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Mechanical properties of mammalian cells in suspension measured by electro-deformation

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Abstract
We describe a planar, micro-fabricated device for generating fringing non-uniform electric fields. We used it to measure the mechanical properties of individual mammalian cells in suspension by deforming them in time-varying, non-uniform electric fields. Electrical stresses generated by the planar microelectrodes were used to trap and stretch cells, while cell deformation was observed using optical microscopy. Two distinct cell types were compared after fitting strain data with a three-parameter ‘standard linear solid’ model of visco-elasticity, and with a two-parameter power-law method. Chinese hamster ovary (CHO) cells were approximately twice as stiff as U937 human promonocytes, and CHO cells displayed an elastic behaviour with recovery of initial shape, while U937 strain data bore witness to plastic deformation. Our results demonstrate that electrical stresses generated by micro-fabricated electrodes permit mechanical characterization of distinct mammalian cell types.

List of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>U</td>
<td>electric potential (V)</td>
</tr>
<tr>
<td>E</td>
<td>electric field strength (V m⁻¹)</td>
</tr>
<tr>
<td>εₖ</td>
<td>dielectric permittivity of a cell (F m⁻¹)</td>
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<tr>
<td>εₘ</td>
<td>dielectric permittivity of suspension media (F m⁻¹)</td>
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<td>κ</td>
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<td>σₙ</td>
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<td>K</td>
<td>Clausius–Mosotti factor (dimensionless)</td>
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<td>F</td>
<td>force (N)</td>
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<tr>
<td>rₑ</td>
<td>cell radius (m)</td>
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<tr>
<td>L</td>
<td>length (m)</td>
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<tr>
<td>Lₑ</td>
<td>length of an ellipsoid major axis (m)</td>
</tr>
<tr>
<td>Lₘ</td>
<td>length of an ellipsoid minor axis (m)</td>
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<tr>
<td>L₀</td>
<td>initial length (m)</td>
</tr>
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<td>γ</td>
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<td>strain of an ellipsoid major axis (dimensionless)</td>
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<td>cell compliance factor (m² V⁻²)</td>
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<tr>
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<tr>
<td>τ</td>
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<tr>
<td>α</td>
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</tr>
<tr>
<td>A</td>
<td>power-law prefactor (Pa⁻¹ s⁻α)</td>
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</table>

1. Introduction

The mechanical properties of mammalian cells in suspension are important determinants of biological functionality in several in vivo and ex vivo contexts. Cells of the circulatory system, for example, have been extensively studied from a mechanical perspective, and increased stiffness of diseased erythrocytes and leukocytes is known to restrict their flow...
through small channels such as capillaries [1, 2]. Recently, some chemotherapy treatments were shown to increase the stiffness of both lymphoid and myeloid leukaemia cells [3]. Measurements of cell stiffness have therefore been essential for determining the biomechanical effects of various drugs and treatments relevant to cells in circulation [4, 5].

Mammalian cells are increasingly being used for the production of recombinant proteins and related products in large-scale bioreactors [6, 7]. Suspension culture permits mammalian cells to grow in bioreactors by methods similar to those used in microbial systems that enable scale-up [6]. The range of available culture conditions is, however, limited by shear and extensional forces, which are present in several types of bioreactors [8–11]. To reduce the harmful effects of mechanical stresses, shear-stress modifiers are often added to cell suspensions [12], although some of these additives can enter through the cell membrane, with unknown consequences to the health and function of the cultured cells [13]. Methods to quantify the mechanical properties of suspended cells can therefore be used to improve the design of new cell types, bioreactors and micro-fluidic devices by predicting the cellular elastic and visco-elastic responses to various forces.

Unlike adherent cells, which remain fixed during the timescales of most mechanical measurement protocols, suspended cells must be stably positioned within a measurement device. Although the requirement for cell-positioning complicates measurements in some cases, suspended cells have nevertheless been mechanically characterized using a variety of techniques. These include optical traps (OT) [14–18], micropipette aspiration (MPA) [19–23], atomic force microscopy (AFM) [3, 24] and electro-deformation (ED) [25–28]. Surprisingly, the most recent review articles that describe methods for mechanical characterization of individual cells did not discuss ED [18, 29], in spite of several important advantages of this technique mentioned in section 4. Furthermore, erythrocytes appear to be the only cell type for which multiple independent ED measurements have been reported to date [25, 27, 30, 31] and thus ED appears to be underutilized. The vast majority of mammalian cell types have not been studied using the ED method.

It is well known that cells in suspension can be trapped in a non-uniform electric field, $E$, by dielectrophoresis (DEP) [32, 33], and increased strength of $E$ can result in cell deformation [25, 34]. Although ED has not been widely reported compared with other techniques, recent advances in micro-fabrication have resulted in increased use of electric fields to manipulate cells [35–37], and ED of protoplasts in a micro-fabricated device has been demonstrated [38]. We hypothesized that new micro-fabricated electrode geometries could be used to produce electrical forces of sufficient magnitude to trap and deform several types of mammalian cells, which have not yet been characterized mechanically. Our objective was therefore to use ED for mechanical characterization of individual mammalian cells. To accomplish this goal we designed a new microelectrode geometry, which permits the capture and deformation of individual cells in suspension by DEP and ED, respectively.

