Optimization of thin-film highly-compliant elastomer sensors for contractility measurement of muscle cells

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Abstract

Test assays capable of providing quantitative characterization of the contraction of cardiac and smooth muscle cells are of great need for drug development and screening. Several methodologies have been proposed for achieving measurement of cell contractile stress or force, however almost all rely on optical methods to detect contraction. Recently, we proposed a test assay method based on the cell-induced deformation of thin-film, elastomeric, capacitive sensors. The method uses an electrical (capacitive) read-out enabling facile up-scaling to a large number of devices working in parallel for high-throughput measurements. We present here a model for the prediction and optimization of sensor performance. Our model shows the following trends: a) a cell region ratio of approximately 0.75 of the culture well radius produces the largest change in capacitance for a given cell contractile stress, b) the change in capacitance generated by cell contraction increases as the Young's modulus, sensing layer thickness and electrode thicknesses of the sensor decrease, following an inverse relationship. A prototype device is fabricated and characterized in cell culture conditions. Mean standard deviations as low as 0.2 pF are achieved ($< 0.05\%$ of the initial sensor capacitance), representing a minimum detectable cell stress of 1.2 kPa, as predicted by our model. This sensitivity is sufficient to measure the contractile stress of smooth and cardiac muscle cell monolayers as reported in the literature.

Keywords: Thin-film elastomer sensors, muscle cell contraction, cell contraction assay, dielectric elastomer sensors.

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1. Introduction

The malfunction of cardiac or smooth muscle cells are responsible for a variety of physical conditions such as arrhythmia, systolic heart failure, asthma and atherosclerosis. In the development of drugs to combat these conditions, high-throughput screening systems capable of characterizing cell contractility *in vitro*, in response to various chemical stimuli, are greatly needed.

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Figure 1: Highly compliant, thin-film, elastomeric capacitive cell stress sensor. (i) Photo of an array of fabricated sensors integrated into a 24-well cell culture plate. (ii) Schematic representation of an individual sensor with cell region radius A' and culture well radius B' indicated (following the naming convention outlined in Appendix A), and description of sensor working principle: A thin and highly compliant elastomer membrane (the sensing layer) is patterned with annular shaped compliant electrodes. The region covered by the compliant electrodes i.e. the sensing region, forms a parallel plate capacitor with capacitance C' . The device is electrically isolated using a passivation layer, onto which cells are patterned. Cell adhesion and patterning is facilitated through surface functionalization e.g. patterning with adhesion proteins. Cell contraction causes a reduction in surface area of the cell region and an expansion in the sensing region, resulting in a change in the measured capacitance to C'' . (Device shown in this configuration for clarity, for a real experiment the device would be placed upside-down relative to the schematic, in order to minimize sagging due to the weight of the cell culture medium, see Figure 5i).

Several solutions have been proposed for the characterization of cell contractile force or stress, which can be less than 10 kPa [1] (or less than 10 nN for individual cells/cell monolayers [2]). These solutions include those based on cell bilayer cantilevers using elastomer or rigid substrates [1, 3, 4, 5, 6], cells cultured on top of elastomer micro-pillars [7, 2, 8, 9] and monolayers patterned between aligned micro-wall arrays [10]. Though these approaches are successful in measuring cell contractility, they all rely upon optical methods to detect contraction. This presents a practical limitation to the scaling up of devices for highly parallelized, real-time measurements, and also limits device modularity due to the need for external apparatus such as optical microscopes.

We previously proposed a novel sensing methodology based on the deformation of thin and highly compliant elastomer capacitive sensors, induced by a monolayer of muscle cells [11]. The methodology enables cell stress measurement with electrical (capacitive) read-out, without the need for optical measurement and the associated post-processing. The flexible fabrication methodology also allows for large-area fabrication of device arrays and integration into apparatus commonly used for cell culturing, such as a 24 well culture plate (Figure 1i).

Here we present a model to predict sensor behavior i.e. the change in capacitance resulting from cell contractile stress, for a given set of sensor and cell properties

(e.g. film thickness, Young’s modulus etc.). The modeling approach uses elastic energy minimization, which handles well the high aspect ratio geometries of the device, to find the equilibrium stretch in the system as a function of the material and biophysical properties. The model serves to establish fundamental design rules for our system, validating our approach and helping to improve the sensitivity of future devices.

