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# Cell stretching devices as research tools: engineering and biological considerations

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Cells within the human body are subjected to continuous, cyclic mechanical strain caused by various organ functions, movement, and growth. Cells are well known to have the ability to sense and respond to mechanical stimuli. This process is referred to as mechanotransduction. A better understanding of mechanotransduction is of great interest to clinicians and scientists alike to improve clinical diagnosis and research into medical pathology. However, the complexity involved in *in-vivo* biological systems creates a need for better *in-vitro* technologies, which can closely mimic the cells microenvironment with induced mechanical strain. This technology gap motivates the development of cell stretching devices for better understanding of the cell response to mechanical stimuli. This review focuses on the engineering and biological considerations for the development of such cell stretching devices. The paper discusses different types of stretching concepts, major design consideration and biological aspects of cell stretching and provides a perspective for future development in this research area.

## 1. Introduction

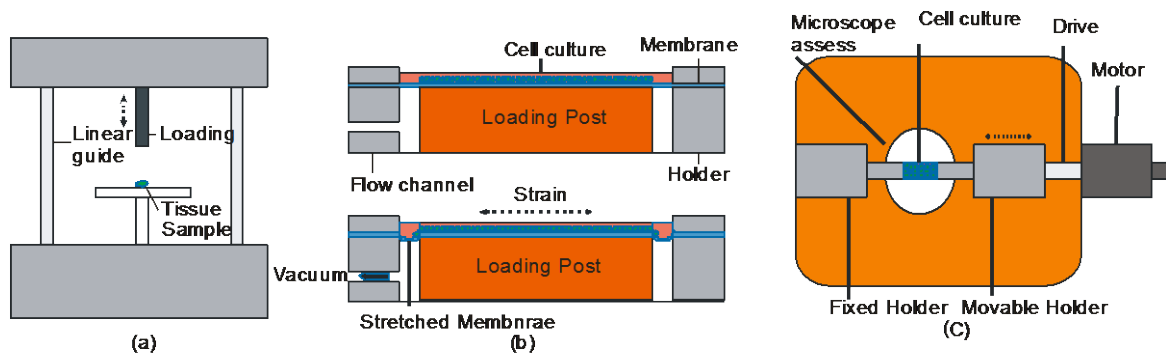
Cells experience various kinds of mechanical forces within a normally functioning body. These forces play an important role for the development of cells as well as for the regulation of their homeostatic activities. For instance, endothelial cells in blood vessels are subjected to both shear stress due to the blood flow and cyclic strain due to the blood pressure.

Mechanical forces acting on cells are well known to induce intracellular biochemical signals, which play important roles in cellular behaviours such as proliferation, growth and migration.<sup>1, 2</sup> A cell can sense mechanical stimuli and convert these stimuli into biological responses through a series of cellular processes, which is known as mechanotransduction.<sup>3-5</sup> Mechanotransduction plays significant roles in the regulation of the cellular activities and can significantly influence cell activities.<sup>6</sup> Abnormalities in these processes may contribute to the pathogenesis of several diseases, such as cancer, asthma, heart failure, etc.<sup>7, 8</sup> Thus, mechanotransduction has been a major research interest in the field of regenerative medicine and bioengineering.

Due to the complexity of the *in-vivo* cellular environment, most mechanotransduction research relies on the development of *in-vitro* techniques with integrated *in-vivo* like stimuli. Thus, *in-vitro* cell-stretching assays are vital for further understanding the dynamics of mechanotransduction.<sup>9-11</sup> The development of *in-vitro* techniques that better mimic the pathological *in-vivo* environment of cells will facilitate researchers to gain greater

insight into mechanotransduction and will significantly improve our understanding of physiology and cellular biology for clinical diagnosis and subsequent treatments.

Micropipette, tweezers, atomic force microscopes (AFM) and micro posts with integrated magnets are some of the most common techniques that have been traditionally used for cell stretching in clinical diagnosis.<sup>12-16</sup> Recently, more elegant techniques have been pursued commercially, such as Flexcell (Flexcell International Corporation); Strex Systems for cell Stretching (STREX Inc.) and ElectroForce (Bose Corporation), (Fig.1). Flexcell's Stage Flexer has been used ubiquitously due to its well-characterised strain profile, homogenous strain pattern and adaptability of stretching modes.<sup>17-19</sup> Apart from these commercial systems, several other custom-made cell-stretching devices have been reported over the last decade.<sup>20-23</sup> However, most of these devices have low throughput and generate a non-linear strain profile. Contemporary research on cell stretching has been significantly influenced by the state-of-the-art technologies of Micro-Electro-Mechanical Systems (MEMS) and microfluidics.<sup>24-26</sup> Recent advances in MEMS and microfluidics technologies have facilitated complex operations such as trapping cells, creating more realistic microenvironments and providing direct observation for quantifying cell behaviour.<sup>27-29</sup> MEMS and microfluidics technologies are both expected to play a significant role in the future to recreate the cellular microenvironment and provide a more accurate physiological model *in-vitro*.



**Fig.1:** Commercially available cell stretchers: (a) ElectroForce 3100 (Bose Corporation) (b) Stage Flexer (Flexcell international Cop.) (c) Stretch System for Microscope STB-150 (STERX Inc.).

