Toward compression of small cell population: Harnessing stress in passive regions of dielectric elastomer actuators

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Toward compression of small cell population: Harnessing stress in passive regions of dielectric elastomer actuators

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ABSTRACT

We present a dielectric elastomer actuator (DEA) for *in vitro* analysis of mm^2 biological samples under periodic compressive stress. Understanding how mechanical stimuli affect cell functions could lead to significant advances in diseases diagnosis and drugs development. We previously reported an array of 72 micro-DEAs on a chip to apply a periodic stretch to cells.

To diversify our cell mechanotransduction toolkit we have developed an actuator for periodic compression of small cell populations. The device is based on a novel design which exploits the effects of non-equibiaxial pre-stretch and takes advantage of the stress induced in passive regions of DEAs. The device consists of two active regions separated by a 2mm x 2mm passive area. When connected to an AC high-voltage source, the two active regions periodically compress the passive region. Due to the non-equibiaxial pre-stretch it induces uniaxial compressive strain greater than 10%. Cells adsorbed on top of this passive gap would experience the same uniaxial compressive stain. The electrodes configuration confines the electric field and prevents it from reaching the biological sample. A thin layer of silicone is casted on top of the device to ensure a biocompatible environment. This design provides several advantages over alternative technologies such as high optical transparency of the area of interest (passive region under compression) and its potential for miniaturization and parallelization.

Keywords: Dielectric elastomer actuator, deformable cell culture system, compressive strain, optically transparent

1. INTRODUCTION

The human body is constantly exposed to a complex set of mechanical forces. Ranging from gravity to muscle contraction, these forces can induce changes in the structure, composition or function of our biological tissues.[1] A good example of this is the bone loss observed in astronauts after a long stay in space.[2] It is now widely accepted that mechanical forces affect various fundamental cellular functions such as differentiation, proliferation and gene expression. However, the mechanotransduction processes by which cells can convert mechanical stress into biochemical reaction are still not fully understood.

Several techniques have been developed to isolate and study the effect of mechanical forces on cellular functions. The most common one is to culture cells on a deformable silicone substrate which can then be subjected to different types of mechanical loads. With this approach, the entire biological sample (a few centimeters in size, with up to several hundreds of thousands of cells) is subjected to the same mechanical strain. Working with such large populations limits the scope of the studies to communal behaviors. In addition, the effect of different strain levels or frequencies are challenging to study due to inevitable variation between biological samples. In response to these limitations, various devices were developed to apply mechanical loads on single cells or small populations.[3][4] Although these approaches enable single cell response monitoring (by opposition so communal behavior), most of them exhibit low screening throughput and requires complex setup and manipulations.

Dielectric elastomer actuators (DEAs) offer various desirable properties such as large actuation strains and fast actuation speeds. In addition they can be made from bio-compatible materials and the fabrication processes are not as complex as for MEMS. Significant advances were achieved in theoretical modeling and material engineering throughout the last years. It led to the development of devices capable of giant actuation strain (>1000%)[5], actuators with sub-millisecond response time[6] and compliant electrodes with self-healing capabilities[7].

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As depicted in Fig. 1(a), typical DEAs consist of a dielectric elastomer membrane sandwiched between two flexible and stretchable electrodes. Dielectric elastomers (DEs) are polymers with relatively low Young's modulus and high elongation at break. When a voltage difference is applied on the device it generates electrostatic forces between the two electrodes which induce Maxwell stresses $\sigma_{Maxwell}$ across the membrane. As can be seen from Eq. (1), Maxwell stresses are a function of the material dielectric permittivity $\varepsilon_r\varepsilon_o$, the thickness of the elastomer membrane t and the actuation voltage V. Due to the elastomer incompressibility, the electrostatic forces results in an out-of-plane compression and an in-plane elongation of the membrane as shown in Fig. 1(b).

$$\sigma_{Maxwell} = \varepsilon_r \varepsilon_0 \left(\frac{V}{t}\right)^2 \quad (1)$$

Application in biomedical studies appears to be an interesting avenue for DEAs. Typical requirements in terms of generated force (mN), actuation strain (5-20%) and actuation speed (<5Hz) can be achieved. In addition, elastomers are good candidates for biomedical applications with their low Young's modulus and good biocompatibility. We previously reported an array of micro-DEAs to periodically apply uniaxial tensile strain (up to 20%) on small areas (100µm x 100µm) of a larger (few square centimeters) biocompatible membrane.[8][9] This approach provides a low-cost and simple solution to enable single cell response monitoring and high screening throughput. This DEA design was however limited to tensile strain. Moreover, the areas under tensile stress were not optically transparent due to the presence of carbon-based electrodes and the biological sample was exposed to fringing electric fields.