A prototype is fabricated in order to assess the signal stability achievable from an ultra-compliant elastomer sensor made using our developed fabrication methodology (based on [11, 12]). Finally, we compare the stability measurement against the expected capacitance change predicted by our model, hence defining the cell contractile stress detection range.

2. Model development

2.1. Device working principle

Our device design and operating principle is shown schematically in Figure 1ii. Compliant electrodes, in the shape of an annulus, sandwich part of a thin, circular, prestretched elastomer membrane, forming a parallel plate capacitor. The top electrode is covered by a passivation layer which is also made of a prestretched elastomer membrane. A confluent monolayer of muscle cells are patterned onto the passivation layer in the central region of the membrane i.e. the transparent region not sandwiched by the compliant electrodes in Figure 1i, referred to as the cell region. The cell region surface is functionalized with fibronectin [11, 13, 14, 15] (Figure 2ii), in order to ensure good cell adhesion and facilitate cell patterning [11]. Contractile stress generated in the cell monolayer are transmitted to the underlying elastomer membrane (assuming no slip between the monolayer and the sensor) causing a change in the dimensions of the sensing region i.e. the region patterned by the compliant electrodes, and therefore result in a change in the device capacitance. The cell contractile stress can be deduced from the change in capacitance of the sensor geometry, mechanical properties and the properties of cell monolayer (either the Young’s modulus or the constraint-free stretch).

2.2. Cell region mechanics

In order to prevent buckling or wrinkles from being produced [16], a tensile pre-stretch λ_{pre} is applied to the passivation and sensing layers. Hence, during the cell monolayer contraction, which is taken to be equi-biaxial in the in-plane dimensions due to the heterogeneous orientation of the cells (any out-of-plane deflections are assumed to have negligible effect on the capacitance change), the cell region of the

Figure 2: Schematic representation of modeling approach and model domains. Elastomer layers shown in yellow, compliant electrode shown in dark grey. (i) The radial and hoop stretches λ_R and λ_θ in the sensing region of the device are determined numerically, as a function of an arbitrary stretch in the cell region λ_A (in the range $0 < \lambda_A \leq 1$), using the shooting method. A solution is found in satisfying the conditions $\lambda_\theta(A'') = \lambda_A \lambda_{pre}$ and $\lambda_\theta(B'') = \lambda_{pre}$. Stretches in the cell region are taken to be equi-biaxial in the in-plane dimensions (any out-of-plane deflection are assumed to have negligible effect on capacitance change). λ_c is the stretch of the cell monolayer and λ_{pre} is the prestretch the elastomer layers. (ii) The thickness stretch λ_z is determined using the solution from step (i), the total capacitance C'' is found by discretizing the sensing regions and summing the elemental capacitances c_i . (iii) The total elastic energy from all the sensor domains U_{total} is found as a function of λ_A . The value of λ_A at which U_{total} is minimum is the equilibrium stretch λ_{equil} .

sensor remains in tension and has radial and hoop stretch equal to $\lambda_A \lambda_{pre}$ (Figure 2i), where $\lambda_A = A''/A'$, $\lambda_{pre} = A'/A$, A is the cell region radius before prestretch, A' is the radius of the cell region after prestretch but before cell contraction and A'' is the cell region radius after cell contraction (following the convention outlined in Appendix A). Assuming elastomer incompressibility i.e. $\lambda_R \lambda_\theta \lambda_z = 1$ [17], the total thickness stretch in these layers is equal to $(\lambda_A \lambda_{pre})^{-2}$.

For the cell monolayer, the situation is different. We assume the cells undergo volume-constant, in-plane contraction, analogous to anisotropic thermal shrinking/expansion [3]. Hence we assume elastic deformations are generated only when the cell monolayer is constrained (neglecting the residual stress described by Ramasubramanian *et al.* [18]) such that,

$$\lambda_R^{cells} = \lambda_\theta^{cells} = \lambda_c = \frac{\lambda_A}{\lambda_{free}} \quad (1)$$

and

$$\lambda_z^{cells} = \frac{1}{\lambda_c^2}, \quad (2)$$

where λ_R^{cells} and λ_θ^{cells} are the in-plane elastic stretches generated in the cell mono-

layer as a result of the mechanical constraint imposed by the sensor, λ_Z^{cells} is the thickness stretch in the cell monolayer and λ_{free} is the constraint-free contractile stretch (assumed to be a property of the cells) that would be observed if the cell monolayer contraction was limited by the cell response only, and not by the mechanics of the sensor [3, 18]. Note that if the sensor mechanics are indeed a limiting factor for the contraction of the cell monolayer (i.e. $\lambda_A > \lambda_{free}$) then the cells are in a state of tension (i.e. $\lambda_c > 1$). It is assumed that there is perfect bonding between the cell monolayer, passivation layer and sensing layers such that there is no slip.