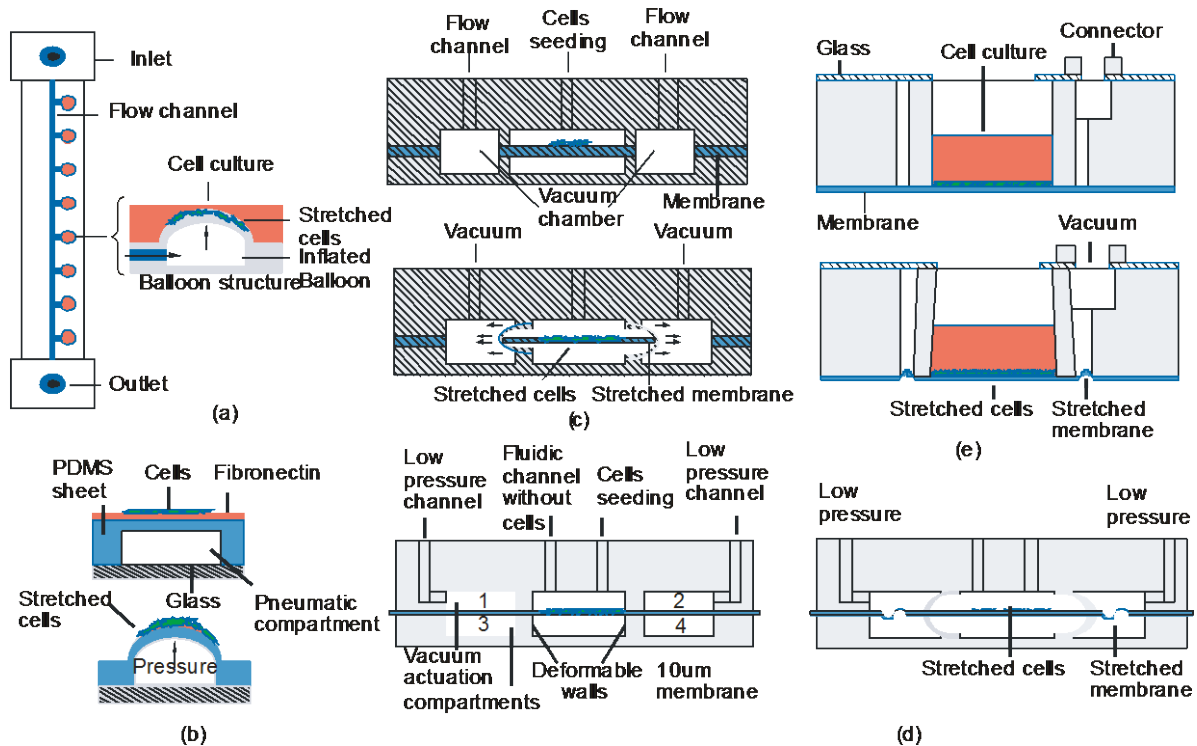
Several review articles have evaluated the different actuation techniques and methods used for cell stretching.<sup>30-33</sup> Although, most of these articles are focused upon different parameters such as cell stretching methods, cell mechanics or actuation, whereas very few discuss the engineering as well as biological considerations involved. Furthermore, most reviews were published more than five years ago, thus warranting an updated review for this exciting field. In the present review, we provide an in-depth exploration of cell stretching systems with a focus on the major design considerations and biological aspects involved in the development of cell stretching device and provide future perspective for the design, fabrication

## 2. Actuation concepts

This section discusses the different types of actuation that have been reported in the literature, where stretching concepts are classified based on the different actuation techniques used for cell stretching. Furthermore, we elaborate on the suitability of the stretching concept and other major design considerations for achieving the delicate task of cell stretching.

### 2.1 Pneumatic actuation

Pneumatic actuators have been widely used to induce



**Fig.2** Typical cell stretching devices with pneumatic actuation: (a) Inflated balloons with positive pressure; (b) Actuation chamber positive pressure; (c) Two-chamber side actuation with negative pressure; (d) Four-chamber side actuation with negative pressure; (e) Radial stretching with circular support and negative pressure.

technologies and materials required for the development of cell-stretching devices.

mechanical stress or strain to cells *in-vitro*. This actuation concept holds substantial advantages such as simple setup,

homogenous strain actuation and no direct contact with the cells and/or the media, which is important to avoid contamination. The majority of devices using pneumatic actuation techniques are based on the deformation of a thin membrane with controlled actuation pressure. The cells are cultured directly onto this membrane.<sup>27,34-36</sup>

Actions with both positive and negative pressure sources have been exploited for cell stretching. For instance, Shimizu, *et al.* (2011) utilized positive air pressure for inflating serially connected balloons, Fig. 2(a).<sup>37</sup> Furthermore, the pressure drop in microchannels was utilized to deliver a wide range of strain magnitudes within a single device. Similarly, Heo, *et al.* (2013) fabricated pneumatic a microactuator consisting of pneumatic chambers.<sup>38</sup> The balloon-like expansion of the chambers stretches the cells cultured on the membrane( Fig. 2b). Huang and Nguyen (2013) developed a multilayered PDMS device, in which vacuum source was used to pull the membrane by deforming the wall attached to the sides of the membrane (Fig. 2c).<sup>20</sup>

Tremblay, *et al.* (2014) further advanced the above concept and designed a similar multilayered PDMS device with four low-pressure compartments for biaxial stretching.<sup>39</sup> In this device, the independently controlled negative pressure was used to pull the membrane and deform each compartment to stretch the cell culture in both horizontal and vertical directions (Fig. 2d). Kreutzer, *et al.* (2014) developed a circular device with a thin membrane and computer controlled vacuum pressure in the cavity between the two PDMS shells to deform the membrane.<sup>40</sup> This induced a symmetrical radial stress onto the inner shell and subsequently stretches the cells grown on the membrane (Fig. 2e).