In order to overcome these limitations we developed a new actuator which can be used to apply compressive stress to cells and which provides high optical transparency while also minimizes fringing electric fields. The second section of this paper details the design's working principle. It also presents and discusses the main geometrical considerations and the role of pre-stretch. The third section presents the fabrication process and experimental measurement of the DEA actuation strain.

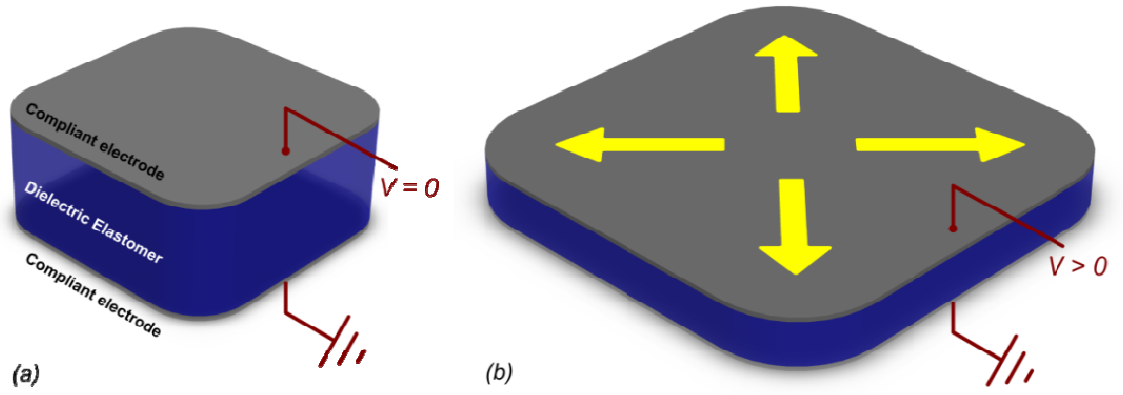

Figure 1. Dielectric Elastomer Actuators (DEAs) working principle.

2. ACTUATOR DESIGN AND WORKING PRINCIPLE

2.1 Actuation principle

Figure 2 presents a conventional DEA design. Two square electrodes are patterned on the top and bottom sides of a dielectric elastomer membrane. The volume bounded by these two electrodes corresponds to the active region whereas the surrounding area corresponds to the passive region. In most cases, DEAs use a support frame for handling or to hold pre-stretch in the dielectric elastomer membrane. As depicted in Fig. 2, using such a frame is the equivalent of having fixed boundary conditions.

For cell mechanotransduction studies the biological sample would be located on top of the device. It can either cover the entire membrane or only the high strain area (active region) by means of surface functionalization. Under actuation, the

active region expands and consequently compresses the passive region. If the ratio between active and passive areas is under 1/100, the boundary condition has essentially no impact on the maximum strain.[10] In this ideal case, the actuation strain is maximum in the active region and minimum in the passive region. Figure 2(c)-(d) shows the mechanical stress distribution in a biological sample that would covers the entire device. As can be seen, the sample would be under high tensile strain and low compressive strain above the active and passive areas respectively.

