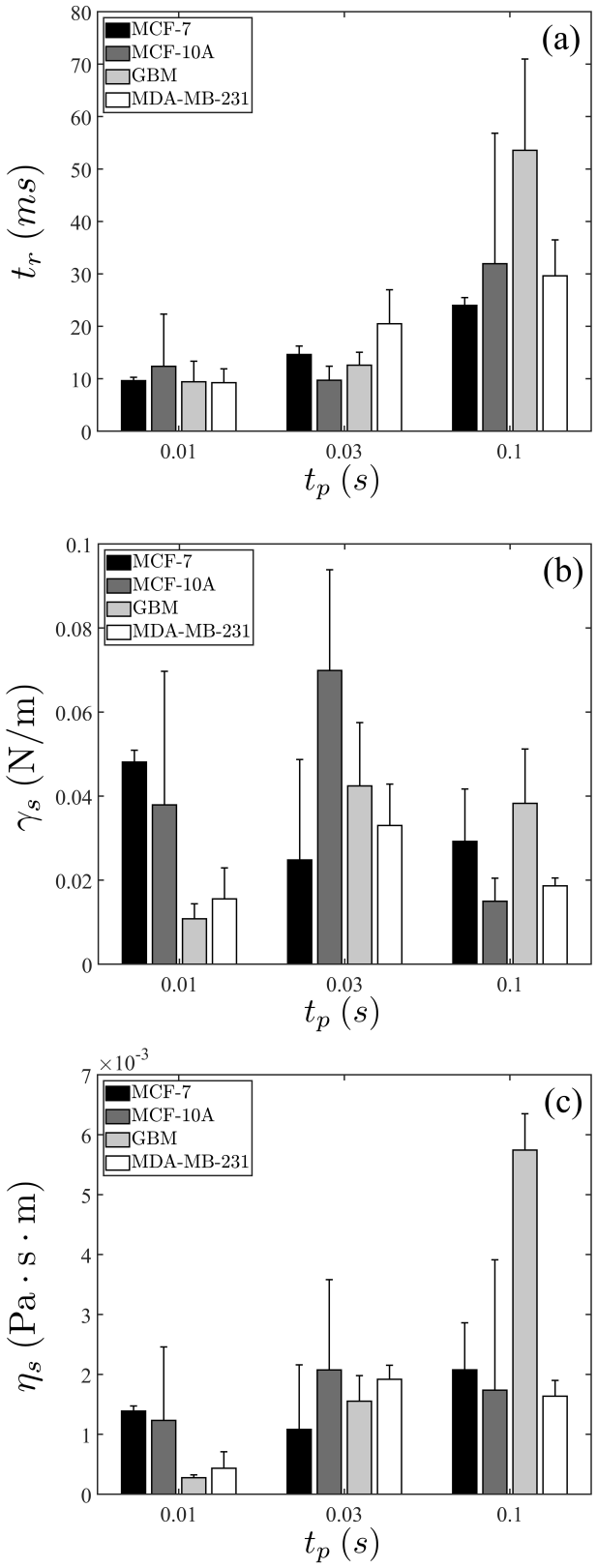

Figure 5: (a) Extracted timescale, t_r. (b) Surface tension, γ_s, and (c) surface viscosity, η_s. Error bars indicate standard deviation. Number of repeats is provided in Table F.1.

is the power exponent, and λ is the corresponding module in power-law regime which is constant. Solution using a Laplace transform leads to the creep response (1), details are elaborated in Appendix B. Data analysis leads to the extraction of α, shown in Fig. 4a. The combination T_0/λ can also be determined, but not independently. We thus again can resort to simulation to compute T_0, and to subsequently extract modulus λ (Appendix E). However, following prior work, we more conveniently transform to the frequency domain so that the storage and loss moduli are

$$E^{'} = \lambda\,\omega^{\alpha}\cos(\pi\alpha/2), \tag{6}$$

$$E^{''} = \lambda\,\omega^{\alpha}\sin(\pi\alpha/2), \tag{7}$$

where the frequency is calculated as $\omega = 2\pi/t_p$, and the magnitude of the complex modulus is

$$E_0 = \lambda\,\omega^{\alpha}.$$

The loss tangent is related to the power exponent via

$$\eta = \frac{E''}{E'} = \tan(\pi\alpha/2). \tag{8}$$

This is a simple, monotonic relationship relating η with α, and hence we do not show results on the former for brevity. On the other hand, extracted values of E_0 for $t_p \geq 0.1$ s are shown in Fig. 6. Despite more significant variabilities are present in the data in this regime, we observe that E_0 values are appreciably greater for $t_p = 0.1$ and 0.25 s, particularly for MCF-7, and decrease to the 1-10 kPa range when t_p assumes longer durations.

The above trends become more apparent when we apply the model analysis to all cell types with all pulse durations (regardless of the relative model accuracy and validity in the pulsing regimes). These results are presented in Fig. 7. In general, both γ_s and E_0 decrease with an increasing t_p while η_s increases. These trends again reflect transitional behavior from the elastic to the SGR regime, where cortical strength weakens and effective viscosity increases. Further discussions on these trends and comparisons with those in literature are found in the next section.