We used ED to measure strain and relaxation of two distinct cell types: (i) Chinese hamster ovary (CHO) cells [39], which are adherent epithelial cells; and (ii) U937 human promonocytes [40], which are non-adherent. The mechanical properties of these cell types are relevant to their use in suspension cultures since CHO cells are used in large-scale bioreactors for the production of recombinant proteins [7, 12], and U937 cells are used to study differentiation along the monocyte–macrophage pathway [41, 42]. To demonstrate the potential use of ED for the mechanical characterization of these two cell types, we fit strain and relaxation data with two well-known models of visco-elastic mechanical properties.

2. Materials and methods

2.1. Cells and media

U937 cells were obtained from ATCC (Manassas, VA), and cultured in RPMI 1640 (Sigma, St Louis, MO) supplemented with 10% fetal bovine serum (FBS; Atlanta Biologicals, GA). CHO-K1 cells were obtained from ATCC (cat. no CCL-61) and cultured in HAM-F12 (Sigma) supplemented with 10% FBS. Both cell types were incubated at 37 °C, in an atmosphere supplemented with 5% CO2. All cells used for ED were selected during the third and fourth weeks of culture, namely passage nos 8–12 for the U937 cells and nos 6–8 for the CHO cells, to ensure that they were healthy and proliferating normally.

The medium used for ED experiments (EDM) was an isotonic buffer with low electrical conductivity, \( \kappa = 15 \text{ mS m}^{-1} \) (EDM: 3.4 mM NaCl, 0.115 mM KH2PO4, 280 mM D-Glucose); pH was adjusted to 7.3 ± 0.1 with NaOH and the osmolality was 285 mOsm kg\(^{-1}\). Low electrical conductivity was required to maximize ED stretching forces, as previously described by others [25–28]. Cell-viability tests (‘Live/Dead’, Invitrogen, Carlsbad, CA) showed no observable differences for either CHO or U937 cells, which were suspended in EDM for up to 30 min, compared with tests performed in media with roughly physiological \( \kappa \) (PBS, \( \kappa \sim 1.5 \text{ S m}^{-1} \)). For EDM, exposure of cells to EDM was limited to 10 min. The use of low-\( \kappa \) media also reduces electrical conductivity-generated (‘Joule’) heat, which scales with \( \kappa \) (see section 3.7 of [35]). In the present work, the maximum temperature increase of the suspension during ED was estimated to be less than 1 °C. Prior to EDM, cells were centrifuged at 190 \( \times \) g for 5 min at moderate cell densities (\(~10^6\) cells mL\(^{-1}\)), and re-suspended in EDM at low densities (\(~1–5 \times 10^5\) cells mL\(^{-1}\)), as measured by the hemocytometer. Low densities were used for ED in order to facilitate the capture of individual cells and to minimize undesirable effects arising from too many neighbouring cells. However, cell density could readily be increased depending on the required number at each electrode tip (see below: \(~10^4\) cells mL\(^{-1}\) led to \(~1\) cell/tip). Cells suspended in EDM were deposited over the electrode surface in 5 \( \mu \)L droplets (total cell number \sim 50–250).
2.2. Fabrication of electrodes and modelling of the electric field

To perform EDM during observation by optical microscopy, we micro-fabricated arrays of planar electrodes on standard microscope glass slides (figure 1). An electric potential, \( U \), applied between opposing planar electrodes resulted in a ‘fringing’ electric field, \( E \) (figure 1(a)), which penetrated into the cell suspension dispensed in droplets over the surface (a droplet of a suspension medium is represented by the circular area in figure 1(b)). The following electrode geometry was used to trap and deform cells: a set of triangularly shaped outer electrodes (e1 in figures 1 and 2) surrounded a central ring electrode (e2 in figures 1 and 2), which was driven at opposite polarity (see figure 1(b)). The triangular tips were rounded (radius of electrode curvature, \( r_e = 12.5 \ \mu \text{m} \)), and their closest approach to the outer perimeter of the ring was \( 50 \ \mu \text{m} \) (figure 2). A third electrode (e3 in figure 1) was not required for the present EDM and was, therefore, not connected to the signal generator.

Planar Ti/Pt electrodes were fabricated using standard lift-off processes, which we have described previously [43]: chromium masks were fabricated on glass (Bandwidth Foundry, Sydney, NSW, Australia) and photolithography was carried out by spin-coating an adhesion promoter, AP300 (Silicon Resources, Chandler, AZ), a lift-off resist LOR5A (MicroChem, Newton, MA) and a final, positive, resist S1813 (Shipley, now part of Rohm & Haas, Philadelphia, PA). UV exposure was done using a Karl Süss MA-4 mask aligner (Süss Microtec, Waterbury Center, VT). The electrodes were deposited by electron beam evaporation (Ti adhesion layer, 10 nm) and sputtering (Pt layer, 70 nm): the former under ultra-high vacuum and the latter in argon at 2.4 Pa. For each glass slide (dimensions: \( 76.2 \times 25.4 \times 1.5 \text{ mm} \)), the lift-off procedure produced 12 sets of electrodes, each with three leads (figure 1(b)) to which electrical connectors were bonded using a combination of a conductive silver epoxy (MG Chemicals, Surrey, BC, Canada) and a standard two-phase epoxy (LePage, Toronto, ON, Canada).