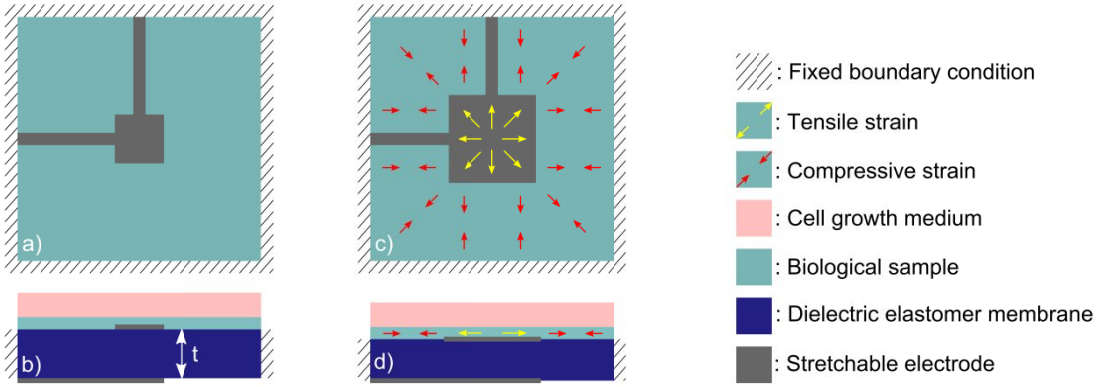

Figure 2. (a) Top and (b) side view of a typical DEA design generating expansion of the central region. (c) Top and (d) side view of a typical DEA under actuation.

The design we present in this paper takes advantage the stress induced in passive regions of the membrane. As can be seen from Fig. 3(a)-(b), two active regions of width *w* are aligned along their length *L* and separated by a gap *g*. The elastomer membrane is under anisotropic pre-stretch in order to achieve uniaxial actuation. [11] (The role of the pre-stretch will be discussed in more details in section 2.3.) The high pre-stretch direction is oriented along *Y* in order for the actuation to occur along the length *L* of the active regions. As the two active regions are bounded on one side by a fixed boundary condition, the resulting actuation strain is unidirectional. As shown on Fig. 3(c)-(d), both active regions expand toward the center of the membrane and induce uniaxial compression in the passive gap separating them. As a result, a biological sample located on top of the actuator would experience high compressive strain and low tensile strain above the passive gap and active areas respectively.

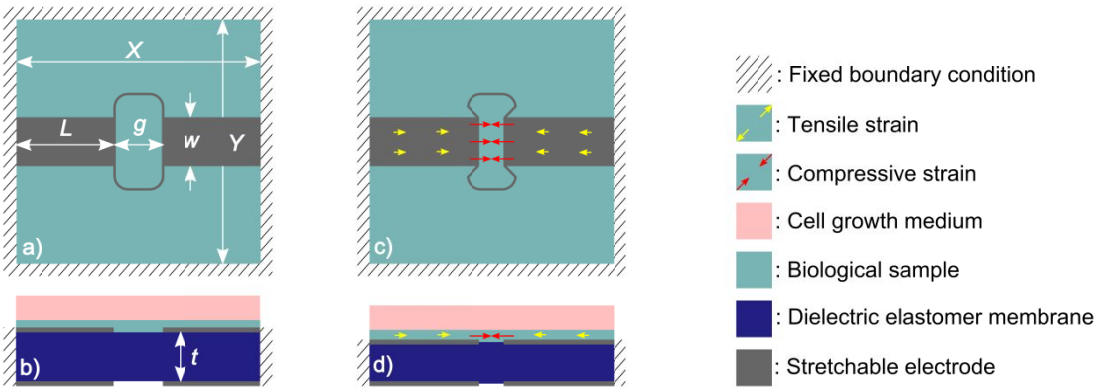

Figure 3. (a) Top and (b) side view of the developed DEA. (c) Top and (d) side view of the developed DEA under actuation.

In addition to the ability of generating compressive stress, other aspects make this design interesting for mechanotransduction studies. First, it can easily be integrated in a matrix configuration. As mentioned earlier, one important aspect in the development of deformable cell culture systems is to achieve high screening throughput. The

ability to have an array of actuators on a single membrane is therefore a great benefit. Various parameters can be studied simultaneously by having for example a gradient of strain across one axis of the array.

Moreover, the region where cells will be exposed to high compressive stress (passive gap) is highly transparent in the visible range. In comparison, the previously reported device had the cells located on top of the active region.[8] As in the device schematize in Fig. 2, the optical properties where therefore limited by the electrodes which resulted in poor optical transparency. This parameter is of great importance for biologists. It allows inspection of cells with high resolution inverted optical microscopes. For example, with optical access to the biological sample the evolution of cell morphology can be monitored under periodic compression.