Finally, it would be of interest to directly compare results from the two models. For this purpose, we first convert surface tension and viscosity to effective, lumped elastic modulus and viscosity via (Appendix C),

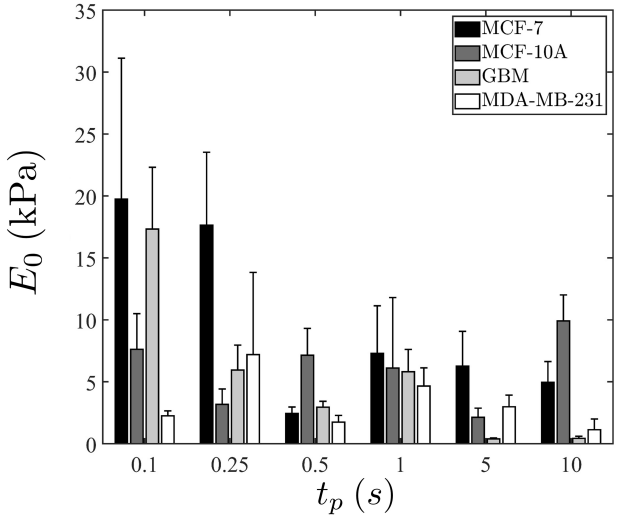

Figure 6: Extracted E_0 for $t_p \geq 0.1$ s. Error bars indicate standard deviation. Number of repeats is provided in Table F.1.

$$E'_{eff} = \frac{24}{23}\frac{\gamma_s}{a_0}, \tag{9}$$

$$\mu_{eff} = \frac{8}{23}\frac{\eta_s}{a_0}. \tag{10}$$

Note that these quantities are effectively averages over the entire cell, which also facilitate comparison with similar bulk measurements from literature below. On the other hand, they are different from the effective cortex modulus which is obtained by scaling with cortical thickness [18, 60]. The magnitude of the complex modulus is

$$E_{0,eff} = \sqrt{{E'_{eff}}^2 + {E''_{eff}}^2}, \tag{11}$$

$$E''_{eff} = \omega\mu_{eff}. \tag{12}$$

Results suggest that both total and loss moduli are in good agreement. On the other hand, the power-law model tends to overestimate the elastic modulus by several times, in particular in the short-pulse regime. We thus conclude that the single timescale, surface-based model (2) is not only appropriate, but necessary for valid quantitative mechanical analysis in the intermediately-high frequency regime.

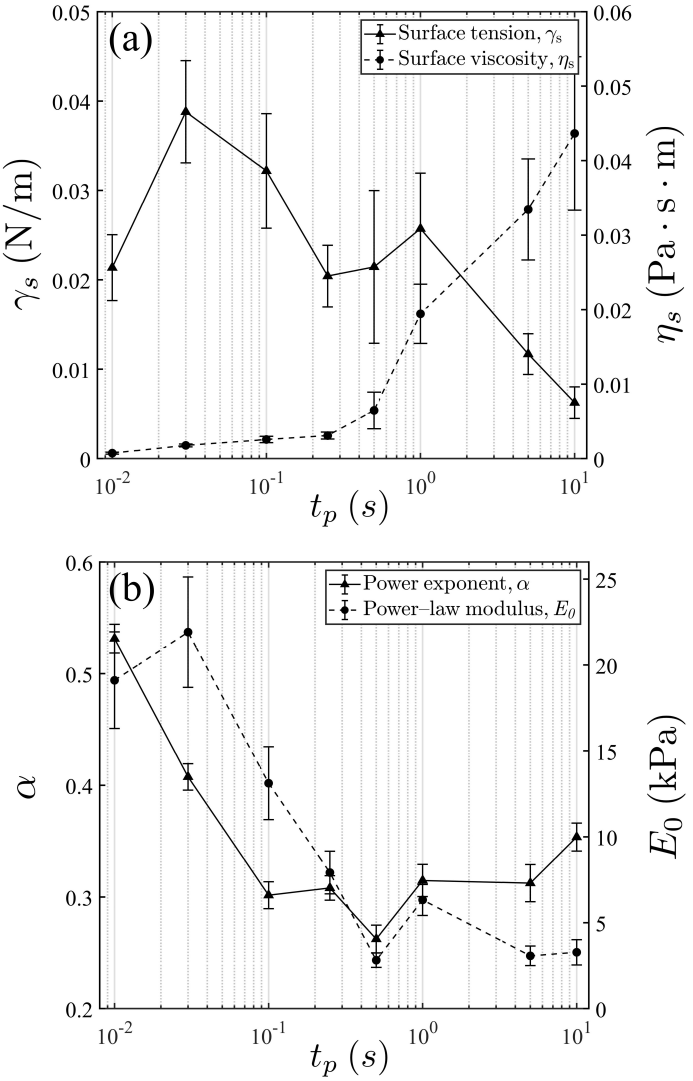

Figure 7: Pooled results for all cell types and pulse durations. (a) γ_s and η_s from the surface tension model. (b) α and E_0 from the power-law damping model. Error bars indicate standard error. Number of repeats is provided in Table F.1.