We use a neo-Hookean strain energy density function (SEDF) (equation 3 below) to model the cell monolayer and sensor. This is a reasonable choice of SEDF given the small elastic deformations expected ($< 10\%$).

$$W = \frac{\mu}{2} (\lambda_R^2 + \lambda_\theta^2 + \lambda_Z^2 - 3), \quad (3)$$

here W is the strain energy density, μ is the shear modulus (assumed to be one third the Young's modulus E , and homogenous over the monolayer), and the subscripts R , θ and Z refer to the radial, hoop and thickness directions, respectively.

For the cell monolayer, the true contractile stresses σ^{cells} can therefore be determined as a function of the derivative of the strain energy density W with respect to the principal stretches, as follows [19]:

$$\sigma_R^{cells} = \lambda_R \frac{\partial W^{cells}}{\partial \lambda_R} - p \quad (4)$$

$$\sigma_\theta^{cells} = \lambda_\theta \frac{\partial W^{cells}}{\partial \lambda_\theta} - p \quad (5)$$

$$\sigma_Z^{cells} = \lambda_Z \frac{\partial W^{cells}}{\partial \lambda_Z} - p \quad (6)$$

where the value of p depends on the kinematic boundary conditions [19]. For planar, equi-biaxial stress we obtain,

$$\sigma_R^{cells} = \sigma_\theta^{cells} = \sigma_c = \lambda_c \frac{\partial W^{cells}}{\partial \lambda_c} - \lambda_Z \frac{\partial W^{cells}}{\partial \lambda_Z}. \quad (7)$$

Solving equation 7 using equation 3 and applying incompressibility, we obtain:

$$\sigma_c = \mu \lambda_c^2 - \mu \lambda_Z^2 \quad (8)$$

$$\sigma_c = \mu (\lambda_c^2 - \lambda_c^{-4}). \quad (9)$$

2.3. Sensing region mechanics and sensor capacitance

Here, we determine the coupled ordinary differential equations which govern the radial and hoop stretches λ_R and λ_θ , in the sensing region, as a result of the contraction in the cell region. The differential equations are found as a function of radial position R in the reference state (before material prestretch and cell contraction). The sensor capacitance is subsequently determined as a function of the stretches λ_R and λ_θ .

In the sensing region, the radial and hoop stretches are nonlinear and dependent on the radial position (shown schematically in Figure 2i) [17]:

$$\lambda_R(R) = \frac{dR''(R)}{dR}, \quad (10)$$

$$\lambda_\theta(R) = \frac{2\pi R''(R)}{2\pi R} = \frac{R''(R)}{R}, \quad (11)$$

where R is the radial position of an arbitrary point in the sensing region before prestretch and cell contraction (the reference state), and R'' is the radial position of the point after cell contraction, such that $R'' = R'\lambda_A = R\lambda_A\lambda_{pre}$ (see Appendix A). Assuming material incompressibility,

$$\lambda_Z = 1/(\lambda_R\lambda_\theta). \quad (12)$$

Considering radial equilibrium in the sensing region (Figure A.1B in Appendix A), we obtain [3, 17]:

$$\frac{ds_R(R)}{dR} + \frac{s_R(R) - s_\theta(R)}{R} = 0, \quad (13)$$

where s_R and s_θ represent the nominal radial and hoop stresses respectively, i.e.

$$s_R = \sigma_R/\lambda_R, \quad (14)$$

$$s_\theta = \sigma_\theta/\lambda_\theta, \quad (15)$$

and where σ_R and σ_θ are the true radial and hoop stresses respectively.