Thin films (\( \sim 550 \text{ nm} \)) of hydrogenated silicon nitride (SiN\(_x\):H, a clear, transparent dielectric) were deposited over the electrodes by plasma-enhanced chemical vapour deposition (PECVD) to prevent electrolysis of the suspension medium during ED, and to enable multiple reuses of the devices. For this, the slides were placed on the grounded electrode of a ‘Reinberg’-type parallel plate radio-frequency (RF, 13.56 MHz) PECVD reactor, in a flowing gaseous mixture of silane (SiH\(_4\)) and ammonia (NH\(_3\))": the flow rates and partial pressures were (SiH\(_4\): 2.5 sccm, 12 mTorr) and (NH\(_3\): 25 sccm, 43 mTorr), and the total pressure was maintained at 55 mTorr. The power delivered to the plasma was 50 W (the power density at the surface of the grounded electrode was \( \sim 150 \text{ mW cm}^{-2} \)), and the substrate temperature was maintained at 125 °C. The deposition time was 45 min and the film thickness, measured by variable-angle spectroscopic ellipsometry (VASE, J. A. Woollam & Co., Lincoln, NE), was \( 550 \pm 25 \text{ nm} \). We have previously described the operation of this PECVD system in more detail, albeit for the case of depositing thin films of a semi-conductor, nano-crystalline silicon [44].

![Figure 1](image1.png)

**Figure 1.** Micro-fabricated planar electrode array. (a) Illustration of the ‘fringing’ electric field (\( E \)), insulating barrier (\( B \)), metallic conductors (\( M \)) and substrate (\( S \)) (dimensions are not to scale). (b) Geometry of the three-electrode (e1, e2 and e3) configuration, with an applied electric potential of arbitrary magnitude (red = max, blue = min). (c) An array of electrodes on a glass microscope slide; the scale bar is 2 cm.

![Figure 2](image2.png)

**Figure 2.** Geometry of planar electrodes and electric field distribution. (a) Planar electrodes, e1 and e2, are driven at opposite polarity; the scale bar is 150 \( \mu \text{m} \). (b) Simulated electric field, arbitrary scale (red = max, blue = min); the scale bar is 150 \( \mu \text{m} \). (c) Electric field in the presence of a polarizable spherical object; the scale bar is 25 \( \mu \text{m} \). (d) U937 monocytes trapped at the electrode tip; the scale bar is 25 \( \mu \text{m} \).
We simulated the electric field distribution using the ‘conductive media mode’ in Comsol Multiphysics v3.2 software (Comsol, Stockholm, Sweden), with the electric potential assigned at boundaries (Dirichlet-type boundary conditions). The non-uniform \( E \) (figure 2(b)) was designed to capture individual cells at each electrode tip. In this region (figure 2(c)), values of \( E \) surrounding a polarized cell are high near the cell poles and low near the equator. For the case of a uniform applied \( E \), the radial component of the induced dipole field has an angular dependence that is proportional to \( \cos \theta \) [45]. In the present (non-uniform) case, \( E \) is highest near the cell pole that faces the electrode tip (figure 2(c)), and cells are therefore trapped in this region (figure 2(d)).

2.3. Cell trapping and stretching protocol

Electrodes were placed within the slide carrier of an inverted optical microscope (Zeiss, AxioVert S100TV) and connected to a signal generator (Agilent, 33220A). A sinusoidal electric potential, \( U \), of frequency, \( f = 5 \times 10^6 \) Hz, was applied and the amplitude was modulated during ED: \( U = 2 \) V (peak-to-peak) was used to capture and hold cells (figures 3(a)–(c)), and \( U = 10 \) V (peak-to-peak) was used to deform cells at constant stress (figures 3(d) and (e)). The \( f \)-dependence of cell polarization (see appendix A) can be approximated using well-known ‘single-shell’ theories, which estimate the effective permittivity of the cell in terms of \( \varepsilon \) and \( \kappa \) for the cell membrane and the internal cytosol [35, 36, 47]. Three distinct \( f \)-regimes occur: (i) at low \( f \) (\( f < ~10^5 \) Hz, in the present cases), a membrane charging by diffuse currents occurs and we observed ‘negative’ DEP of cells; (ii) at intermediate \( f \) (\( 10^6 \) Hz < \( f < 10^7 \) Hz), the cytosolic \( \kappa \)-term is dominant (compared with \( \kappa_m \)), and we observed ‘positive’ DEP (and ED); (iii) at high \( f \) (\( f > ~10^7 \) Hz), similar \( \varepsilon \)-values of the cytosol and the suspension medium (both aqueous) resulted in negligible force. The three operating regimes outlined above have been previously described in detail by others [28], and we too have previously described the \( f \)-dependent DEP of U937 cells in various suspension media [43].