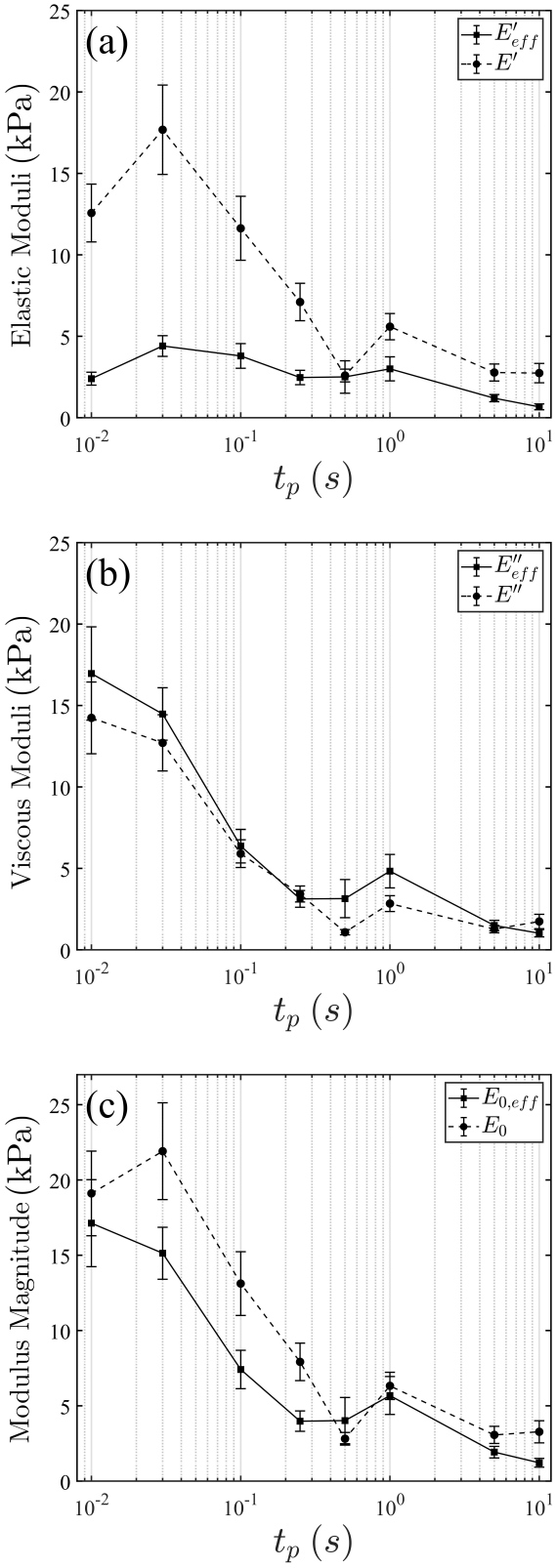

Figure 8: Direct property comparison between the two models. Error bars indicate standard error. Number of repeats is provided in Table F.1.

Discussion

Cortical tension in the short-pulse regime

In the above, we observe two regimes consistent with understanding in the literature: an SGR regime that is characterized by a low power exponent α at the long pulse durations (lower frequencies), and a regime at the short pulse durations (higher frequencies) where the response is characterized by a single timescale. Indeed, an interpretation is provided by prior work that this is because in this regime unbinding is not initiated, modes longer than cross-link spacing are suppressed, so that "only small-scale bending fluctuations between cross-links can relax" [20]. Consequently, the theory also predicts a plateau in E' [20], which is observed in, for example, [61]. In our data (Fig. 8a), even though such a plateau is not rigorously seen, we do observe a slight decrease in E'_{eff} toward the shortest pulse, $t_p = 0.01$ s. It is unclear from the data whether this is due to its intrinsic large variability, or this decrease is actually mechanistically driven. We are not observing the high-frequency regime where $\alpha = 3/4$, even if we force a power-law analysis (Fig. 4a). Similar to [28], we speculate that our shortest pulse duration, $t_p = 0.01$, is not sufficient to reach that regime, although we do see α values are higher around 0.48-0.58. On the other hand, relaxation of the bending-fluctuations may be a cause of the weak dependence on shown t_p in Fig. 7a at the short pulse times.

One particular thesis of the current work is that cortical tension is more faithfully quantified in the short-pulse regime. The rationale is straightforward, given the above data trend as well as previously established theories. We aim to establish that under short-pulse ($\sim$0.01 s), small-amplitude (several percents of strain) electrodeformation, the extracted tension/prestress reflects that in a state where the cortical structure is close to the undisturbed state.

Comparison with literature values

A vast body of literature exists on measuring mechanical properties of cells. Importantly, a recent study by Wu *et al.* systematically examined the properties of MCF-7 with various techniques, and observed that moduli vary by as much as 3 orders of magnitude, depending on the particular method, the state of the cells (attached or suspended), the target (partial membrane, cortex, or whole-cell), and interrogation strength and frequency [62]. Indeed, this reflects the very complex and adaptive nature of cells as a living mechanical entity.

In the literature, the cortical tension is commonly measured with the micropipette aspiration technique, which reports values in the range of 30-3000 pN/μm for various cell types

Source	Cell type	Properties (kPa)	Timescale (s)	This Work (kPa)	t_p (s)
[62]	MCF-7	$E'_{eff} = 0.018 \pm 0.024$	8	1.87 ± 0.23	10
[62]	MCF-7	$E' = 0.95 \pm 0.15$	1	6.38 ± 3.38	1
[37]	MCF-7	$E'_{eff} = 0.358 \pm 0.053$	25	1.87 ± 0.23	10
[37]	MDA-MB-231	$E'_{eff} = 0.327 \pm 0.052$	25	0.35 ± 0.21	10
[51]	MCF-7	$E'_{eff} = 2.1 \pm 0.1$	0.1	7.10 ± 1.52	0.1
[51]	MDA-MB-231	$E'_{eff} = 0.8 \pm 0.19$	0.1	0.70 ± 0.11	0.1

Table 1: Comparison with prior data in similar frequency ranges. Here timescales refer to force application times in optical stretching/parallel-plate rheometry (first and second row, respectively) [62], electrodeformation (third and fourth rows) [37], and residence time translocating a constriction (fifth and sixth rows) [51]. For properties, notations follow those of this work (the last two columns).

[14, 60, 63, 64], which is in general weaker than extracted values by this work. Trends from the current work suggest that this may be due to the much longer force application times, e.g., a few hundred seconds for typical aspiration measurements [16, 60, 65]. On the other hand, measurements from Real-time deformability cytometry do reveal tension of 0.02 N/m, matching the current results [66]. Note that interestingly, an upper cut-off time for the power-law regime was also observed in [18].

Against those here we only selectively compare our results on elastic modulus with the most similarity in configuration, namely, whole-cell measurements in similar frequency range, and with the same cell types. The results are summarized in Table 1, and depends on cell type. For MDA-MB-231, our data is in good agreement with prior work measured with different techniques [37, 51]. On the other hand, properties for MCF-7 are greater in value when compared with those from other work, by several times or even order of magnitude. The cause of this difference is unknown, yet one possible difference lies within force distribution on the whole-cell level, e.g., when comparing optical streching and plate rheometry with electrodeformation. On this aspect, the latter has a comparative advantage: both the stress and strain fields have a relatively simple, cosinusoidal distribution to the leading order, and hence allow spatially-resolved model construction (Appendix A).

Note that although at each pulse duration we do observe differences in the cell types, a consistent trend is not seen at all pulse times. On the other hand, the variation with respect to t_p provides the major variability in the system, and such is the rationale of pooling data from all cell types as a function of pulse time in Fig. 7. Further controlled study via various drug treatment such as those following [18, 33, 51] will help shed light on the biological regulators of cortical tension and genotype similarities and/or differences.