A third benefit to this design is the electric field confinement. As can be seen from Fig. 3, the electrodes are completely overlapping which minimizes the fringing electric field outside the dielectric elastomer membrane. It ensures that the biological sample is protected from the electric field generated by the actuator. Biological samples are essentially composed of living cells and cell culture medium (ionic liquid). Strong electric field reaching this region could affect cell development or lead to ionic diffusion from the cell culture medium into the elastomer membrane and modifies the actuators behavior and performances.

Cells adhere to bio-compatible surface by the mean of protein adsorption [12] and exhibit low Young's modulus. It can therefore be assumed that the actuation strain generated in the device membrane will also be experienced by the biological sample. The main performance metric to consider for the design optimization is the maximum compressive strain that can be induced in the passive gap during actuation. Cell mechanotransduction studies typically require 5-20% compressive strain. It is also important to ensure good strain uniformity over a given area. Our goal is to achieve better than 1% uniformity over a 500μm x 500 μm area. Sub-section 2.2 and 2.3 will discuss the geometrical considerations and the role of pre-stretch in the design optimization.

2.2 Geometrical considerations

There are a few geometrical parameters to consider when optimizing this design. The first and most important one is the ratio between the length L of the electrodes and the width g of the passive gap. Equation 2 gives the relation between these geometrical parameters and the strain generated in the membrane. The variables $S_{tensile}$ and $S_{compressive}$ correspond to the tensile strain generated in the active area and the compressive strain induced in the passive gap respectively. The most interesting scenario is when the ratio between L and g is higher than ½. In this specific case there is an amplification effect and the generated tensile strain can induce a much larger compressive strain. For example if the active regions length is 5 times longer than the passive gap, the amplification factor α is equal to 10. This means a 1% tensile strain in the active area would induce a 10% compressive strain in the passive gap.

$$\frac{L}{g} = \frac{1}{2}\frac{S_{Compressive}}{S_{Tensile}} = \frac{1}{2}\alpha \qquad (2)$$

A second geometrical parameter to consider is the ratio between the widths of the active area w and the membrane Y respectively. The maximum ratio would be equal to one, which corresponds to the case where the electrodes are as wide as the membrane. The closer the ratio gets to unity, the more the actuation strain is affected by the fixed boundary conditions. First, it limits the maximum actuation strain of the device which is one of the main figures of merit used for DEAs. Second, it induces strain non-uniformity in the active regions which indeed results in non-uniform compression of the passive gap. In the extreme case of a w/D ratio equal to unity, the strain would be maximal in the center of the passive gap and gradually decrease to zero along the direction perpendicular to the actuation. This strain non-uniformity might not be a critical issue for most applications but it is definitely an important aspect to consider in the frame of this project. The strain uniformity in the passive gap should be maximized in order to ensure a good control over the strain that will be experienced by the biological sample.

2.3 Role of the pre-stretch

Another important design parameter is the pre-stretch applied on the dielectric elastomer membrane. Its amplitude and orientation can be adapted to significantly modify the behavior and performances of the device. Equation (3) defines pre-stretch λ and presents the notation that will be used in this paper. The sets of variables (X, Y) and (X', Y') correspond to the initial and final dimensions of the membrane respectively.

$$\lambda = (\lambda_x;\ \lambda_y) \tag{3}$$

$$where\ \lambda_X = \frac{X'}{X}\ and\ \lambda_y = \frac{Y'}{Y}$$

The first role of pre-stretch is to improve the maximum actuation strain of the device. It can be used to suppress electromechanical instabilities which are usually the first failure mode to occur in DEAs.[13] The optimal parameters in terms of actuation strain can be calculated based on the dielectric elastomer physical properties. It is important to note that pre-stretch doesn't need to be equibiaxial but its optimal amplitude ($\lambda_x \cdot \lambda_y$) indeed depends on its orientation. For pre-stretch greater than this optimal value there is performance degradation. It increases the membrane stiffness which results in higher working voltage and lower actuation strain.