2.4. Strain measurement

Images were captured with a CCD camera (Model QIC-F/-M2, QImaging, Burnaby, Canada) at a rate of approximately one frame per second, during EDM, and saved for subsequent analysis using commercial software (Northern Eclipse v.7, Empix Imaging, Mississauga, Canada). Measurements of cell dimensions were carried out manually, using the ellipse-fitting and measurement tools of the Graphic Image Manipulation Program (GIMP v.2). Strain in the directions parallel or perpendicular to \( E \) (\( \gamma_x \) and \( \gamma_y \), respectively; see figure 3(f)) was calculated as

\[
\gamma(t) = \frac{L(t) - L_0}{L_0},
\]

where \( L(t) \) was the length of the cell at time, \( t \), and \( L_0 \) was the original length. The apparent Poisson’s ratio of the cell was then given by \( \nu = -\gamma_y/\gamma_x \). Mechanical behaviour of ‘simple’ solids, for example visco-elasticity, involves the (linear) relationship between an applied stress, \( \sigma \), and the resulting strain, \( \gamma \); the material-specific property linking the two is known as the solid’s compliance, \( J \) [46]:

\[
\gamma = \sigma J.
\]

In other words, \( J \) is the strain per unit stress, and it is a measure of the solid’s ‘stiffness’.
2.5. Calculation of the applied stress

To simplify data fitting, we derived an expression for the average value of uni-axial stress, \( \sigma \), applied to a whole cell during a typical EDM. We estimated the magnitude of the total force acting on the cell, using the following well-known expression for the time-averaged DEP force [47]:

\[
(F_{\text{DEP}}(t)) = 2\pi r_c^3 K \varepsilon_m|E_{\text{rms}}|^2
= 4\pi r_c^3 K \varepsilon_m|E_{\text{rms}}|\nabla|E_{\text{rms}}|,
\]

(3)

where \( r_c \) is the cell radius, \( \varepsilon_m \) is the dielectric permittivity of the suspension medium, \( E_{\text{rms}} \) is the root-mean-squared value of \( E \) and \( K = \text{Re}\{K(f)\} \) is the real part of the dielectric polarization (Clausius–Mosotti) factor of the cell (see appendix A). For trapped cells, we assumed that \( \pm F_{\text{DEP}} \) were the forces acting on each half-sphere, which stretched the cell approximately uni-axially. The actual distribution of \( E \), and therefore of \( \sigma \) and \( F \), was more complicated (figure 2(c)); the present simplifications are discussed in section 4.

Equation (3) can be written in one dimension as

\[
(F_{\text{DEP}}(x, t))_{1=2} = 4\pi r_c^3 K \varepsilon_m|E_{\text{rms}(x)}| \frac{d}{dx}|E_{\text{rms}(x)}|,
\]

(4)

and the following approximation for \( E(x) \) used

\[
(\langle |E_{\text{rms}(x)}| \rangle) = 0.7 E_0,
\]

(5a)

\[
\left( \frac{\frac{d}{dx}|E_{\text{rms}(x)}|}{E_0} \right) = \frac{n E_0}{r_c},
\]

(5b)

where \( E_0 = U/d \) is the peak magnitude of the electric field, with \( U \) being the applied potential and \( d = 50 \mu m \), the electrode gap; \( n \) is a geometry- and material property-dependent factor and \( r_c \) is the cell radius. Good estimates of \( E_0 \) during ED are \( E_0 = 4 \times 10^4 \text{ V m}^{-1} \) during trapping (\( U = 2 \text{ V} \)), and \( E_0 = 2 \times 10^5 \text{ V m}^{-1} \) during stretching (\( U = 10 \text{ V} \)). The approximations in equation (5) result in the following expression for the force on each half-sphere:

\[
F = \pm 2\pi r_c^3 n K \varepsilon_m E_0^2.
\]

(6)

The average value of stress on the surface of the sphere can then be found by dividing equation (6) by the half-cell area:

\[
\langle \sigma \rangle = n K \varepsilon_m E_0^2.
\]

(7)

The non-uniformity of \( E_0 \), near the tip of e1, was determined by FEM simulations and found to be of the same order of magnitude as that induced around a typical cell: \( d/dx(E_0) \sim 1.5 E_0/r_c \) (\( n \sim 1.5 \) in equation (5b)). Assuming a maximum value of \( K = 1 \) (see appendix A), and \( r_c = 7.0 \mu m \) (measured optically), equation (7) yields \( \langle \sigma \rangle = 0.85 \text{ Pa} \) during trapping (\( U = 2 \text{ V} \)), and \( \langle \sigma \rangle = 21.2 \text{ Pa} \) during stretching (\( U = 10 \text{ V} \)).