## 2.2 Piezoelectric actuation

Another popular actuation approach to induce stretching has been piezoelectric micro- and nanomanipulators. Piezoelectric manipulators using a high displacement resolution have been included in a number of studies to induce cell stretching, where the key advantages is the precision and broad range of controllable strains. Whilst the high resolution of displacement further provides an active tool to control loading during the process of cell stretching. However, some piezoelectric actuators require a direct physical contact with cells, which severely limits its applications. Although indirect stretching through microstructures can be used to overcome this problem, loading limitations still remain. Nonetheless, it is common to use Micro-Nano manipulators which are externally linked to the on-chip microstructures such as micro intendant or microplate for on-chip cell stretching.<sup>41-44</sup>

Kamotani, *et al.* (2008) developed a device with an array of miniature cell stretching chambers which included microwells with a flexible bottom membrane placed over a piezoelectrically actuated pin.<sup>45</sup> Each pin was independently actuated by individual piezoelectric actuators using a customised computer program that pushes the bottom membrane to achieve radial strain on the cells (Fig. 3a). Deguchi, *et al.* (2015) used two tandemly arrayed piezoelectric actuators to stretch a PDMS membrane,<sup>46</sup> with each consisting of a chamber with a base membrane (Fig. 3b). Fior, *et al.* (2011) fabricated a MEMS device consisting of microstructure linkages connected to the cell stretching area.<sup>22</sup> This device used an externally controlled piezoelectric actuator to displace the micro linkages and subsequently displace the membrane (Fig. 3c). Sato, *et al.* (2010) designed and fabricated a device consisting of elastic transparent micro-chambers and a MEMS micro-linkage

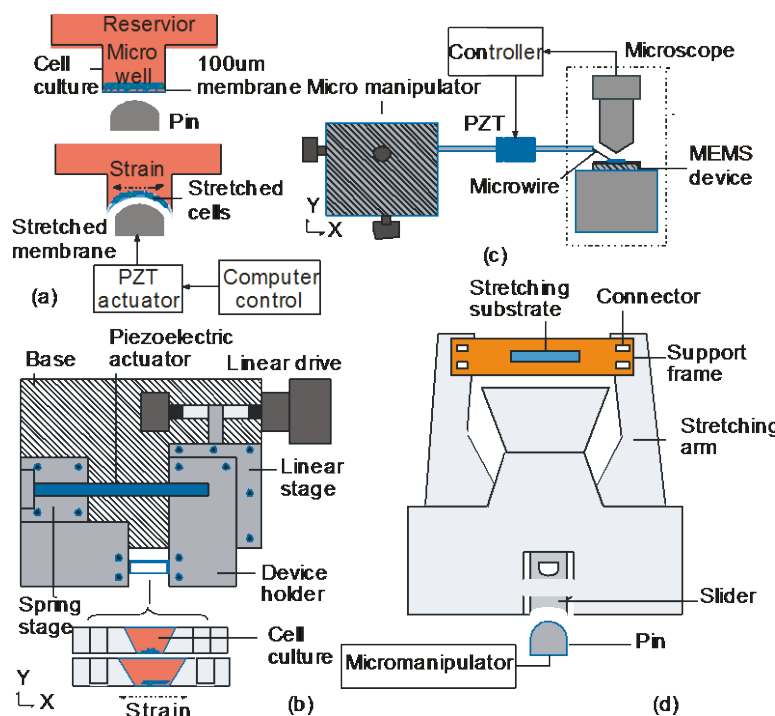


Fig. 3. Typical cell stretching devices with piezoelectric actuation: (a) Radial strain with pushing pin; (b) Linear stretching with piezoelectric linear drive; (c) MEMS translation stage with external actuator; (d) MEMS linkage mechanism.

mechanism,<sup>47</sup> where six 2mm×2 mm devices were fabricated on a 22mm glass plate. Each device consisted of a silicone rubber microchamber, stretching arms, a slider to drive stretching arm, bearing to transmit linear motion into rotation and micro-needle connected to micro-actuators to push the slider and to drive stretching arm (Fig.3d).

### 2.3 Electromagnetic actuation

Electromagnetic actuators provide another sophisticated alternative for inducing mechanical stress on cells. Controlled electromagnetic motors have been used to achieve the desired stretching effect,<sup>48-50</sup> however, the ongoing need for lubrication and the possibility for device erosion are major concerns for cell contamination. Yet, advantages such as high precision and intuitive programmability with a relatively simple setup for both static and dynamic loading are both attractive features with this actuation concept.

Because of the above advantages, many custom-made cell-stretching devices have used electromagnetic actuators. Huang, *et al.* (2010) designed a mechanical cell stretching system based on the indenter design and drove it with a servomotor.<sup>51</sup> The device consists of a membrane holder ring fixed to a mobile plate, where the indenter ring placed on the base is smaller than the membrane holder ring. The controlled vertical downward motion of the mobile plate leads to the stretching of membrane (Fig. 4a).