The second role of pre-stretch is to ensure that pure compressive strain is induced in the passive gap when the device is under actuation. This requirement is motivated by the final application of the device. Mechanotransduction studies usually try to isolate the effect of a single mechanical stimulus on biological cells. It is therefore important to achieve a uniform and pure compressive strain distribution in the passive gap. For an equibiaxial pre-stretch, both the length *L* and width *w* of the electrodes increase under actuation. As a result, the passive gap is exposed to compressive stress along one direction and tensile stress along the orthogonal direction (in the place of the membrane). This configuration is indeed not generally desirable for mechanotransduction study.

One consequence of pre-stretch is mechanical stiffening of the elastomer membrane. This effect is directional and consequently non-equibiaxial pre-stretch can be used to induce anisotropy in the mechanical properties of the membrane. One solution to eliminate the tensile stress component in the passive gap is to apply a non-equibiaxial pre-stretch for which λ_y is much larger than λ_x. In that configuration the entire membrane becomes very stiff along the width *w* of the electrodes. The active areas only expand along the length *L* of the electrode and the passive gap is therefore exposed to pure uniaxial compressive stress.

The value of λ_x should be chosen carefully in order to maximize the compressive strain in the passive gap. For uniaxial pre-stretch we have $\lambda_y > 1$ and $\lambda_x^{uniaxial} = 1/\sqrt{\lambda_y} < 1$ (unconstrained and completely relaxed). Due to the small dimensions of the passive gap, the mechanical loss of tension in the active regions occurs at a very low strain. In order to avoid this and to achieve reasonable compressive strain, a small pre-stretch should be applied in the actuation direction. As a design rule, λ_x should be equal to or greater than the targeted compressive strain multiplied by $\lambda_x^{uniaxial}$. It is important to realize that pre-stretch along the actuation direction also stiffens the membrane which tends to lower the actuation strain. There is therefore a tradeoff here which limits the maximum compressive strain that this design can generate.

3. EXPERIMENTAL RESULTS

3.1 Fabrication process

An important part of the development work resides in the fabrication process. Figure 4 presents a schematic view of the device at every major steps of the fabrication process. First, a 75 μm thick Dow Corning Sylgard 186 silicone elastomer membrane is casted on a high quality PET film using a Zehntner ZAA 2300 automatic film applicator coater. After curing, a 3cm x 10 cm section of the membrane is peeled from its substrate and pre-stretched λ=(0.9;2.75). (a) Circular Plexiglas frames with a 44mm inner diameter are used to hold the pre-stretch and ease the handling of the membrane. Adhesives Research Inc. ARclear® 8154 adhesive and Dow Corning® 734 Flowable Sealant are used to ensure strong adhesion between the membrane and the frames throughout the entire process. (b) Compliant electrodes are patterned on the top surface of the membrane: An uncured conductive carbon black-elastomer composite is printed on the membrane by stamping using a Teca-Print Pad Printing Machine TPM 101. After curing, (c) the device is flipped and the printing process is repeated in order to pattern electrodes on the bottom side of the membrane. At this point of the process flow, the device is already a working DEA. Electrodes are patterned on both sides and the membrane is in the optimal pre-stretch state in terms of compressive strain that can be generated in the passive gap. It is however not ready for the intended biological applications. (d) Using again the same stamping technique, a few microns thick silicon encapsulation layer is printed over the electrodes on one side of the actuator. This encapsulation layer plays two roles. First, it provides an electrical insulation between the electrodes and the cell culture medium that will eventually cover them. Second, the encapsulation material is chosen to be bio-compatible and ensure the cells viability. Once the encapsulation layer is cured, (e) a new set of Plexiglas frames are fixed to the membrane using Adhesives Research Inc. ARclear® 8154 and

Dow Corning 734 Flowable Sealant. As can be seen, the sealed area fits inside the inner opening (22mm x 22mm) of the Plexiglas frames. In addition, the frames have two additional small openings that align over the extending section of the electrodes. This is designed to easily connect the actuator to an external voltage source. (f) Finally the membrane is cut to release the inner frames.