2.6. Visco-elastic properties

The simplest ‘lumped-parameter’ visco-elastic model, which fits our data reasonably well, is the three-parameter standard linear solid (SLS) model, characterized by two elastic constants, \( k_1 \) and \( k_2 \), and one viscous constant, \( \eta \). In a well-known mechanical analogue, the so-called Zener model, these correspond to a parallel combination of a spring (\( k_1 \)) with a series combination of a second spring (\( k_2 \)) and a dashpot (\( \eta \)). The compliance function, \( J(t) \), now describes the time-dependent response of the material to an applied time-varying \( \sigma \), in terms of these model parameters. For the SLS, \( J(t) \) is given by [19 (equation (12)), 4 (section 2.11, equation (12))]:

\[
J(t) = \frac{1}{k_1} \left[ 1 - \left( \frac{k_2}{k_1 + k_2} \right) e^{-t/\tau} \right] I(t),
\]

(8)

where \( I(t) \) is the unit step function and \( \tau \) is the time constant (or ‘relaxation time’) at constant stress:

\[
\tau = \frac{\eta}{k_1 k_2}.
\]

(9)

In response to a step-wise (either increasing or decreasing) change in \( \sigma \), the SLS model predicts (i) an instantaneous change in \( J \), \( J(t = 0) = J_0 = 1/(k_1 + k_2) = 1/E_0 \), where \( E_0 \) is the initial elastic modulus; (ii) a limiting value of \( J \), \( J(t = \infty) = J_\infty = 1/k_1 = E_R \), where \( E_R \) is the relaxed elastic modulus; and (iii) a single time constant, \( \tau_\sigma \), given by equation (9), which determines the relaxation time.

2.7. Power-law model

We also use a power law to model \( J(t) \), as was done previously by others for uni-axial stretching of single C2–7 cells, derived from skeletal muscle of adult CH3 mice [48]:

\[
J(t) = A t^\alpha,
\]

(10)

where \( A \) and \( \alpha \) are constants. The power law has been proposed as a general fitting procedure, which considers the cell to be a material with a continuum of relaxation times. The parameters, \( \alpha \) and \( A \), can be related to the low-frequency storage modulus, \( G'(\omega) \), using the following equation [48]:

\[
G'(\omega) = \left( \frac{\cos \left( \frac{\pi \alpha}{2} \right)}{\Gamma(1 + \alpha)} \right) (2\pi f)^\alpha,
\]

(11)

where \( \Gamma \) is the gamma function and \( f \) is the frequency.

2.8. Data fitting

Strain data were fit using equation (2) shown above, with the stress \( \sigma \) given by equation (7), and the material’s compliance function \( J(t) \) given by either equation (8) or (10). The differences between measured and calculated values of \( J \) were minimized using a nonlinear least-squares algorithm (lsqnonlin, Matlab v. 7.2, The MathWorks, Natick, MA).

3. Results

3.1. Strain and relaxation of cells

Our ED protocol permitted time-dependent strain, \( \gamma(t) \), measurement of individual cells over several cycles of strain and relaxation (figures 4 and 5). CHO cells were observed to be stiffer than U937 cells, and \( \gamma \) of the latter showed more significant long-term residual strain or plastic deformation than the former cells. Maximum values of \( \gamma \) for CHO cells were limited to \( \sim 0.2 \) (figure 4(c)) whereas, for a typical U937 cell, \( \gamma > 0.5 \) was observed after the third cycle (figure 5(c)). In some cases, contact with neighbouring cells imposed a limit on \( \gamma \) (indicated by ‘CL’ in figure 5(c)).
Figure 4. CHO stretching and recovery. (a) CHO cells in a DEP ‘holding potential’ of $U = 2\, \text{V}$. (b) The same cells as in (a) 60 s after applying a potential step function, $U = 10\, \text{V}$. (c) strain, $\gamma(t)$, of the middle cell (see the arrow in (a) and (b)) during the first three cycles; circles: $\gamma_x$, or dots: $\gamma_y$ refer to the cell’s major or minor axes; the applied electric potential, $U(t)$, is shown on the same time scale as $\gamma$. The scale bar is 25 $\mu\text{m}$.

Figure 5. U937 stretching and recovery. (a) U937 cells in a DEP ‘holding potential’ of $U = 2\, \text{V}$. (b) The same cells as in (a) 30 s after applying a potential step function, $U = 10\, \text{V}$. (c) strain, $\gamma(t)$, of the middle cell (see the arrow in (a) and (b)); circles: $\gamma_x$, or dots: $\gamma_y$ refer to the cell’s major or minor axes; the applied electric potential, $U(t)$, is shown on the same time scale as $\gamma$. Contact between cells (b) limits the maximum value of $\gamma$, which is indicated in (c) by ‘CL’. The scale bar is 25 $\mu\text{m}$.

Table 1. Model parameters for the ‘standard linear (visco-elastic) solid’ (SLS).

<table>
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<tr>
<th>Cell type</th>
<th>$k_1$ (Pa)</th>
<th>$k_2$ (Pa)</th>
<th>$\eta$ (Pa s)</th>
<th>$\tau_\sigma$ (s)$^a$</th>
<th>Reference</th>
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<td>CHO</td>
<td>193 ± 130</td>
<td>1379 ± 930</td>
<td>2905 ± 1958</td>
<td>17.2 ± 11.6</td>
<td>Present work$^b$</td>
</tr>
<tr>
<td>U937</td>
<td>99 ± 44</td>
<td>798 ± 353</td>
<td>608 ± 269</td>
<td>6.2 ± 2.8</td>
<td>Present work$^b$</td>
</tr>
<tr>
<td>Neutrophil</td>
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<td>73.7 ± 35</td>
<td>13.0 ± 5.4</td>
<td>0.65 ± 0.3</td>
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<td>~180</td>
<td>~7500</td>
<td>~75</td>
<td>[23]</td>
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</tbody>
</table>

$^a$ Relaxation time given by equation (9).