We also model the elastomer material using the neo-hookean SEDF such that,

$$\sigma_R = \lambda_R \frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_R}, \quad (16)$$

$$\sigma_\theta = \lambda_\theta \frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_\theta}. \quad (17)$$

Substituting equations 14, 15, 16 and 17 into equation 13 we obtain:

$$\frac{\partial}{\partial R} \left[\frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_R} \right] = \frac{1}{R} \left[\frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_\theta} - \frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_R} \right]. \quad (18)$$

Expanding the left side of equation 18 using the chain rule and rearranging we obtain:

$$\frac{\partial \lambda_R(R)}{\partial R} = \frac{1}{R} \left[\frac{\partial^2 W(\lambda_R, \lambda_\theta)}{\partial \lambda_R^2} \right]^{-1} \left[\frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_\theta} - \frac{\partial W(\lambda_R, \lambda_\theta)}{\partial \lambda_R} \right]. \quad (19)$$

Combining equations 10 and 11 gives;

$$\frac{\partial \lambda_\theta(R)}{\partial R} = \frac{1}{R} (\lambda_R - \lambda_\theta). \quad (20)$$

For equation 20, the values at the culture well wall ($R = B$) and at the cell region-sensing region interface ($R = A$), are known (Figure 2i);

$$\lambda_\theta(A) = \lambda_A \lambda_{pre}, \quad (21)$$

$$\lambda_\theta(B) = \lambda_{pre}. \quad (22)$$

The capacitance C'' of the device is found by integrating equation 23 over the sensing region, i.e.:

$$C'' = \int_A^B \frac{\varepsilon_0 \varepsilon_r 2\pi R \lambda_\theta(R) \lambda_R(R) dR}{t \lambda_Z(R)}, \quad (23)$$

where t is the initial sensing layer thickness before prestretch, ε_r is the relative permittivity of the sensing layer (assumed constant and independent of prestretch) and ε_0 is the permittivity of free space equal to $8.854 \times 10^{-12} \text{ F m}^{-1}$ (fringe field capacitance at the extremities of the sensing region are ignored). Substituting equation 12 into equation 23 we obtain,

$$C'' = \varepsilon_0 \varepsilon_r \int_A^B \frac{2\pi R dR}{t \lambda_Z^2(R)}. \quad (24)$$

2.4. Elastic energy minimization

We use an elastic energy minimization approach [17] to predict the performance of our sensor design for a given geometry, set of mechanical properties (of both the sensor and cell monolayer) and the biophysical properties of the cells, with the aim of maximizing the change in capacitance ΔC produced for a given contractile stress. The model is also used to find the optimal cell region radius to well radius ratio $D = A/B$ that produces the maximum ΔC .

We use the following procedure:

1. The stretch distribution in the sensing region is calculated by solving numerically the system of ordinary differential equations 19 and 20. This boundary value problem (where λ_θ is known at $R = A$ and $R = B$, see equations 21 and 22) is transformed into an initial value problem using the shooting method (with the values of λ_θ and λ_R at $R = A$ as initial conditions). To solve this system of equations, an initial guess G is made for the stretch value λ_R at $R = A$ and equations 19 and 20 are solved using an ODE solver (MATLAB, MathWorks). If the solution does not satisfy equation 20, the guess value G is iterated using the secant method until equation 20 is satisfied within a specified tolerance.
2. The total capacitance C'' of the sensor is determined by discretizing the sensing region into N number of elements, applying equation 24 to each element and summing the parallel element capacitances (Figure 2ii). The thickness stretch λ_Z in each element (assumed to be uniform within each element and equal to the value at the center of the element) is determined using equation 12 and the solutions for λ_R and λ_θ found in step 1.
3. The total energy in the system U_{total} is defined as the sum of the elastic energy in the system as a function of λ_a in the range $0 < \lambda_a \leq 1$ (all other parameters being fixed):

$$U_{total}(\lambda_A) = U_c + U_p^{CR} + U_s^{CR} + \sum_{i=1}^N U_{pi} + \sum_{i=1}^N U_{si} + \sum_{i=1}^N U_{ei}, \quad (25)$$

where the subscripts c , p and s refer to the cell monolayer, passivation layer and sensing layer in the cell region, respectively, and the subscripts pi , si and ei refer to elements of the passivation, sensing and electrode layers in the sensing region, respectively (depicted in Figure 2iii). U is the energy in each domain of the sensor given by,

$$U = V.W(\lambda_A), \quad (26)$$

where V is the initial volume of each domain or element and W is the neo-hookean strain energy density, defined in equation 3. The λ_A stretch at which U_{total} is a minimum is the equilibrium stretch λ_{equil} , for the system (Figure 2iii). C'' is calculated using equations 24 and 12 at $\lambda_A = \lambda_{equil}$. ΔC is then found as the difference in the sensor capacitance before and after cell contraction i.e. $\Delta C = C'' - C'$.