Ursekar, *et al.* (2014) adapted a similar construction with a stepper motor as the actuator.<sup>23</sup> The authors addressed the issue of heterogamous strain and fabricated a 1-mm thick and 10-mm tall cylindrical wall on the membrane to confine the cells and to

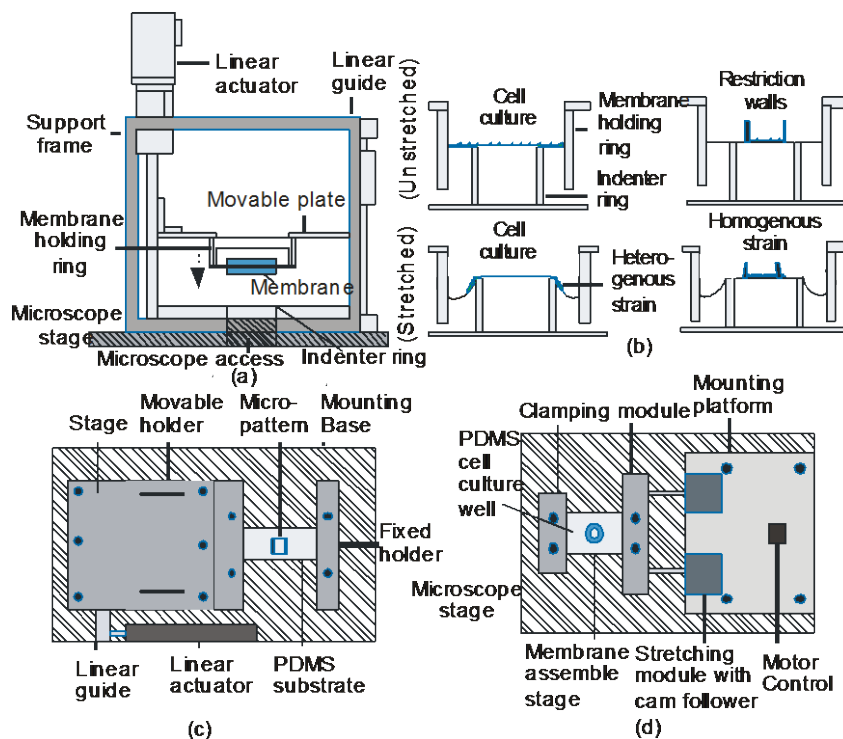
induce a homogeneous strain (Fig. 4b). Chang, *et al.* (2013) used a translation stage for motorised stretching of neural stem cells,<sup>52</sup> where the flexible stretching substrate with micropatterns was clamped with one end fixed to the base and other end connected to the translation stage. Linear motion of translation stage actuated by the motor transfers the strain to the substrate and results in the stretching (Fig. 4c).

Shao, *et al.* (2013) introduced a cam-follower mechanism, driven by an electrical motor,<sup>53</sup> this device consisted of a clamping module with one fixed and one movable L-shaped plate. The L-plate-cam-follower linkage stretches the PDMS membrane clamped to the movable L-shaped plate (Fig. 4d).

### 2.4 Optical actuation

Optical actuation is another approach for cell stretching. Being a non-contact actuation technique, optical actuation avoids interference with the cell culture and maintains sterility. Optical tweezers or optical stretchers have been reported in many clinical studies,<sup>54-57</sup> where a simple optical stretcher incorporates a high power diode laser with a wavelength and beam width smaller than the cells. The laser beam induces a force onto the cells along the axis of the laser which causes deformation of the cells.<sup>21, 58, 59</sup> A major limitation of this type of technique is that the flow in the microchannel cannot be controlled precisely. Therefore the cells will settle in the microchannel and may form clusters, which disturb flow, ultimately causing non-uniform stretching and quantification difficult.

Sraj, *et al.* (2010) demonstrated a method to stretch swollen red blood cells (RBCs) with an optical trap by a single-beam, diode laser.<sup>60</sup> The authors fabricated a microfluidic channel in PDMS bonded to glass with a cross section of 15 μm × 150 μm, using



**Fig. 4.** Typical cell stretching devices with electromagnetic actuation: (a) Linear pushing with a servo motor; (b) Radial strain with pushing by a stepper motor; (c) Stretching with linear translation stage; (d) Stretching with cam follower mechanism.

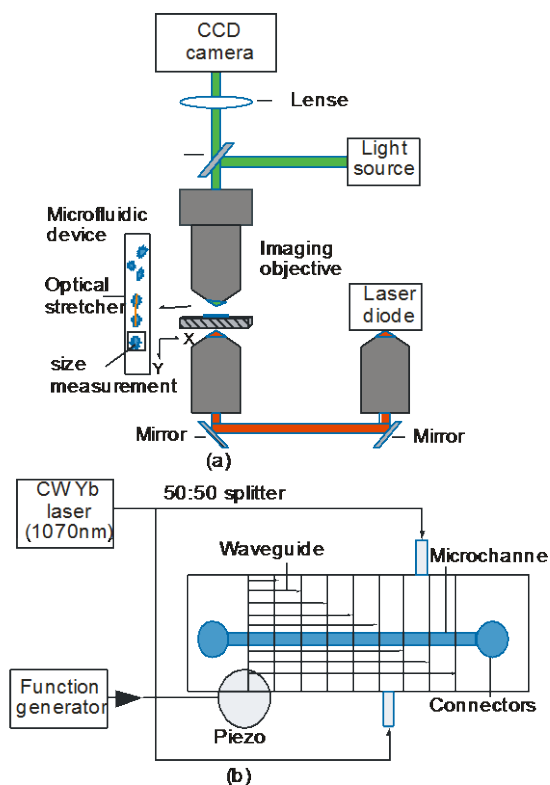


Fig. 5 Typical cell stretching devices with optical actuation: (a) Stretching in a microchannel with free-space laser beam

a single-mode laser diode (830 nm, 200 mW) to optically trap the RBCs in the microchannel and to induce the deformation. The shape and orientation of the cells were determined by taking a measurement in a trap and after exiting the trap (Fig. 5a). Whereas, Nava, *et al.* (2015) employed an acoustophoretic method using a piezoelectric transducer to pre-focus the flow in a microfluidic device by integrating optical waveguide separated by a distance of 25  $\mu\text{m}$  for cell manipulation and stretching (Fig. 5b).<sup>61</sup>