$^b$ All values from the present work are mean ± SD (CHO: $N = 5$, U937: $N = 10$).

3.2. Modelling of cell strain

Although contact between cells could limit the maximum strain in some cases (figure 5(c)), data were always taken from the first cycle of strain and relaxation of freely deformable cells (figure 6), where strain was not limited by the presence of other cells. Both the SLS and PL models (equations (8) and (10), respectively) were found to fit $\gamma$-data reasonably well (tables 1 and 2), although better fits were encountered for the
Table 2. Model parameters for the ‘power-law solid’ (PL).

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$\alpha$</th>
<th>$A \times 10^{-3}$ (Pa s$^{-1}$)</th>
<th>$G'(1$ Hz) (Pa)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>0.301 ± 0.20</td>
<td>3.7 ± 2.49</td>
<td>466 ± 314</td>
<td>Present work$^b$</td>
</tr>
<tr>
<td>U937</td>
<td>0.356 ± 0.11</td>
<td>7.0 ± 3.10</td>
<td>262 ± 116</td>
<td>Present work$^b$</td>
</tr>
<tr>
<td>C2–7 myoblast</td>
<td>0.24 ± 0.01</td>
<td>2.4 ± 0.3</td>
<td>~660</td>
<td>[50]</td>
</tr>
</tbody>
</table>

$^a$ Low-frequency storage modulus, $G'(\omega)$, given by equation (11).

$^b$ All values from the present work are mean ± SD (CHO: $N = 5$, U937: $N = 10$).

Table 3. Mechanical properties of the cells derived using the SLS model.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$\nu^a$</th>
<th>$E_R$ (Pa)</th>
<th>$G_R$ (Pa)</th>
<th>$E_0$ (Pa)</th>
<th>$G_0$ (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHO</td>
<td>0.37 ± 0.07</td>
<td>193 ± 130</td>
<td>70.3 ± 60.7</td>
<td>1572 ± 1059</td>
<td>574 ± 495</td>
</tr>
<tr>
<td>U937</td>
<td>0.39 ± 0.14</td>
<td>99.4 ± 44.0</td>
<td>35.8 ± 28.7</td>
<td>898 ± 397</td>
<td>323 ± 259</td>
</tr>
</tbody>
</table>

$^a$ Apparent Poisson’s ratio of the cell; all values are mean ± SD (CHO: $N = 5$, U937: $N = 10$).

3.3. Measured material properties

Deformations of CHO and U937 cells were rendered reasonably well by both the SLS and PL models, when the maximum value of strain was limited to $\gamma < \sim 0.2$ (figure 6). Long-term plastic deformation of U937 cells was observed for higher $\gamma$ values (figure 5(c)), but the maximum applied stress ($\sigma \sim 20$ Pa) was insufficient to induce comparable high strain in the case of CHO cells (figure 4(c)).

The three parameters of the SLS model ($k_1$, $k_2$ and $\eta$) can be related to the following material properties [4 (section 2.11, equation (8))]: $k_1 = E_R = 2(1 + \nu)G_R$, and $(k_1 + k_2) = E_0 = 2(1 + \nu)G_0$, where $E_R$ is the relaxed elastic modulus, $G_R$ is the relaxed shear modulus, $E_0$ is the initial elastic modulus and $G_0$ is the initial shear modulus [4 (section 2.11, equation (8))]. Their numerical values are listed in table 3.

4. Discussion

4.1. Assessment of cell mechanical behaviour by ED and comparison with other techniques

The objective of this work was to use micro-fabricated planar (micro-)electrodes to generate (non-uniform) electric fields, $E$, of sufficient magnitude to induce substantial deformations to mechanically assess both CHO and U937 cells. These cell deformations, observed by optical microscopy, enabled us to fit measured $\gamma$ data with well-known SLS and PL models: our results clearly demonstrate that ED may indeed be used to evaluate the biomechanical properties of individual (CHO and U937) cells in suspension; the preliminary work with other cell types (not shown here) further suggests that even broader applicability of ED is possible. The advantages of flexibility and modularity of micro-fabricated devices would further lend themselves to more widespread application of ED for mechanical assessment of cell behaviour. Some advantages of ED over other techniques that have also been used, by other workers, for mechanical characterization of individual cells in suspension, are the following: (i) ED obviates the need for moving parts or for micro-beads in the cell.
in suspension; (ii) mechanical contact between cells and device structures is minimized; (iii) planar electrodes for ED are easily micro-fabricated as arrays, which enables simultaneous measurements on several cells, and simple integration within biochips; and (iv) programmable ranges of $U$ values enable the study of mechanical properties over multiple timescales.

