Integrative Biology

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Cite this: DOI: 10.1039/c1ib00056j

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CRITICAL REVIEW

# (Micro)managing the mechanical microenvironment

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***Received 7th June 2011, Accepted 5th August 2011***
**DOI: 10.1039/c1ib00056j**



Mechanical forces are critical components of the cellular microenvironment and play a pivotal role in driving cellular processes *in vivo*. Dissecting cellular responses to mechanical forces is challenging, as even "simple" mechanical stimulation *in vitro* can cause multiple interdependent changes in the cellular microenvironment. These stimuli include solid deformation, fluid flows, altered physical and chemical surface features, and a complex transfer of loads between the various interacting components of a biological culture system. The active mechanical and biochemical responses of cells to these stimuli in generating internal forces, reorganizing cellular structures, and initiating intracellular signals that specify cell fate and remodel the surrounding environment further complicates cellular response to mechanical forces. Moreover, cells present a non-linear response to combinations of mechanical forces, materials, chemicals, surface features, matrix properties and other effectors. Microtechnology-based approaches to these challenges can yield key insights into the mechanical nature of cellular behaviour, by decoupling stimulation parameters; enabling multimodal control over combinations of stimuli; and increasing experimental throughput to systematically probe cellular response. In this critical review, we briefly discuss the complexities inherent in the mechanical stimulation of cells; survey and critically assess the applications of present microtechnologies in the field of experimental mechanobiology; and explore opportunities and possibilities to use these tools to obtain a deeper understanding of mechanical interactions between cells and their environment.

## 1. Introduction

The effects of mechanical forces on biological systems are commonplace. Muscle development, bone remodeling, and fibrous skin callus formation, for example, are all influenced by external mechanical loading. These organ-level phenomena can be traced to cellular behaviour, prompting interest in understanding how cells mechanically interact with their surroundings. Mechanical forces have been identified as critical components of the cellular microenvironment, regulating cytoskeletal structure[1] and consequently apoptosis,[2] differentiation,[3] adhesion, polarity, contractility and migration,[4,5] gene transfection,[6] protein expression, secretion and metabolic activity.[7,8] Non-mechanical stimuli such as soluble cytokines or extracellular matrix ligands may also affect cytoskeletal structure,[9–11] thereby altering cellular fate and function[12] *via* an intrinsically mechanical pathway. Hence, the cell functions as a *mechanical* entity, both in terms of its behaviour in the surrounding environment, and in being responsive to surrounding

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### Insight, innovation, integration

Mechanical features of the cellular microenvironment provide critical cues in driving cell function, and are important parameters in designing *in vitro* culture models. However, multiple complex and often-overlooked environmental changes result from even 'simple' mechanical stimulation on the macroscale, confounding such studies. In addition, conventional systems typically lack experimental throughput and spatial resolution necessary for certain experiments. In this review, we highlight insights into cellular mechanobiology that arise from exploiting microtechnology-based approaches to mechanobiology; and assess the utility of these approaches through defining critical feature sizes, precisely manipulating physical cues, and increasing experimental throughput. We further discuss future opportunities and challenges in using these tools to obtain a deeper understanding of the mechanical interactions between cells and their environment.

mechanical conditions in a variety of contexts, including in disease,[13–15] development[16–18] and regeneration.[19]

The recognition of the critical role played by mechanics in regulating biological form and function has given rise to two distinct areas of research: biomechanics and mechanobiology.[20,21] *Biomechanics* is "*the application of the principles of mechanics to study living organisms and their components*".[20] Both passive and active mechanical behaviours of the cell are highly complex,[1] reflecting[9–11] and forecasting[12] cell phenotype, and may be useful markers in disease progression.[22] Adhesion, migration and contractility of cells are mechanically-oriented processes through which cells manipulate and remodel the environment, and are hence of critical importance in wound healing,[23] progression of certain diseases and development.[13] In contrast, *mechanobiology* is "*the application or analysis of the role of mechanical forces in eliciting a molecular response, leading to a quantifiable change in form and/or function*".[20,21] The importance and influence of environmental mechanics on cell fate and function has been thoroughly established and is the subject of multiple reviews.[24–26] Mechanobiology is a key component in pathobiology;[13,27,28] development and morphogenesis;[16] and in many specialized tissues such as bone,[29] tendon,[30] the heart valve,[31] intervertebral disc[32] and cartilage.[33,34] The ability to precisely manipulate the mechanical microenvironment to understand the mechanisms and processes by which mechanical forces regulate cell function requires novel experimental approaches.

Microfabricated technologies can provide viable solutions to some of the problems associated with understanding both cellular biomechanics and mechanobiology. Applications of microtechnologies in this field have prompted a few recent reviews on the subject, focused primarily on devices designed to characterize cellular biomechanics.[35–38] In this review, we categorically survey and assess microtechnology-based strategies that have provided key insights in cellular mechanobiology. Future directions in which technological development can enhance our understanding of the cell as a mechanical entity will also be suggested.