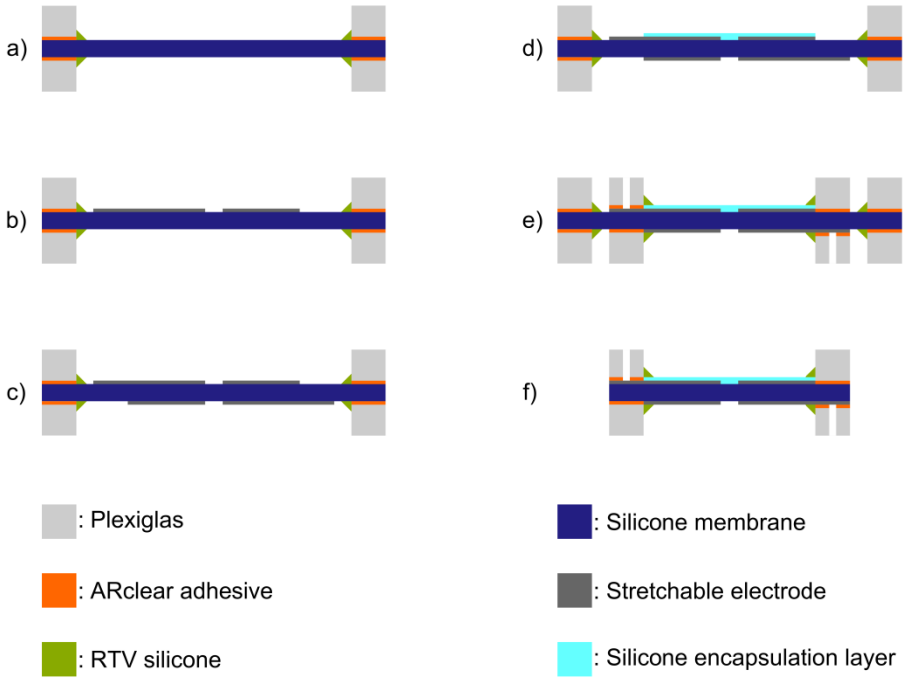

Figure 4. (a)-(f) Fabrication process flow of the DEA.

3.2 Characterization of the actuation strain

Figure 5 presents (a) a picture of a fabricated single actuator device (sample CS003) and (b) a close-up of the passive gap where uniaxial compressive strain is generated. The electrode geometry and the pre-stretch parameters are given in Table 1. These values are based on the geometrical considerations and the role of pre-stretch that were discussed in section 2.

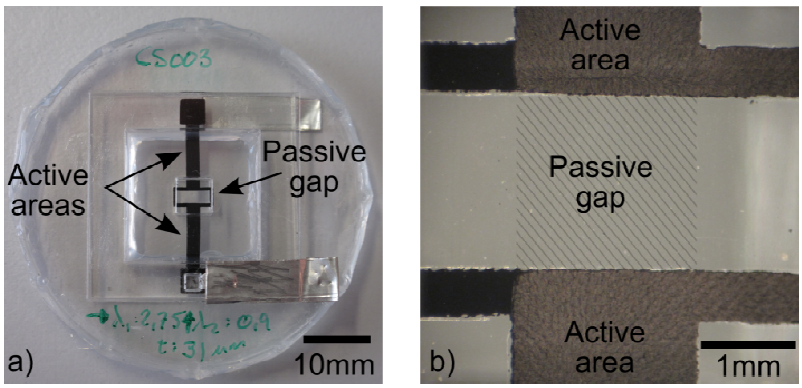

Figure 5. (a) Picture of the fabricated single actuator device (sample CS003) and (b) close-up of the passive gap where compressive strain in generated.

Table 1. Design parameters of the sample CS003.

Membrane geometry			
Membrane width	X	[mm]	22
Membrane height	Y	[mm]	22
Membrane thickness after pre-stretch	t	[μm]	31
Electrodes geometry			
Electrodes length	L	[mm]	10
Electrodes width	w	[mm]	10
Passive gap length	g	[mm]	2
Pre-stretch parameters			
Pre-stretch along the membrane height	λ_y		2.75
Pre-stretch along the membrane width	λ_x		0.9
Effective pre-stretch along the membrane width	$\lambda_x/\lambda_x^{uniaxial}$		1.2

The actuation strain of the device was measured experimentally. The DEA was connected to a DC high-voltage source and placed under an Olympus SZ40 Zoom Stereo Microscope. One of the eyepieces was removed and replaced by a camera adapter and a camera. Images of the actuator were captured at 0V and then at every 50V from 3kV up to 4.6kV. Optical zooms of the microscope and the camera, as well as the position of the sample were kept constant during the experiment to maintain the same reference scale.