Conclusions

In this work, we present an electrodeformation-relaxation assay to probe mechanical properties of whole, suspended cells. We vary pulse duration by 4 orders of magnitudes, from 0.01 to 10 s, which is equivalent to a frequency range of approximately $\omega \sim$ 0.6-600 rad/s (or $f \sim$ 0.1-100 Hz). Expectedly, mechanical properties depend strongly on pulsing time. We observe an SGR regime characterized by a low-exponent power-law behavior in the long-pulse regime, whereas we are able to capture a single-timescale deformation-relaxation behavior with sub-second pulse durations. Within the simplifications and using a rigorous, spatially-resolved (versus lumped) mathematical model, we extract cortical tension that closely approximates that in the naïve cell state - the state that is the least mechanically disturbed. This work demonstrates that electrodeformation can be developed as a contactless technique to rapidly assay cell mechanical properties in a wide frequency range, and to analyze tension statistics using its short-pulse capability.

Appendix A. The surface tension model

Consider a spherical cell of radius a_0 in a fully relaxed state and the displacement is denoted by $\mathbf{u}(\cdot, t) : \partial B \to \mathbb{R}^3$ ($B = \{\mathbf{x} \in \mathbb{R}^3 : |\mathbf{x}| = a_0\}$). The elastic energy, $\mathcal{E}$, and the dissipation potential, $\mathcal{D}$, of the cortex are given by:

$$\mathcal{E}[\mathbf{u}] = \int_{\partial B} \frac{1}{2} \nabla_s \mathbf{u} \cdot \mathbb{C}_\mathrm{s} \nabla_s \mathbf{u}, \tag{A.1}$$

$$\mathcal{D}[\mathbf{u}] = \int_{\partial B} 2\eta_s \left| \frac{1}{2} \left[\nabla_s \dot{\mathbf{u}} + (\nabla_s \dot{\mathbf{u}})^{\mathrm{T}} \right] \right|^2 \mathrm{d}S, \tag{A.2}$$

where $\mathbb{C}_\mathrm{s}$ is the surface elasticity tensor which is proportional to surface tension, γ_s, η_s represent surface viscosity associated with the cortex (Fig. A.1), and ∇_s is surface gradient [59]. Moreover, assuming conservation of the cell interior volume and local surface area at the leading order imply the constraints:

$$\nabla_s \cdot \mathbf{u} = \nabla_s \cdot \dot{\mathbf{u}} = 0 \qquad \text{on } \partial B. \tag{A.3}$$

In terms of spherical harmonic modes and assuming axisymmetry, E and D are given by:

$$\mathcal{E}[\mathbf{u}] = \sum_{l=2,4,6,\ldots}^{\infty} \gamma_s \left(\frac{l(l+1)-2}{2}\right) X_l^2, \tag{A.4}$$

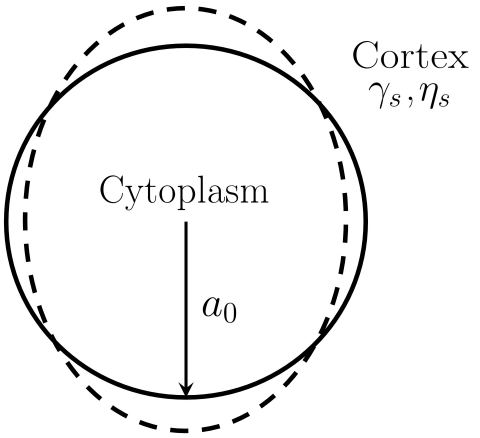

Figure A.1: A model schematic: The cell is simplified as an infinitesimally thin, viscoelastic cortex with cortical tension, γ_s, and surface viscosity, η_s.

$$\mathcal{D}[\mathbf{u}] = \sum_{l=2,4,6,\ldots}^{\infty} \eta_s \frac{2(l(l+1)-2)}{l(l+1)} \dot{X}_l^2, \tag{A.5}$$

where X_l is the l^{th}-mode coefficient of the radial displacement, u^r,

$$X_l = \frac{1}{a_0^2} \int_{\partial B} u^r(R,\theta) Y_l(\theta)\, \mathrm{d}S, \tag{A.6}$$

$$u_r = \sum_{l=2,4,6,\ldots}^{\infty} X_l(r) Y_l.$$

Let $\mathbf{t}$: $\partial B \to \mathbb{R}^3$ be the surface traction on the cell. The rate of work done by the force is given by:

$$\dot{\mathcal{W}} = \int_{\partial B} \mathbf{t} \cdot \dot{\mathbf{u}}\, \mathrm{d}S = \sum_{l=2,4,6,\ldots}^{\infty} \left[t_l^r + \frac{2}{l(l+1)} t_l^\theta \right] \dot{X}_l, \tag{A.7}$$

where t_l^r and t_l^θ are the l^{th} modes in radial and tangential traction, respectively,

$$t_l^r = \int_{\partial B} t^r(R,\theta) Y_l(\theta)\, \mathrm{d}S, \tag{A.8}$$

$$t_l^\theta = \int_{\partial B} t^\theta(R,\theta) Y_l(\theta)\, \mathrm{d}S. \tag{A.9}$$