## 2. 'Large' issues in mechanobiology

In addition to the costs associated with animal models, the mechanical complexity of *in vivo* environments makes identifying the specific effects of a mechanical stimulus challenging. For example, cells in the aortic heart valve leaflet undergo complex deformation cycles as the leaflet opens and closes. Blood flow exerts shear stresses on endothelial cells, and the transient pressure differentials necessary to establish pumping may alter cellular function. In addition, matrix stiffness, composition, and cell phenotype each influence mechanical response.[31] Independently manipulating these parameters is not possible *in vivo*, and hence, effectively probing such complex mechanical environments benefits from the development of *in vitro* culture models.

Determining the effects of external mechanical parameters on cell function has been achieved through the use of several macroscale experimental approaches, which have been reviewed in detail elsewhere.[39] Briefly, a variety of commercial and custom platforms have been developed to apply mechanical

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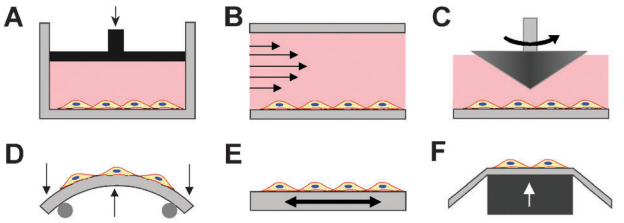


**Fig. 1** Approaches to studying cellular mechanobiology using conventional equipment. (A) Hydrostatic pressure applied to cultured cells. (B, C) Flow-induced shear stress exerted on cells in (B) a parallel plate flow chamber, and in (C) a rotating cone-and-plate shear device. (D–F) Mechanical forces applied to cells by means of substrate deformation *via* (D) out-of-plane bending of the substrate, (E) uniaxial or biaxial in-plane deformation, and (F) in-plane deformation caused by deforming the substrate with a loading post. Each approach can be applied to cells encapsulated in a three-dimensional biomaterial, but this requires careful consideration and analysis of the mechanical stimuli arising in such a system.

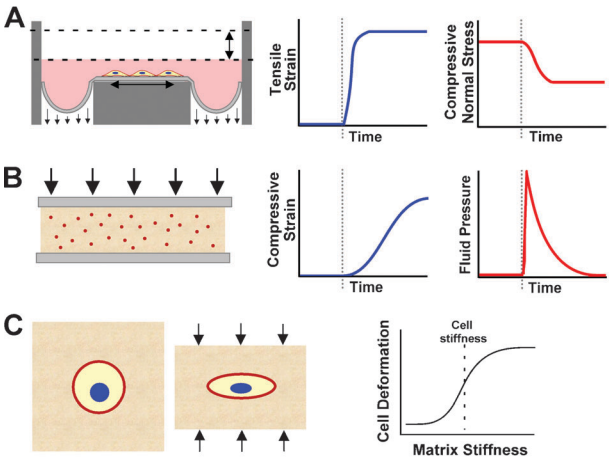


**Fig. 2** Complex mechanical behaviour arising from 'simple' mechanical stimulation. (A) Deformation of two-dimensional cell culture substrates results in displacement of fluid over the cell surface, creating a changing normal force profile for mechanical stimulation with this apparatus. (B) Compression of cells encapsulated within a porous three-dimensional matrix exhibits biphasic deformation behaviour, in which the fluid transiently bears the loads and applies a hydrostatic pressure to cells, before it is squeezed out of the deforming matrix. (C) Load transfer between cells and the matrix in three dimensions is dependent on the mechanical properties of both the cell and the matrix. Deformation increases for matrices stiffer than the cell itself, and *vice versa*.

loads to cells and culture, through the manipulation of fluid pressures, fluid flows and substrate deformation. Compression chambers actuated by a displacing platen can be used to apply hydrostatic pressures to biological samples (Fig. 1A). Shear forces can be applied to cells cultured on glass slides using a parallel plate flow chamber in which liquid is forced between two closely-spaced rigid plates (Fig. 1B); or with a cone-and-plate shear chamber, in which a rotating cone applies uniform shear stresses to cells cultured on an underlying plate (Fig. 1C). Cells cultured on deformable substrates can be mechanically stimulated by applying bending moments to the culture substrates (Fig. 1D), or by applying in-plane stretches (Fig. 1E and F). These methods can be extended to cells cultured in a three-dimensional (3D) matrix and can be applied in combination to mechanically manipulate multiple microenvironmental parameters.

However, the 'simple' mechanical stimulation described in macroscale systems is often tightly coupled to other stimulation modes, making it difficult to isolate mechanosensing mechanisms, and to attribute biological response to a specific mechanical stimulus. Applied substrate deformations cause movement of cells within a fluid environment, which can cause transient changes in fluid height, creating transient reactive normal forces and shear stresses.[40] Since most cell types require a sustaining nutrient liquid, decoupling the fluid–solid interactions in two-dimensional (2D) systems cannot be easily achieved (Fig. 2A). 3D culture systems can better simulate many *in vivo* mechanical conditions, but are also prone to exhibit coupled mechanical behaviours. Cells cultured in mechanically active hydrogel biomaterials experience deformation of the surrounding matrix, complex fluid shears caused by liquid movement within the hydrogel, and hydrostatic transient pressure waves resulting from deformation of the biphasic material.[41] Initially applied loads are borne by the fluid component of the matrix. The increased pressure causes fluid flow out of the matrix, the rate of which is dependent on fluid viscosity and matrix porosity. As fluid leaves the system, the applied load is transferred to the solid component of the matrix (Fig. 2B). Moreover, load transfer between the deforming matrix and encapsulated cells is governed by a complex relationship between the mechanical properties of the matrix and of the cells. Stiff cells encapsulated within a soft deforming matrix would experience little physical deformation, but soft cells in a stiffer matrix would undergo large strains (Fig. 2C).[41] Likewise, cellular adhesion characteristics play a critical role in transferring load,[42] and these combinations of factors can result in local deformation profiles distinctly different from those suggested by macroscale observations. Decoupling the effects of various modes of mechanical stimulation remains a challenge in the design of mechanically dynamic *in vitro* culture platforms.