2.5. Model results

In this section, the results of the model are given for a set of input parameters representative of those found in the literature (summarized in the caption of Figure 3) [1, 3, 11].

An example of the elastic energy summation described in section 2.4 is shown in Figure 3i with the contributions from the different device domains. The energy contribution from the cell monolayer decreases as λ_A approaches λ_{free} (equal to 0.7 in the example), and similarly the contributions from the passivation and sensing layers in the cell region decrease as λ_A decreases. Conversely, the elastic energies from the domains in the sensing region (i.e. the passivation, sensing layer and compliant electrodes) increase as λ_A decreases, as these domains go further into tension.

Figure 3ii shows the change in capacitance ΔC and cell contractile stress σ_c as a function of the cell region ratio D , normalized by their maximum values. As can be seen from the figure, the optimum value of D , at which ΔC is maximum, is approximately 0.75. Moreover the trend remains same irrespective of the model input parameters. We explain this behavior as the result of two competing phenomena: 1) the increasing force generated by the cell monolayer as the cell region radius increases - due to an increasing number of cells. 2) The increasing influence of the fixed boundary at the culture well wall as the cell region approaches it. The cell wall constrains the sensor effectively making it stiffer in the region close to the wall, leading to a lower equilibrium stretch as the cell region ratio becomes larger than the optimum value. Additionally, the use of the neo-hookean SEDF to describe the various sensor domains and the small equilibrium strains which are typically produced ($\ll 10\%$, see Figure 3i), means that the energy minimum is simply scaled linearly when different input parameters are used, leaving the trend, as a function of D , unchanged.

The energy minimization dictates that the stress in the cell monolayer does not change appreciably in the the range $D < 0.5$, and only slightly increases for $D \geq 0.5$

Figure 3: Results of the model for various input parameters. (i) Example of the energy summation process showing the equilibrium stretch λ_{equil} , at which the total energy U_{total} is minimum ($t' = 10 \mu\text{m}$). (ii) The capacitance change ΔC and cell contractile stress σ_c as a function of cell region radius ratio D (ΔC and σ_c normalized by their maximum values). The trends remain the same irrespective of the model input parameters. (iii) ΔC against constraint-free stretch λ_{free} , and (iv) ΔC against contractile cell stress σ_c , for cell Young's modulus $E_{cells} = 30, 15$ and 7.5 kPa (ΔC normalized by its value at $D = 0.75$ from (ii)) - demonstrates the effect of using different cell types (represented by the different Young's moduli) on sensor output and contractile stress prediction. (v) $\Delta C/C'$ normalized against t' , E and t_e (normalized by $5 \mu\text{m}$, 100 kPa and $5 \mu\text{m}$, respectively) for a fixed $\lambda_{free} = 0.4$ (All parameters follow an inverse relationship on $\Delta C/C'$ of the form $f(x) = a/(x + b)$). Input parameters for all sub-figures are the following unless otherwise stated: cell monolayer thickness $t_c = 5 \mu\text{m}$ [3], prestretch in the elastomer membranes (passivation and sensing) $\lambda_{pre} = 1.1$, passivation layer thickness $t_p = 6.05 \mu\text{m}$ ($t'_p = 5 \mu\text{m}$), sensing layer thickness $t = 6.05 \mu\text{m}$ ($t' = 5 \mu\text{m}$), the combined electrode thickness $t_e = 5 \mu\text{m}$, Young's modulus of the cell monolayer $E_{cells} = 30$ kPa [1, 3], Young's modulus of the elastomer membranes (passivation and sensing) $E = 100$ kPa [11], Young's modulus of the electrodes $E_e = 2E$, the ratio of cell region radius to culture well radius $D = 0.75$, the constraint-free prestretch $\lambda_{free} = 0.7$ [3], the relative permittivity of the sensing layer $\epsilon_r = 2.75$, the initial cell region radius $B = 5.1$ mm and the culture well radius $A = 6.9$ mm, the number of discretization points in the sensing region $N = 500$ per mm of radial length of the sensing region i.e. $500(B' - A')$.