By neglecting the higher modes of spherical harmonics, the balance of work for the system

in the second mode (ellipsoidal) leads to:

$$\dot{\mathcal{W}} - \frac{\mathrm{d}}{\mathrm{d}t}\mathcal{E}[X_2(t)] = \mathcal{D}[X_2(t)], \tag{A.10}$$

or

$$\frac{4}{3}\eta_s \dot{X}_2(t) + 4\gamma_s X_2(t) = t_2^r + \frac{1}{3}t_2^\theta = F_0\, H(t_p - t) \tag{A.11}$$

where F_0 denotes the total electrostatic force exerted on the cell cortex, and H is the Heaviside step function to capture the effects of a finite pulse time. The following relationship converts between δ, the shape factor, and X_2,

$$X_2 = \sqrt{4\pi/5}\, a_0 \delta. \tag{A.12}$$

In (A.11), the traction terms are to be evaluated from the Maxwell stress tensor induced by the applied electric field [67, 68],

$$\mathbf{T} = \epsilon(\mathbf{E}\mathbf{E} - \frac{1}{2}|\mathbf{E}|^2\mathbf{I}), \quad \mathbf{t} = \mathbf{T}\mathbf{e}_r, \tag{A.13}$$

where ϵ is electrical permittivity. Solving Eq. (A.11) with constant traction (applicable to our studies) yields

$$\delta(t) = \frac{F_0}{4\gamma_s a_0}\sqrt{\frac{5}{4\pi}}[(1 - e^{-\frac{t}{t_r}})H(t) - (1 - e^{-\frac{t-t_p}{t_r}})H(t - t_p)], \tag{A.14}$$

where the deformation-relaxation timescale is given by

$$t_r = \frac{\eta_s}{3\gamma_s}. \tag{A.15}$$

The calculated values with this model for $t_p \leq 0.1$ for each cell type is provided in Fig. 5 in the proper text. However, to provide complete data, we provide the trends of changes for these properties in relatively longer pulse durations ($t_p > 0.1$) in Fig. A.2.

Appendix B. The power-law model

In the power-law regime, the lumped stress-strain relation is given by:

$$T(t) = T_0 H(t_p - t) = \lambda D_t^\alpha(\delta(t)), \tag{B.1}$$

where $D_t^\alpha(f)$ is the Riemann's fractional derivative, T_0 is a constant applied stress, δ is strain

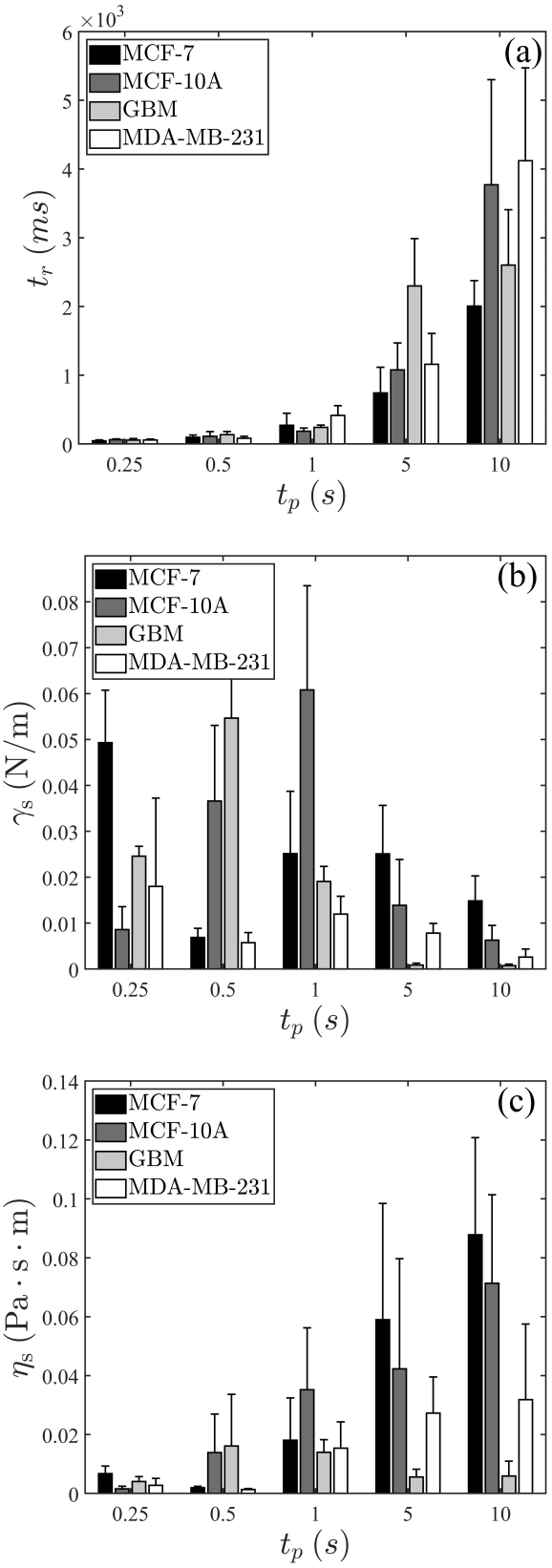

Figure A.2: (a) Extracted timescale, t_r. (b) Surface tension, γ_s, and (c) surface viscosity, η_s for $t_p > 0.1$. Error bars indicate standard deviation. Number of repeats is provided in Table F.1.

(quantified by $a/a_0 - 1$ in our case), α is the power exponent, and λ is the modulus. The creep response is obtained using a Laplace transform.

$$\mathcal{L}\{T(t)\} = \lambda \mathcal{L}\{D_t^{\alpha}(\delta(t))\}, \tag{B.2}$$

$$\delta(t) = \frac{T_0}{\lambda \Gamma(\alpha+1)}[t^{\alpha}H(t) - (t-t_p)^{\alpha}H(t-t_p)], \tag{B.3}$$

where T_0 is determined via

$$T_0 = \frac{F_0}{\pi a_0^2}. \tag{B.4}$$

Rewriting Eq. (B.1) in the frequency domain using Fourier transform provides the storage ($E^{'}$) and loss moduli ($E^{''}$),

$$E^{'} = \lambda\, \omega^{\alpha} \cos(\pi\alpha/2), \tag{B.5}$$

$$E^{''} = \lambda\, \omega^{\alpha} \sin(\pi\alpha/2), \tag{B.6}$$

where ω is frequency ($\omega = 2\pi/t_p$ for our case). The loss tangent or structural damping coefficient, η, is given by:

$$\eta = \frac{E^{''}}{E^{'}} = \tan(\pi\alpha/2). \tag{B.7}$$

The modulus E_0 is related to λ via

$$E_0 = \lambda\, \omega^{\alpha}. \tag{B.8}$$

Appendix C. Effective elastic and viscous modulus

Based on a general model in [59], if the cell is to be considered a bulk material with an effective elastic modulus, E'_{eff}, and bulk viscosity, μ_{eff}, the energy functionals are

$$\begin{aligned} \mathcal{E}_{bulk}[\mathbf{u}] &= \int_B E^{'}_{eff} \left| \frac{1}{2}\left[\nabla \mathbf{u} + (\nabla \mathbf{u})^{\mathrm{T}}\right] \right|^2 \mathrm{d}V = \int_{\partial B} \frac{1}{2} E^{'}_{eff} |\nabla_s \mathbf{u}|^2 \,\mathrm{d}S \\ &= a_0 \sum_{l=2,4,6,\ldots}^{\infty} E^{'}_{eff} \left(\frac{2l^3+3l^2-5}{2l(l+1)}\right) X_l^2, \end{aligned} \tag{C.1}$$

$$\mathcal{D}_{bulk}[\mathbf{u}] = \int_B 2\,\mu_{eff}\left|\frac{1}{2}\left[\nabla\dot{\mathbf{u}} + (\nabla\dot{\mathbf{u}})^{\mathrm{T}}\right]\right|^2 \mathrm{d}V = \int_{\partial B} \mu_{eff}|\nabla_s\dot{\mathbf{u}}|^2\,\mathrm{d}S$$
$$= a_0 \sum_{l=2,4,6,\ldots}^{\infty} \mu_{eff}\left(\frac{2l^3+3l^2-5}{l(l+1)}\right)\dot{X}_l^2. \tag{C.2}$$

Comparing the above with (A.4, A.5) for second mode of spherical harmonics, $l = 2$, we have

$$E'_{eff} = \frac{24}{23}\frac{\gamma_s}{a_0}, \quad \mu_{eff} = \frac{8}{23}\frac{\eta_s}{a_0}.$$

Note that as the coefficient of 24/23 is very close to 1, one can conveniently convert between storage modulus and surface tension via a simple estimate, $E'_{eff} \sim \gamma_s/a_0$.

Appendix D. Deformation vs. applied voltage

We performed limited experiments to confirm the scaling law of deformation and electrostatic forcing. The Maxwell stress scales with $|\mathbf{E}|^2$ per (A.13), therefore we expect that deformation scales with V_{pp}^2, although the field distribution is non-uniform (see Appendix E below). Exemplary results are demonstrated with 4 MDA-MB-231 cells in Fig. D.1, where indeed a general correlation between the maximum strain, δ_{max}, and V_{pp}^2 is observed.

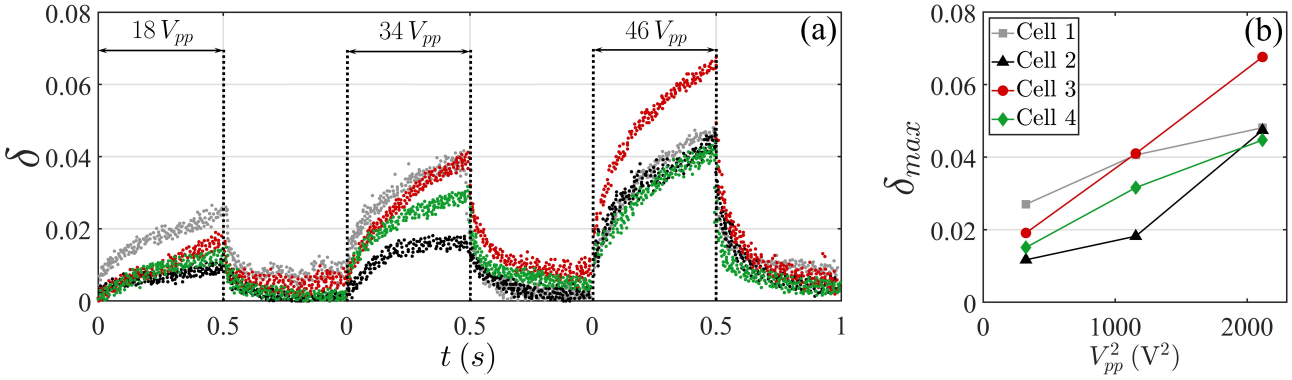

Figure D.1: (a) Shape factor changes of four different MDA-MB-231 cells under consecutively increasing pulsing, each 0.5 s at 7 MHz. (b) The maximum deformation achieved at the end of pulsation, δ_{max}, shows approximately a linear correlation with V_{pp}^2.

Table E.1: Parameters for simulation

Domain	Relative permittivity	Conductivity (S/m)	Thermal Conductivity (W/m·K)	Heat Capacity (J/kg·K)
media	80	10^{-2}	0.611	4180.9
cytoplasm	80	0.4	0.611	4180.9
membrane	2	5×10^{-9}	—	—

Appendix E. Numerical simulation

Simulation is performed with COMSOL Multiphysics (COMSOL Inc, Burlington, MA), and includes studies on electric field, Maxwell stress, and Joule heating. Electric Currents and Heat Transfer in Fluids modules with both transient and frequency domain studies are employed. A $350 \times 350 \times 150\,\mu$m-size-box with electrodes on the bottom surface, spacing $35\,\mu m$ apart, is used as geometry to realistically simulate the chip setup (Fig. E.1). A spherical cell of given radius is seated on the bottom near one of the electrodes; the location is not arbitrary, but is found as a translational force equilibrium for dielectrophoresis, approximating realistic physics. In the simulation, deformation is not considered: electric field distribution in ellipsoids with small deformations (a strain of only a few percents, $\delta \sim 10^{-2}$) presents a negligible deviation [36, 69, 70].

Properties and parameters are listed in Table E.1. The electrical conductivity of the suspending media is measured as described in the proper text. The membrane and cytoplasmic properties are chosen from literature [71–76]. Permittivity, density, and thermal properties of the media and cytoplasm are assumed to be that of water.