## 2.5 Other actuators

Apart from the major actuation techniques discussed above, other concepts such as electro-thermal, dielectrophoretic and electrostatic actuations have also shown potential

Electrothermal actuation incorporates a V-shaped beam which is subjected to thermal expansion stretching along the apex.<sup>62-64</sup> Although this technique has been extensively used for micromanipulation in MEMS technology, it has not yet been exploited due to the thermosensitivity of cells. However, a number of researchers have employed the thermal expansion properties of shape memory alloy (SMA) for cell stretching. For instance, Iwate and Yumura (2009) used SMA actuators for cyclic cell stretching, this system consists of four parallel

connected SMA coils with one end fixed and the other connected to the stretched substrate.<sup>65</sup> A power supply and a fan were used to heat and cool the SMA for cyclic stretching. A sequential square wave of 2.6 V was applied to the SMA to induce cyclic heating and subsequently cyclic stretching of the membrane (Fig. 6a).

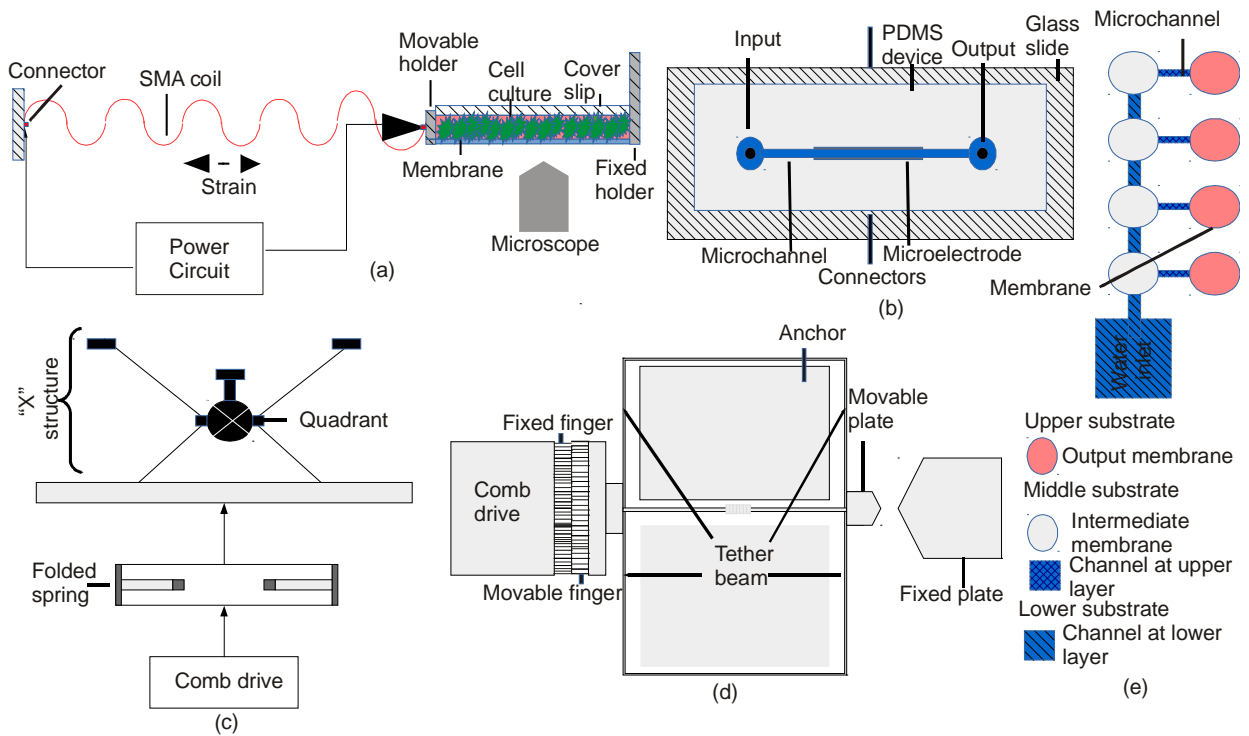
Dielectrophoretic actuation is based on cells experiencing a force when exposed to the high-frequency electric field. This actuation method has been used for cell separation and positioning, but only a few studies reported cell stretching using this method. Guido, *et al.* (2012) fabricated a microfluidic device incorporating two 250- $\mu\text{m}$  wide indium tin oxide microelectrode separated by a microchannel of 20- $\mu\text{m}$  width.<sup>66</sup> Cells were trapped in the channel, and an electric field (6 V<sub>rms</sub> square wave, 15 MHz) was applied to microelectrodes to induce cell deformation (Fig. 6b).