(total variation $< 3\%$), as Figure 3ii shows. This result is analogous with the result presented by Alford *et al.*, based on moment and force equilibrium, where cell stress was relatively independent of the curvature produced by their cell-elastomer bilayer cantilevers for a constant λ_{free} [3].

Figure 3iii shows ΔC (normalized by the maximum value in the figure) against the constraint-free contraction of the cell monolayer λ_{free} (in the range $0.4 \leq \lambda_{free} < 1$), for various cell Young's moduli E_{cells} , and a fixed cell region ratio $D = 0.75$. The figure shows that ΔC increases with increasing cell Young's modulus for a fixed value of λ_{free} i.e. that stiffer cells will generate a larger ΔC . Figure 3iv plots the same data against the contractile cell stress σ_c (instead of λ_{free}) and shows that for different E_{cells} , the data all lie on the same line with the respect to the ΔC - σ_c axis, however the range of stress they produce decreases as E_{cells} decreases.

Figure 3v shows the effect of varying different sensor parameters on the sensor output for a fixed $\lambda_{free} = 0.4$. The following parameters are investigated; t' the thickness of the sensing layer after prestretch (normalized by $t' = 5 \mu\text{m}$), E the Young's modulus of the elastomer membranes (passivation layer and sensing layer, normalized at $E = 100 \text{ kPa}$) and the combined electrode thickness t_e (normalized at $t'_e = 5 \mu\text{m}$, electrode Young's modulus $E_e = 2E$ - assumes the elastomer used for the sensing layer is also used in the electrode). Each line in the figure represents the effect of scaling the parameter with respect to their normalized values, holding all other variables constant, and plotted against the change in sensor capacitance divided by the initial capacitance C' (in order to eliminate the effect that the thickness after prestretch t' has on the initial capacitance value). The figure shows that each of the parameters has an inverse relationship on $\Delta C/C'$ and can be approximated by the expression $f(x) = a/(x+b)$. This result highlights the need for highly compliant materials, fabricated in very thin films, in order to maximize device sensitivity. Figure 3v also shows that ΔC is more sensitive to changes in the elastomer Young's modulus than the sensing layer thickness or the thickness of the electrode layers. For example, increasing the Young's modulus of the elastomer by a factor of two decreases $\Delta C/C'$ by a factor 0.44, where as increasing t or t_e by a factor two decreases $\Delta C/C'$ by 0.75 and 0.65, respectively.

3. Sensor stability characterization

3.1. Device fabrication

We fabricate a functional sensor prototype to assess the signal stability achievable from a fabricated device under cell culture conditions, and hence its usability in real experiments. The prototype fabrication process is outlined in Figure 4. Thin

Figure 4: Fabrication process for prototype sensor. (i) PDMS mixture is cast on a PET substrate coated with a PAA water-soluble sacrificial layer. (ii) Electrode mixture is cast onto a PET substrate coated with a 50 μm PVA water-soluble sacrificial layer. (iii) The cured PDMS membrane is released and prestretched equi-biaxially in-plane. (iv) The cured electrode-PVA film is cut to the desired shape using a CO_2 laser cutter. (v) The cut electrode is placed on the prestretched elastomer membrane to achieve bonding. Ethanol is placed on the membrane before the contact step to prevent the formation of air-pockets at the interface. (vi) After 30 mins the PVA backing of the electrode is removed by submersion in hot de-ionized water. The second electrode is made by repeating steps (v) and (vi) on the reverse side of the PDMS membrane. (vii) A passivation layer is placed on one side of the membrane and electrical connections made via a purpose built PCB.

silicone membranes are made by diluting the two components of soft PDMS (MED-4901, NuSil) in a solvent at a weight % ratio 2:1, then casting the mixture onto a poly(ethylene terephthalate) (PET) substrate coated with a water soluble sacrificial layer, following the process outlined in [20] (Figure 4i).

The compliant electrode is made by ball mixing a 10:1 wt% mixture of the soft PDMS elastomer (MED-4901) and carbon black powder (Ketjenblack 300, AkzoNobel), and then casting the mixture onto a PET substrate coated with a 50 μm thick poly(vinyl alcohol) (PVA) water-soluble sacrificial layer, following the approach described in [12]. After curing in an oven at 100°C for 3 hours, the elastomer membrane is released and prestretched equi-biaxially by 1.1 times using a purpose built biaxial prestretcher [20]. After the electrodes are cured in an oven at 80°C for 3 hours, the electrode and water-soluble film are patterned to the desired shape using a CO_2 laser engraver (Speedy 3000, Troctec) and subsequently peeled away from the PET substrate [12]. Bonding between the elastomer membrane and compliant electrodes is achieved by the natural adhesive properties of the elastomer, which provide sufficient adhesion for our application. A few drops of ethanol are placed on the surface of the prestretched elastomer membrane prior to placing the electrode in order to minimize formation of air-pockets at the interface [12].