Total traction, F_0, as defined in Eq. (A.11) is computed as a function of cell radius, applied voltage, and frequency, and results are shown in Fig. E.2. Variations in parameters are based on the reference case of $a_0 = 7.5$ μm, $V_{pp} = 100\,V$, and $f = 5$ MHz. Note that the relationship with respect to radius and V_{pp}^2 are almost linear (this is expected), and the results allow us to use the correlations as a quick "lookup" table without repeated, additional simulations.

The effect of Joule heating is also evaluated, and an exemplary temperature map for $a_0 = 7.5$ μm, $V_{pp} = 50\,V$, $f = 5$ MHz, and $t_p = 10$ s is shown. The maximum temperature rise is 3.4 °C and is considered insignificant in modifying cortical mechanical response.

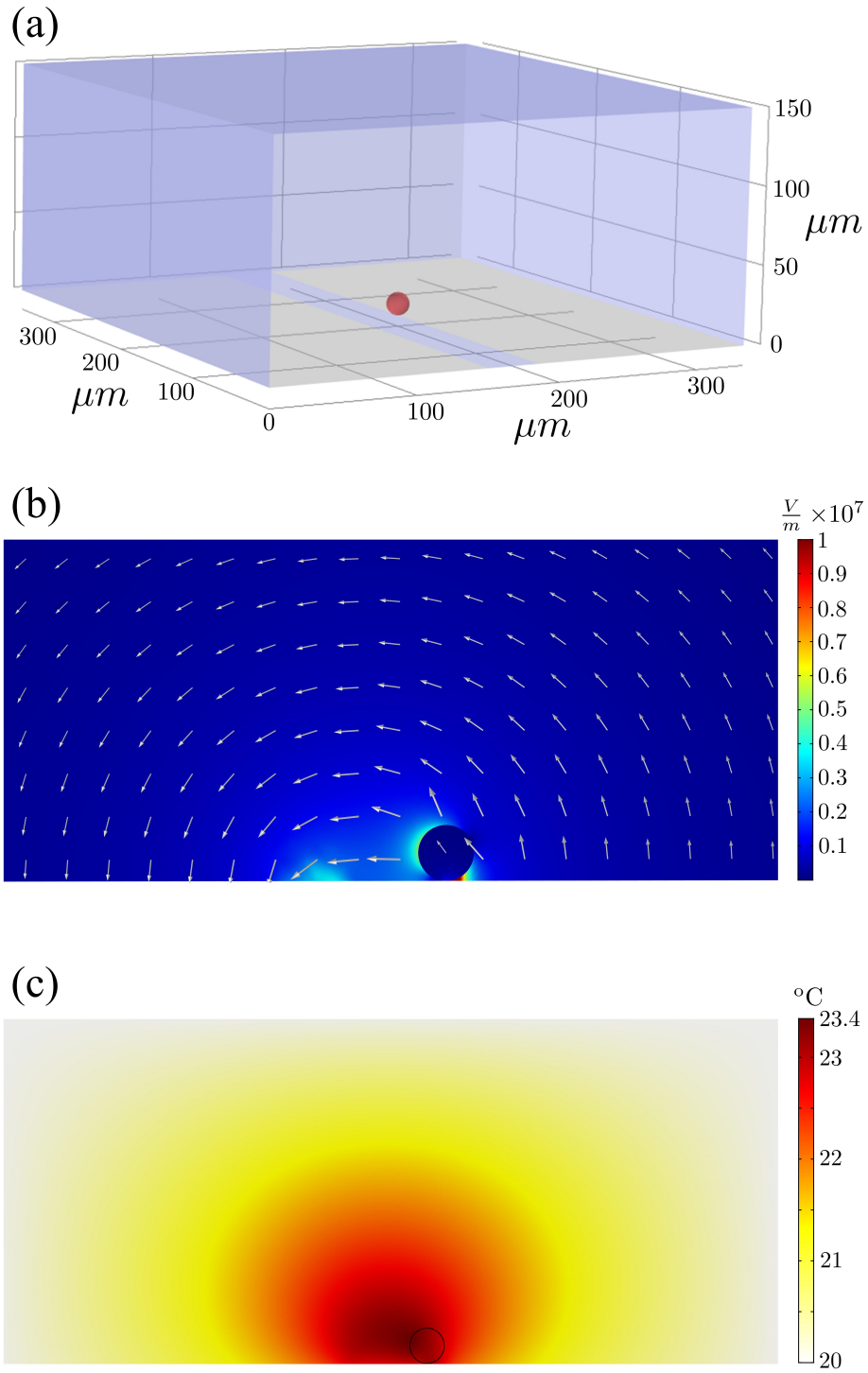

Figure E.1: (a) Model geometry. (b) Exemplary electric field distribution. (c) Exemplary heat map due to Joule heating; the ambient temperature is assumed to be 20 °C . The cross-section is taken perpendicular to the electrode edges, and at the cell equator. Simulation parameters are $a_0 = 7.5$ μm , $V_{pp} = 50$ V, and $f = 5$ MHz.