Electrostatic forces were utilised for micromanipulation in many MEMS applications. An electrostatic actuator primarily consists of a comb drive with a linear range of few micrometres.<sup>67-70</sup> Scuur, *et al.* (2006) designed and fabricated a biaxial cell stretcher, which included a circular four quadrant sectional disk and linkages.<sup>73</sup> This device was designed in such a way, that the quadrants moved simultaneously in a horizontal and vertical direction to achieve biaxial stretching with the comb drive connected with linkages to provide linear actuation (Fig. 6c). Whilst Shen, *et al.* (2008) used a comb drive to induce uniaxial tension on hydrated collagen fibrils.<sup>74</sup> The MEMS device includes collagen fibrils mounted between the fixed pad and the movable pad, which was further connected to a comb drive. Upon actuation, the movable fingers of the comb drive pull the movable pad and induce tension to the collagen fibrils (Fig. 6d). In another study utilising electrostatic forces, Wang, *et al.* (2014) designed and fabricated a bi-layered microfluidic device made of PDMS and selected water as the medium for hydraulic actuation. PDMS is porous and permeable to gas, pneumatic actuation may not create consistent strain on the cells.<sup>75</sup> Their device consisted of four outlet channels and intermediate membranes connected to the fluidic inlet with a single pump to achieve identical strain patterns, with an intermediate membrane that subsequently deforms the cells on the outlet channels (Fig. 2e).

## 3. Design considerations

Thus far it is evident that several actuation techniques and customised designs can be used to induce strain to cells *in vitro* for mechanotransduction analysis. Stretching parameters such as: magnitude; direction of strain; frequency and time intervals have a different influence on cell behaviour.<sup>70-72</sup> In order to design and develop a well-characterised cell stretching device, it is important to consider all factors contributing towards these aforementioned parameters. Apart from these key parameters, other factors will be discussed in the following section.

### 3.1 Biocompatibility



**Fig. 6** Other actuation schemes for cell stretching: **(a)** Thermomechanic actuation with shape memory alloy; **(b)** Dielectrophoretic actuation; **(c, d)** Electrostatic actuation; **(e)** Hydraulic actuation.

Designing a cell-stretching device must take into account factors that are required to maintain viable cells such as sufficient fresh culture media. Moreover, the biocompatibility of the device must be warranted to avoid contamination, especially in the case of contact-based cell stretching techniques. For instance, cell-stretching techniques with electrostatic and electrothermal actuators<sup>65,68</sup> face serious problems with the exposure to the cell's aqueous environment. Electrostatic actuators such as comb drives are susceptible to reduction in the initial stroke when exposed to cell culture due to the electrically conductive media. In the presence of liquid media, an electrothermal actuator may also present limitations due to the fluctuations in temperature it causes to the cell culture.

Therefore, non-contact based stretching techniques such as pneumatic, magnetic and optical all seem advantageous options. Although no actuation method can be considered completely exemplary for stretching, as each technique has its inherent advantage and disadvantages. Thus, the suitable actuation technique depends on the specific characteristics of the cells tested.

### 3.2 Substrate properties

Substrate properties such as elasticity, roughness and wettability are factors which have to be considered when designing cell stretching devices. It is known that some cells such as a fibroblasts or a smooth muscle cells have sensitive surface adhesion mechanisms and thus require a desirable surface stiffness in order to attach and proliferate.<sup>73</sup>

Gray, *et al.* (2003) reported a distinct behavioural change in fibroblast cells between soft and hard substrate stretching devices.<sup>80</sup> While Palchesko, *et al.* (2012) described phenotypical changes within cells in response to the surface's roughness and wettability.<sup>81</sup> Although many surface adhesion proteins such as collagen, fibronectin, laminin, and matrigel etc. have been used to maintain the desired adhesion mechanisms, substrate properties still play an important role in cell behaviour. The best practise is considering substrate properties and cell adhesion mechanisms when choosing a suitable substrate. Moreover, adhesion of cells to the substrate also contributes towards the magnitude of strain experienced by the cells.

### 3.3 Control strategies

The force applied to the cells is the main factor that directly affects mechanotransduction, and is therefore one of the most important parameters. The magnitude of the required force depends on the cell type being investigated. Techniques such as optical tweezers and atomic force microscopy provide an actuation stress ranging from  $\text{pN}/\mu\text{m}^2$  to  $\mu\text{N}/\mu\text{m}^2$ .<sup>12, 16</sup> An inaccurate strain could change the behaviour, morphology of the cells and lead to irreversible damage.<sup>70</sup> Thus, for any cell stretching system, it is imperative that the stress induced by the actuators has high accuracy, resolution, and repeatability. System control plays an important role to maintain these parameters in the desirable range. The controller needs to induce a desirable magnitude of the force to maintain the well-controlled mechanical stimulation of living cells. For instance, when designing a pneumatic actuator system, apart from the general loading control factors: such as air compressibility; leakage; and gas permeability; the elasticity of the membrane also needs to be considered. Therefore, conceding these control

factor and comparing the data of various actuation techniques indicate that pneumatic and piezoelectric actuators hold a major advantage over the other actuation techniques. Control systems with a high resolution, precision and repeatability are readily and commercially available for these actuation techniques.

### 3.4 Stretching direction

, Stretching direction is a further important parameter when designing a cell-stretching device. Common examples include uniaxial, biaxial and radial stretching. However, depending on the assay requirement, different directional types can be included in the same design. As cells randomly oriented themselves over the *in vitro* membrane and not always in the direction of strain, they may experience less strain than that actually induced. Thus, cell behaviour varies according to the stretching direction. Ahmed, *et al.* (2010) reported the response of skeletal myoblasts after cyclic stretching at  $0^{\circ}$ ,  $45^{\circ}$ ,  $90^{\circ}$  directional orientation using micro contact printed fibronectin lines.<sup>49</sup> The strongest development was observed after cyclic stretching of myoblast at  $45^{\circ}$ . Myoblast at  $0^{\circ}$  showed a random orientation, and myoblast at  $90^{\circ}$  to pattern did not show any significant alignment. These results highlight that stretch direction should be considered when designing a device.