After 30 minutes the PVA backing of the electrode is dissolved by submersion

in hot de-ionized water and the process repeated on the reverse side of the sensing layer for the second electrode. Once the PVA of the second electrode is dissolved a second prestretched elastomer membrane is placed on one side of the sensing layer (using the natural elastomer adhesive properties to achieve bonding), serving as the passivation layer. Electrical connection to the two electrodes is facilitated using two purpose built printed circuit boards (PCB) which sandwich the membrane. The PCBs are held in place using plastic screws and a room temperature vulcanized (RTV) biocompatible adhesive (Silpuran 4200, Wacker) (Figure 5i), which also makes the device water-tight. Toxicity tests were performed on all the materials used in the device fabrication, all proved non-cytotoxic to human bronchial smooth muscle cells for a test duration of up to 72 hours [11].

3.2. Sensor stability

The fabricated prototype is shown in Figure 5ii (inset). The passivation and sensing layers, as well as the electrodes are approximately 6 μm thick after prestretch. The outer radius of the annular electrodes is 7.7 mm (the same as the radius of the hole in the centre of the PCBs), matching the dimensions of a single well of a standard 24-well cell culture plate. The inner diameter of the electrodes was chosen to be 5.7 mm i.e. approximately 0.75 times the outer diameter. The sheet resistance of the electrodes is $60\text{ k}\Omega\text{ }\square^{-1}$. Capacitance measurements were made by measuring the complex impedance of the device over various frequencies using an Agilent LCR meter (1 V excitation voltage), and calculating the capacitance C' using the relationship $C' = 1/(\omega X)$, where ω is measurement frequency and X is the reactance (series resistor-capacitor model assumed). During the stability measurement the device was placed upside down in a petri dish filled with cell culture medium, as schematically represented in Figure 5i, and subsequently placed in a climate chamber (ESPEC SH-661, ThermoTec) which regulated the chamber environment, simulating cell culture conditions (average humidity = 97 % relative humidity maximum variation 0.7 % rel. humidity, average temperature 36 $^{\circ}\text{C}$, maximum variation 0.42 $^{\circ}\text{C}$).

After an initial transient lasting approximately 4 hours (attributed to thermal effects and changes in the culture medium surface tension) the capacitance signal reached a stable value of approximately 410 pF (Figure 5ii). The mean of the moving standard deviations of the capacitance signal is shown in Figure 5iii, as a function of the measurement frequency and the measurement time window in minutes (measurement times representative of those required to observe contraction in vascular smooth muscle cells [3] and in human bronchial smooth muscle cells (Appendix B)). We use this metric as a measure of the device stability (baseline noise). Figure 5iii shows that the mean standard deviations in the capacitance signal lower than 0.2 pF (0.05% of

Figure 5: Characterization of electrical stability of prototype sensor. (i) Schematic representation of experimental setup. (ii) Sensor capacitance signal over 10 hours (measurement frequency ≈ 180 Hz). Inset: Image of fabricated device. (iii) Mean of moving standard deviations for measurement frequencies ≈ 180 , 240, 320 and 420 Hz, and time windows = 30, 60 and 120 minutes. (iv) Model prediction for the change in capacitance ΔC (and $\Delta C/C'$) produced by cell contractile stress σ_c for the prototype sensor. Mean standard deviation of 0.2 pF (from (iii)) overlaid as an error band (shaded green). Detection range is suitable for the contractile stresses reported in the literature [1, 3] (highlighted in orange).

initial capacitance) were achieved with the prototype device for a 120 minute measurement duration, which is excellent given the challenging measurement conditions. The figure also shows that the device signal stability is reasonably constant over the different measurement durations and measurement frequencies selected (mean deviations < 0.5 pF), and is more stable at lower measurement frequencies than at higher ones.