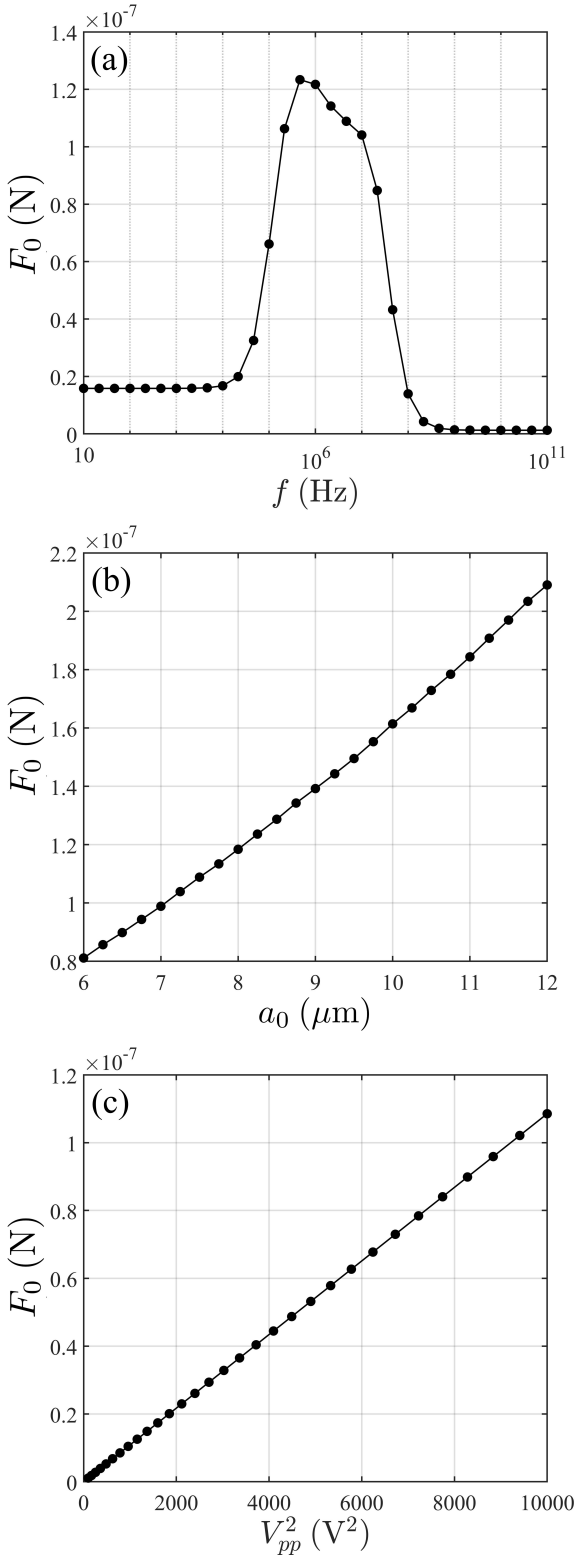

Figure E.2: Dependence of F_0 on (a) frequency, (b) cell radius, and (c) applied voltage. The reference case is $a_0 = 7.5$ μm, $V_{pp} = 100\,V$, and $f = 5$ MHz.

Appendix F. Error quantification

RMS error for MDA-MB-231, MCF-10A, and GBM cells are shown in Fig. F.1 to compare the two analytical methods; the number of cells measurements were made in are shown in Table F.1.

Table F.1: Number of cases (n) for each pulse duration.

t_p(s)	0.01	0.03	0.1	0.25	0.5	1	5	10
MCF-7	6	3	9	6	7	5	5	6
MCF-10A	7	7	6	8	3	3	5	3
MDA-MB-231	8	6	5	8	6	3	6	10
GBM	9	5	4	7	4	3	4	6
n_{total}	30	21	24	29	20	14	20	25

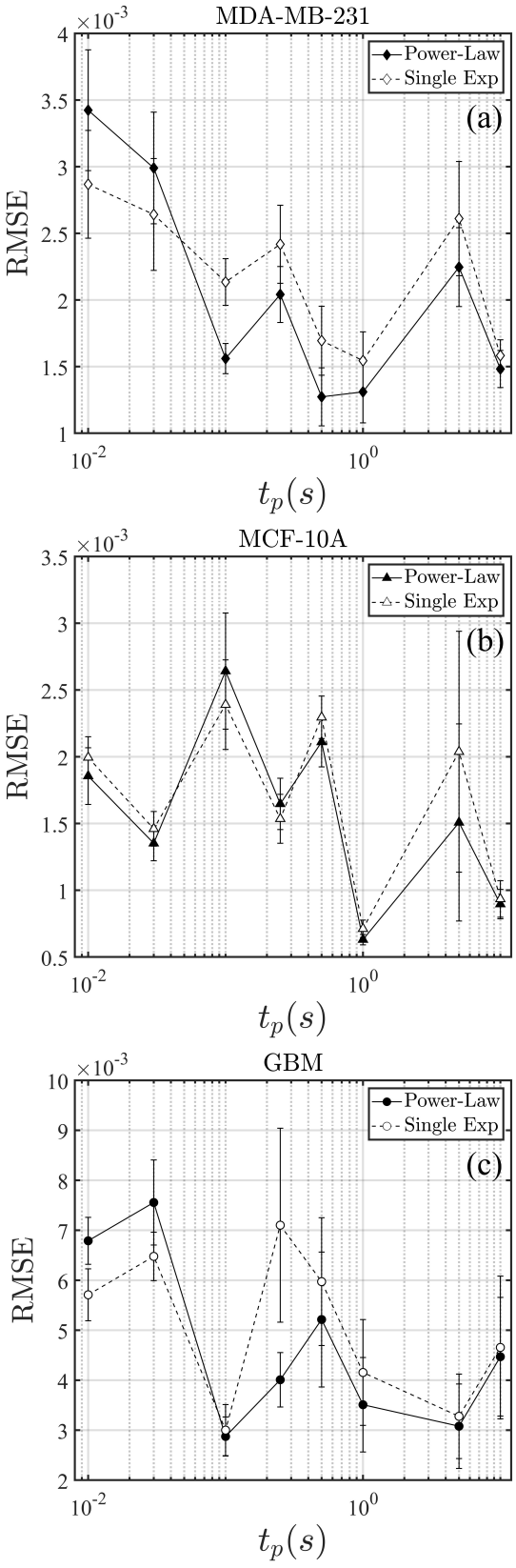

Figure F.1: RMS error (RMSE) for the two analytical approaches. Number of repeats is provided in Table F.1.

Author contributions

HL, LL, RAF, JWS, JDZ, and DIS designed research. YD, SM, and MY performed experiments. SM, YD, and MY analyzed data. SM, LL, and HL developed theory. SM performed numerical simulations. All wrote the paper.

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