### 3.5 Size of the actuation system

The size of the stretching membrane also needs to be considered during the designing phase. Although macro scale devices provide high resolution and precision, unfortunately they consume significant amounts of reagent. On the other hand, microdevices require less culture media but have limited precision and loading capability. Thus, in many reported works, microactuators with macroscopic control systems are often preferred. The advancement in MEMS and microfluidics will lead to high throughput on-chip actuation systems, which might overcome the existing the problems.<sup>29, 36</sup>

## 4. Biological considerations

Cells in the human body are constantly interacting and adapting to a variety of physical forces placed upon them. Some of these forces include: the compressive forces in a loaded joint; the shear forces inside the pumping heart or blood vessel; the tensile forces driven by contracting muscles and the episodic expansion forces within the lungs. Therefore, to maintain tissue homeostasis cells within tissue must proliferate, differentiate, migrate, modulate gene expression and remodel, subject to these forces and thus prevent injury or detrimental pathologies, such as tendinopathies, cardiomyopathies and atherosclerosis.<sup>77</sup> But it is unclear how cells actually respond to such mechanical forces (mechanobiology) and how they are able to transduce these biophysical forces into biomolecular events (mechotransduction) for adaption. Comprehension of how mechanical force influences tissue homeostasis will ultimately lead to advancements in biological therapeutics i.e. bioengineering, biomaterials and regenerative medicine. The following section will provide a brief review on some research that has been explored in these two areas of cellular biology, while providing some specific examples of *in vitro* cellular and tissue/organ mechanobiological research.

### 4.1 Mechanobiology

In a way, Empedocles over 2 millennia ago was right, all sensations we perceive from the world is by physical touch. Yet biological science is only beginning to understand how physical

forces influence cellular physiology, while physicians and scientists are just uncovering the mechanical basis aetiologies for some diseases such as osteoporosis, stroke and asthma. However, before we can understand mechanobiology, we must first explore the architecture of tissue itself. Tissue is a complicated sandwich of cells, reinforced by a lattice work of protein fibres (extracellular matrix: ECM), which is mirrored inside each individual cell. Thus, being a sandwich of organelles inside a membrane held together by a sea of filamentous cytoskeletons. This intracellular cytoskeletal framework is not a passive device,<sup>8</sup> rather a complex transducer for the forces that pass onto and through it.

The cell's cytoskeleton can further distribute and balance physical forces through their adherence to the protein fibres in the ECM, which creates a complicated viscoelastic phenomenon within cells and tissues. Trepap *et al* (2007). demonstrated that the cytoskeleton can actually transform between fluid and solid phases depending on the mechanical loads exerted on it.<sup>78</sup> Therefore, mechanical loading of the cytoskeleton and the ECM will affect the appearance and behaviour of cells. Ingber (2003), reinforces this notion and suggests that physical forces play a critical role in the development, differentiation and maintenance of all living cells/tissue.<sup>8</sup> For example, chondrocyte ECM gene expression and proliferation was the highest under cyclic compressive loads compared to constant loads.<sup>79</sup> Osteoblasts proliferate and lay down more tissue under a constant frequency (1Hz) and low strain.<sup>80, 81</sup> Repetitive stretch and relaxation of skeletal muscle cells increased their elasticity and bulk.<sup>82</sup> Endothelial cells respond most efficiently to shear forces,<sup>83</sup> and stretch force differentiates ligamentous fibroblasts.<sup>77</sup> But how does a mechanical force result in these phenotypic changes? This leads us to mechanotransduction.

### 4.2 Mechanotransduction

As previously mentioned, the ability of the cell to maintain its integrity when subjected to mechanical forces is considerably due to the cytoskeleton's viscoelastic nature. Mechanical loads are reciprocally displaced through the cytoskeleton and across the cell membrane into the ECM via integral mechanoreceptors known as "integrins". Integrins function as load elements adhering to actin cytoskeletal elements at focal points on the membrane which connect to the cell's nucleus.<sup>84</sup> The ECM-integrin-cytoskeletal pathway is currently the most researched and understood of the mechanotransduction pathways,<sup>77</sup> which connects to cellular components such as: G-proteins; cadherin complexes; GTPases; mechanical-gated ion channels; protein kinases; and transcription factors etc., thus converting physical stimuli into biochemical signals.

It has been demonstrated experimentally that when integrins are mechanically deformed, the cellular response is to produce a localised intracellular biochemical transduction, upregulate and recruit focal adhesion proteins to the point of stress and re-orientate the cytoskeletal architecture to withstand additional stress.<sup>85</sup> Thereby, the application of an external stress will directly affect the morphology and sensitivity of the focal adhesions for the characteristics of that particular stress, subsequently creating a more efficient biochemical modulation for intracellular signal transduction.<sup>7</sup> For example, when endothelial cells were exposed to a mechanical stress, similar to the fluid shear forces found within a blood vessel, the cells elongated and the focal adhesions along with the cytoskeleton orientated themselves perpendicular to this stress.<sup>86</sup> In this case,



a subset of the GTPase proteins played a pivotal role in the signal transduction pathway and thus facilitated the production of focal adhesion complexes. Different stretch magnitudes and frequency by the pulsating vessel will result in different signalling properties by endothelial cells. Therefore, the actual mechanical forces themselves are necessary to maintain a proper functioning of the vascular system and irregularities can cause inappropriate cellular activities and consequential cardiovascular abnormalities.<sup>87-89</sup>

Whereas, the highly localised arrangement of focal adhesions within the cytoskeletal projections (cilia) of hair cells within the vestibular apparatus, respond ideally to ECM alterations to fluid dynamics, although in this case the focal adhesions are linked to mechanical ion gates.<sup>8</sup> Many *in vitro* studies have reported the different effects strain has on a monoculture. However, due to the complex *in vivo* biological environment, with respect to mechanical forces in such systems as the respiratory system organ-on-a-chip research has become an exciting contemporary avenue of research.