The model developed in section 2 is used to predict the performance of the prototype device. The Young's modulus of the elastomer membranes E , and the carbon electrodes E_e , were determined experimentally to be approximately 0.30 MPa and 0.60 MPa, respectively. A Young's modulus of 30 kPa and thickness $t_c = 5$ μm is assumed for the cells [1, 3]. The relative permittivity of the sensing layer is taken to be 2.75 and all other parameters are as stated in the caption¹ of Figure 3. The results of the model are plotted in Figure 5iv, the mean standard deviation at a measurement frequency of 180 Hz and a measurement duration of 120 minutes (from Figure 5iii) is shown as an error band on the model prediction. Figure 5iv shows that contractile stresses as low as 1.2 kPa would be detectable using the fabricated sensor. This sensitivity is sufficient to measure the contraction of cardiomyocytes as reported by Grosberg *et al.* ($\sigma_c = 20$ kPa average systolic, $\sigma_c = 8$ kPa average diastolic) and human umbilical arterial smooth muscle as reported by Alford and colleagues ($\sigma_c = 13$ kPa).

Higher sensitivity could yet be achieved by using a PDMS with a lower Young's modulus, though this may affect device manufacture, making the sensor more difficult to fabricate and reducing yield. Moreover, reducing the PDMS Young's modulus will likely also increase the susceptibility to mechanical noise, and may therefore be a practical limitation to further improvement in sensitivity. Reducing the sensing layer thickness, however, should reduce susceptibility to electrical noise (due to the increase in the sensor initial capacitance) simultaneous to the increase mechanical noise, and so may allow for greater overall gains in sensitivity. With the manufacturing processes described here, electrode thickness as low as 3 μm were achievable, however, signal stability could decrease as a result of the increased resistance and subsequent increase in the RC time constant (reducing the effective capacitance). Future work will involve the optimization of the compliant electrode mixture in order to improve resistance without significantly increasing the Young's modulus, enabling the use of thinner electrode layers.

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¹ $\lambda_{pre} = 1.1$, $D = 0.75$, $\lambda_{free} = 0.7$, $\varepsilon_r = 2.75$, $N = 500$ mm^{-1} .

4. Conclusion

High-throughput test assays capable of characterizing cardiac and smooth muscle cell contractility are required for drug screening and development. Many of the approaches currently being developed rely on optical methods to measure cell contraction, limiting their scalability to multiple wells for highly parallelized measurement. Our approach measures cell contractile stress using the cell-induced deformation of thin-film, elastomeric capacitive sensors. This approach offers an electrical read-out (capacitive) scheme, as opposed to an optical one, increasing the potential throughput and scalability compared to currently available test assays. Moreover, with our approach and versatile fabrication methodology, large-area arrays of devices can be fabricated. The development of a model to predict the cell-induced change in capacitance for a given device geometry, material properties and cell parameters, is described. The model solves the nonlinear stretch field in the region of the sensor patterned by the compliant electrodes numerically using the shooting method, and the equilibrium stretch of the system is found by elastic energy minimization. The model shows that a cell region ratio of approximately 0.75 of the total culture well diameter is optimal for maximizing the change in capacitance with respect to cell contraction. Device sensitivity increases with decreasing sensor Young's modulus, sensing layer thickness and electrode thickness, following an inverse relationship, highlighting the necessity for the use of thin and highly compliant elastomer films. A prototype device was fabricated to demonstrate the stability of a fabricated device under realistic cell culture conditions. Mean standard deviations as low as 0.2 pF ($< 0.05\%$ of device capacitance) were attained from the device prototype, representing a device sensitivity of 1.2 kPa. This sensitivity is sufficient to measure the contractile stresses of cardiomyocyte and smooth muscle cell monolayers reported in the literature.

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Appendix A. Device geometry and mechanics

For radial force equilibrium of a infinitesimally small material element of the device (as schematically represented in Figure A.1B), we obtain:

$$(s_R + ds_R)(R + dR)td\theta - s_R Rtd\theta - 2s_\theta \sin(d\theta/2)tdR = 0 \quad (\text{A.1})$$

For small angles $\sin(d\theta/2) \simeq d\theta/2$, hence,

$$(s_R + ds_R)(R + dR)td\theta - s_R Rtd\theta - s_\theta d\theta tdR = 0 \quad (\text{A.2})$$

Expanding the first term of equation A.2 and dividing by $td\theta$ gives,