4.2. Mechanical properties of CHO, U937 and other cell types

CHO cells were found to be stiffer than U937 cells, and less easily deformed by electrical stresses during ED. The nearly twofold higher elastic modulus of CHO cells compared with that of U937 cells (table 3) can be partly explained by differences of their cytoskeletons (work being reported in a separate study). To our knowledge, neither of these cell types had previously been studied by ED, nor have their mechanical properties been estimated using either the SLS or PL models. It is therefore of interest to compare the present results with those of other workers who did use these models with other cell types and other measuring techniques. Comparison of the present data with those for neutrophil- or articular chondrocyte deformation, the latter two using the MPA technique, demonstrates a large range of biomechanical properties among the different cell types: CHO and U937 cells both appear to be stiffer than neutrophils, but less stiff than suspended chondrocytes (table 1). The viscous constant, $\eta$, does not represent the cytosolic viscosity, but rather an ‘apparent’ viscosity of the whole cell, so that high values reported here are not unexpected. The apparent Poisson’s ratio values for CHO and U937 cells were both less than 0.5 (table 3), suggesting that the cells are slightly compressible. As mentioned above, both SLS and PL models were found to fit our data reasonably well, but this agreement is not expected to hold generally; predictions based on these models tend to diverge for small $\gamma$ values, and at shorter timescales than the $\sim 1$ s used in the present work [48].

Our findings are consistent with previous reports, which suggest that healthy suspension cells are generally less stiff than anchorage-dependent ones [22, 29]. Furthermore, higher $E_R$ values have been reported for spread than for spherical morphologies, for the cases of osteoblasts, chondrocytes, adipose-derived adult stem cells and mesenchymal stem cells [24]. We cite the morphology-dependent stiffness of cells in suspension, but we also acknowledge that $E_0$ and $E_R$ values previously reported by others were found to increase as a function of seeding time for the case of superficial chondrocytes, but to decrease for that of chondrocytes taken from middle/deep cartilage layer depths [49]. The above-described observations and the large statistical variances within the estimated mechanical properties for a given cell type, reported both by the present and by other workers (see the large SD values in tables 1 and 3), emphasize the need to refine methods for biomechanical characterization of cells on an individual basis.

Non-lethal (but physiologically significant) responses to hydrodynamic shear stresses have been reported for a variety of cell types in suspension, when the stress was in the range of 1–10 Pa [9]: lethal values of stress for anchorage-dependent CHO-K1 cells were on the order of 1 Pa, but exceeded 200 Pa for suspension-cultured cells. In the present work, $\gamma \sim 0.1–0.3$ for suspended CHO and U937 cells rarely resulted in their rupture or lysis. Our calculated value of $\sigma \sim 20$ Pa is therefore in good agreement with previous measurements of non-lethal, physiologically relevant mechanical stresses. We conclude that ED, although not capable (at least, in the present configuration) of generating maximum $\sigma$ values comparable to their MPA-based counterparts (up to $\sim 1 \text{ kPa}$ [22, 23]), can nevertheless be used to study the biomechanical properties of several mammalian cell types in suspension.

4.3. Potential methodological improvements and future work

Reliable use of the ED technique requires experimental calibration of the forces and stresses generated. For example, cell-sized synthetic microspheres with well-characterized mechanical properties might be used as reference materials; some candidates that come to mind are commercially available polystyrene or hollow silica microspheres. This type of measurement would increase the precision of estimating $\sigma$, which has been calculated analytically in the present work (equation (7) and appendix B).

ED requires the use of low-$\kappa$ media to maximize $\sigma$, which depends on the differential polarizability of cells and media (appendix A). In the present experiments, this was accomplished by reducing salt concentrations to sub-physiological values. EDM were always performed for less than 10 min and the effects of low salt concentrations on U937 or CHO metabolism was not considered; future improvement of low-$\kappa$ media for long-term culture of cells is expected to benefit DEP and ED methods.

In deriving our expression for $\sigma$ (equation (7)), we simplified $E$ by defining a normalized average value of electric field non-uniformity (equation (5b)) and assumed that $\sigma$ was normal to the cell surface. It is also worth noting that equation (7) results in an expression for $\gamma$ (equation (2)), which is similar to the following expression reported previously by others for the case of ED [28, 38]:

$$\gamma = CKE^2,$$

where $C$ accounts for cell compliance, and is related to $J$ in our model by $C = n\varepsilon_0J$. Electrically induced stresses of comparable magnitude to those estimated using equation (7) can exist at the cell/medium interface, even if $E$ is initially uniform (see appendix B). Although theoretical work has been developed by others to explain deformation of liquid drops in (initially uniform) E-fields [55], further work seems appropriate to clearly identify all sources of electrical stresses in the case of complex dielectric objects such as cells.

More realistic modelling of cell mechanics is also required: although the SLS and PL models were useful for rapid characterization of the investigated cell types, we must acknowledge that they over-simplify cell structure. Finite-element modelling (FEM) can potentially alleviate some of these problems, since more realistic cell and electrode geometries can be simulated within dynamic environments [17, 50]. Although we have used FEM to simulate the
distribution of $E$, in the future we also plan to use FEM to study the distribution of $\sigma$ and to model cell deformation dynamically.