Boccafoschi, *et al.* (2007) observed the effect of different magnitude (1-25%) and frequency (0.25-3 Hz) of cyclic stretch on human lung fibroblast.<sup>107</sup> Results showed that at a magnitude of 1%, fibroblast aligned to stretch direction, but at and 2% achieved the highest orientation to strain whilst no significant alteration was seen from 5-20%. Also it was observed that the frequency change did not influence the percentage of cells oriented perpendicular to the stretch direction. Further increase in strain at 25% resulted in cellular death. Recently, Cui, *et al.* (2015) showed that 1-5% cyclic stretching at frequencies of 0.01-10 Hz increased spreading and stress fiber formation of fibroblasts on soft substrates ( $k=2.3$  nN/ $\mu$ m).<sup>9</sup> It was also found that these stretched-induced cellular behaviours were linked to biochemical responses by two related transcription factors, MRTF-A (myocardin-related transcription factor-A) and YAP (Yes-associated protein). The lung-on-a-chip device has an ideal platform for mechanotransduction studies. The platform is a microfluidic device consisting of two micro-channels separated by 10  $\mu$ m PDMS membrane. Epithelial and endothelial cells can be seeded separately in microchannel and cultured on ECM coated porous PDMS membrane stretched using pneumatic actuation to mimics the alveolar capillary interface.<sup>27</sup>

## 5. Conclusions and perspective

In this paper, we first discussed the possible actuation concepts for cell stretching devices. Pneumatic actuation is the most common concept that relies on external vacuum or pressure to stretch a flexible membrane with cells cultured on it. The main advantage of pneumatic actuation is that it induces a homogenous mechanical strain to the cells whilst the device only needs tubing to connect the actuation chambers on the device to the external vacuum/pressure supply, and therefore does not interfere with the cell culturing process. Piezoelectric actuation is another viable method as it does not induce heat although due to the need of a relatively high voltage, piezoelectric actuation is not suitable for an integrated solution where the actuators may be in contact with wet and conducting medium. Electromagnetic actuators such as servomotor or stepper motor can be precisely controlled, however, the ongoing need for lubrication and the possibility for device erosion are major concerns for cell contamination.

Besides these three major actuation concepts, other actuation schemes such as electrothermal, electrostatic, optical and dielectrophoretic concepts can all be utilised for designing cell stretching devices. Electrothermal and electrostatic actuators could be implemented in the same way as piezoelectric and electromagnetic actuators. Due to the heat and high voltage involved, these actuators have to be placed outside of the cell culture chamber, e.g. coupled mechanically with a linkage mechanism. Optical and dielectrophoretic actuations produce relatively small forces and are therefore suitable for *in-situ* single-cell stretching. Monitoring and controlling single-cell stretching is a big challenge as the culturing condition has to be met at the same time. Furthermore, these actuation concepts may induce optical and electrostatic stimuli to the cells, which cannot be separated from mechanical stretching and make designing mechanotransduction experiments difficult.

Both state-of-the-art commercially available and experimental stretching devices are limited to simple induction of mechanical stretching to the cells. Both microfluidics and MEMS technologies have not been fully utilised for making these devices yet. Following a few directions for their improvement to make cell-stretching devices more versatile and enabling tools.

First, most devices reported in the literature are only able to induce one strain rate in a single experiment. To improve the throughput, the stretching device should have the same format as a standard well plate array with a range of programmable strain rate. A 3x5 array of pneumatic cell stretching devices with three different strain rates have been demonstrated before.<sup>20</sup> In the future, the same concept could be scaled up to suit the standard format of 96-well, 384-well and 1536-well microplates.

Second, all reported devices relied on the geometry and applied force/pressure to generate a fixed strain pattern on the cells. As the response to mechanical stimuli may help cells to differentiate and to form functional tissue, a programmable two-dimensional or possibly three-dimensional strain map could be designed to create different tissue type on the same stretching device. The programmability of the strain distribution would allow for a new method of bottom-up tissue printing where cells differentiate themselves based on mechanical cues.

Third, most of the reported devices used manual processes for seeding of cells and delivering and exchanging culture media. Although most stretching devices based on microfluidics have the capability to manipulate cells and liquids in microchannels, none of them actually have pumping and valving integrated for automated handling of cells and culture medium. In the future, if the handling process can be automated, cell-stretching devices can be set up and used at a low cost and minimum manual labor.

Finally, the complexity of cell stretching devices may be enhanced with an additional microfluidic network for creating different concentrations of chemicals such as growth factors. The ability to induce both mechanical and chemical stimuli to the cells would bring the capability of these devices to the next level. Cell-stretching devices with these improvements will

enable us to understand better the important processes of many diseases associated with defects in mechanotransduction.

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## Notes and references

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