The images were post-processed using the NI Vision Assistant. Filtering and border tracking tools were used to locate the borders of the passive gap and calculate its width g. The data were then normalized over its initial value. Figure 6 presents the compressive strain in the passive gap as a function of the electric field in the active regions. The electric field is calculated as the ratio between the applied voltage and the membrane thickness. A maximum compressive strain of 12.5% was obtained at 4.6kV which corresponds to a 153V/μm electric field. It was limited by the mechanical loss of tension which could be observed at higher voltages. Higher pre-stretch along the actuation direction could potentially lead to greater compressive strain.

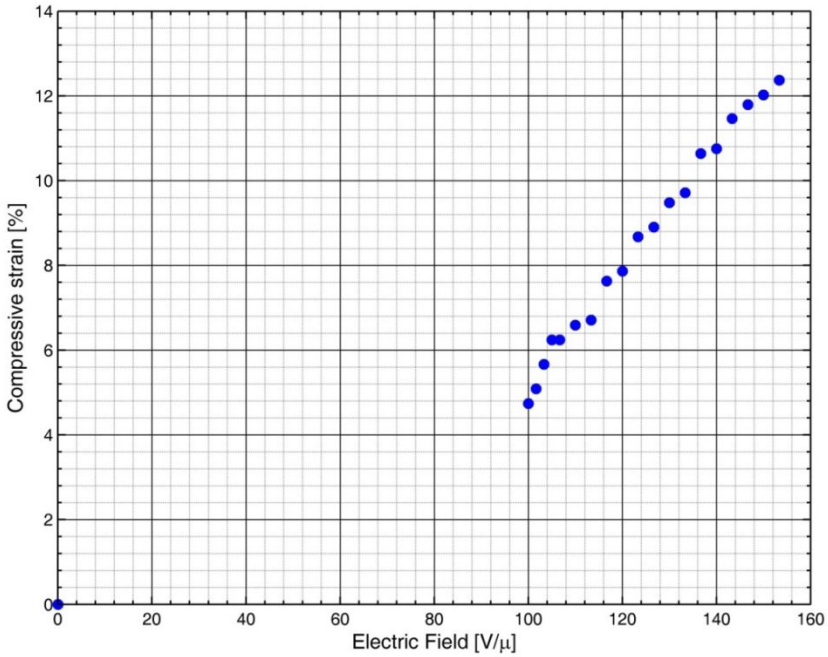

Figure 6. Experimental characterization of the sample CS003. Compressive strain generated in the passive gap as a function of the applied electric field.

4. CONCLUSION

We presented a novel DEA design that harnesses stress in passive regions of the membrane. The device was developed for cell mechanotransduction studies and presents notable advantages, namely its high optical transparency, its good electric field confinement (minimal stray electric field) and its compatibility with matrix configuration which would enable high screening throughput experiments.

The important geometrical design parameters were presented and discussed. It was shown that with suitable designs, small tensile strain in the active areas can be used to induce much larger compressive stress in the surrounding passive region. The importance and roles of pre-stretch were also presented and discussed. It was shown that non-equibiaxial pre-stretch is the best option and that both λ_x and λ_y should be chosen carefully in order to optimize the maximum compressive strain that can be induced in the passive gap. A device based on this design was fabricated and characterized. A maximum compressive strain of 12.5% was achieved under 4.6kV which corresponds to a 153V/μm electric field.

On the short term, future work will focus on demonstrating that biological samples can be cultured on top of the DEA and periodically compressed. On the longer term, the design will be miniaturized (500μm x 500μm passive gap) and integrated into a matrix configuration for parametric studies with high screening throughput. It is anticipated that this will lead to improved deformable cell culture systems for cell mechanotransduction studies. Advances in that field of research could lead to better diagnosis and treatment of various diseases ranging from dystrophy to cancer progression and metastasis.

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