The maximum average $\sigma$ values, applied to cells by ED, were calculated to be $\sigma \sim 20$ Pa, using the maximum available $U$ (10 V peak-to-peak, into a 50 $\Omega$ load at 5 MHz). It would be relatively simple to increase these values, because $\sigma$ scales with the square of $U$ (equation (7)), and the true limits to $\sigma$ are therefore presently unknown. Threshold values of $\sigma$ required to stretch cells have been reported in the range of 10–100 Pa [20, 21], and increasing $U$ while maintaining small inter-electrode distances (50 $\mu$m in the present case) would therefore increase the number of cell types and synthetic materials which could be studied by ED.

Our ED devices were micro-fabricated directly on microscope slides to permit observation by optical microscopy during ED; improved imaging methods can therefore be used to study the mechanics of sub-cellular structures such as the cytoskeleton. It may also be possible to include imaging arrays directly in an ED device for increased portability. So-called cell-on-chip micro-fluidic devices, which permit multimodal analysis and manipulation of cells [51, 52], can be readily made to incorporate microelectrodes for ED-based mechanical measurements. Using the ‘low-temperature’ micro-fabrication methods described here, we have fabricated ED test devices on transparent plastic (polymer) substrates (figure 7), but in order to resist the 125 °C substrate temperature during PECVD (see above), clear polymers with high glass-transition temperatures are required, for example cyclic polyolefin or poly(sulfone) [44]. Based on the above, we therefore expect diverse future implementations of the methods we have described in this paper.

5. Conclusions

Electrical stresses, generated by planar microelectrodes with relatively low values of the applied potential ($U < 10$ V), have been used to electro-deform CHO and U937 cells in suspension. Our electrode geometry and ED methodology have permitted biomechanical characterizations of individual cells of both these cell types. Electrode structures required for novel ED implementations can readily be micro-fabricated and included within micro-fluidic devices, for increased automation of measurements in computer-controlled biochips. The results presented here therefore suggest that ED should become an increasingly favoured technique for biomechanical measurements.

Acknowledgments

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Appendix A. The Clausius–Mosotti factor

The complex Clausius–Mosotti factor of a (‘spherical’) cell driven at a frequency, $f$, is given by

$$K(f) = \frac{\varepsilon^* - \varepsilon_m^*}{\varepsilon^* + 2\varepsilon_m^*}$$

(A.1)

where $\varepsilon^* = \varepsilon + i(\kappa/2\pi f)$ is the complex permittivity, $\varepsilon$ is the dielectric permittivity, $\kappa$ is the electrical conductivity, $i = \sqrt{-1}$ and the subscripts, $c$ and $m$, refer to the cell and medium, respectively. Values of $K$ are bounded by $-0.5 < K < 1$, and are maximized ($K > 0$) for EDM, by an appropriate choice of $f$ and $\kappa$. We selected $f = 5 \times 10^6$ Hz and $\kappa = 15$ mS m$^{-1}$ to assure ‘positive’ DEP forces ($K > 0$), which can be calculated in the quasi-electrostatic regime [25, 27, 32]. We have previously described the $f$-dependence of $K$ for U937 cells [43].

Appendix B. The Maxwell stress on a dielectric sphere in a uniform applied electric field

A dielectric sphere of radius, $r_c$, and dielectric permittivity, $\varepsilon_{c}$, is placed within a medium with dielectric permittivity, $\varepsilon_m$, and a uniform electric field, $E_0$, is applied in the positive $x$-direction (figure A1). Using the polar coordinates ($r$, $\theta$), with $r = 0$ at the centre and $r = r_c$ at the surface, the electric fields induced inside, $E_{in}$, and outside, $E_{out}$, the sphere can be obtained by solving Laplace’s equation. They are found to be [53, 54]

$$E_{in} = \frac{3\varepsilon_m}{\varepsilon_c + 2\varepsilon_m} E_0 (\cos \theta \hat{r} - \sin \theta \hat{\theta}),$$

(B.1)
Equations (B.1) and (B.2) apply when the driving potential is sinusoidal, with the dielectric permittivities, $\varepsilon_c$ and $\varepsilon_m$, replaced by their respective complex permittivities, $\varepsilon^*$. We define the factors $K_1 = 3\varepsilon_m/(\varepsilon_c + 2\varepsilon_m)$ and $K_2 = (\varepsilon_c - 2\varepsilon_m)/(\varepsilon_c + 2\varepsilon_m)$, where $K_1 + K_2 = 1$; the values of $K_1$ and $K_2$ are bounded by $0 < K_1 < 1.5$ and $-0.5 < K_2 < 1$, respectively. The radial components of the electric fields can then be written as

$$E_{\text{out}} = E_0(\cos \theta \hat{r} - \sin \theta \hat{\theta})$$
$$+ \frac{\varepsilon_c - \varepsilon_m}{\varepsilon_c + 2\varepsilon_m} \varepsilon_0(2 \cos \theta \hat{r} + \sin \theta \hat{\theta}).$$  \hspace{1cm} (B.2)

References