Cellular activity itself also plays a significant role in mechanobiological response. The cytoskeletal structure plays an integral role in transducing external mechanical signals to internal responses.[1] Since cytoskeletal structure is strongly influenced by mechanical and chemical factors, a defining feature predicting cellular response is conditioning of the cytoskeleton. The cytoskeleton is an active structure, mechanically interrogating the environment by exerting internally-generated traction forces on the surrounding matrix.[43] Easily deformable microenvironments do not resist these traction forces, resulting in low cytoskeletal tension. More rigid microenvironments enable higher tensions within the cell, which can have an impact on cell fate and function.[3,44] Hence, it is neither desirable nor possible to isolate the study of biomechanics from mechanobiology, but combined studies require novel experimental approaches. These mechanobiological systems are made more complex in that cell-generated traction forces deform the surrounding matrix, which can cause the activation of matrix-immobilized mechanically sensitive reservoirs of biochemical factors,[45] altering cellular response. Moreover, cells

are sensitive to these small localized matrix deformations,[46] and can sense mechanical activities of a neighboring cell from a distance.[47,48] Finally, the exquisite sensitivity of cells to small variations in environmental mechanics[49] places stringent requirements on experimental platforms for cellular mechanobiology.

Resolution limitations in patterning, accuracy, sensitivity, and experimental density of macroscale technologies inherently limit conventional mechanobiology platforms to studying population-based phenomena using relatively coarsely-defined mechanical stimulation parameters. For example, the most widely used systems for mechanical stimulation of cells through substrate deformation are a range of platforms produced by Flexcell International Corporation,[39] which are based on a standard 6-well plate format. These systems tend to be relatively large in size due to limitations in manufacturing are expensive, require large quantities of expensive reagents, and are low in throughput.

As such, deciphering the complex feedback loops that exist between cells and the mechanical microenvironment requires the ability to precisely manipulate mechanical stimulation parameters at the colony, cellular and sub-cellular scales, suggesting the need for technologies and approaches characterized by precision and repeatability. Furthermore, the interdependency and cross-talk between cell signalling networks, and the variety of cues present in the *in vivo* environment requires stimulation platforms that combine multiple stimulation modes and mechanical cues. Given the vast number of experimental conditions arising from systematically manipulating multiple mechanobiological parameters, high-throughput approaches to combinatorially manipulate the cellular microenvironment are required to study mechanobiological phenomena.

Microfabricated devices may be well-suited to address some of the technical limitations of conventional equipment in better understanding cellular mechanobiology. Control of micrometre-scale features enables precise definition of the microenvironment, and the ability to manipulate culture systems at multicellular, cellular and sub-cellular scales enables studies that would not be possible with standard experimental techniques. To date, microdevices have made substantial contributions to cell biology studies, particularly in the area of cellular biomechanics, through component integration and miniaturization; reduction in experimental complexity; improvements in usability; reductions in reagent and operation costs; and in making rapid measurements with greatly improved spatial and force resolutions.[50] While these advantages also apply to using microfabricated systems to study cellular mechanobiology, more specific advantages can be realized, which will be discussed with relevant examples in the following sections.

## 3. 'Small' steps forward

Specific to studies in cellular mechanobiology, the utility of microdevices in elucidating relevant biology has been established in the following key functions: (1) an ability to precisely define critical microenvironmental features; (2) precise manipulation and decoupling of mechanical stimulation parameters; and (3) possibilities for combinatorial and high-throughput studies of multiple systematically manipulated mechanobiological parameters.

### 3.1 Defining critical features

Critical mechanobiological cues can be provided by designing the interface between culture materials and the cell itself. Surfaces can be engineered with natural or synthetic matrix proteins to control cell adhesion with sub-cellular resolution in length, enabling fundamental studies of the relationships between mechanical cell spreading area, matrix composition and cell fate and function. Physical surface topography and substrate mechanics are also critical components of the interface between cells and environment, and play an important role in modulating cell behaviour.

**3.1.1 Spatial control of adhesion.** Control of cell adhesion area has been shown to be a critical determinant of cell function. Selected works demonstrating the importance of this technique in mechanobiological studies are reviewed here, but the interested reader is directed to recent comprehensive reviews on using micropatterning approaches to determine cell function.[51,52]

In seminal works using micropatterning approaches to confine cell spreading, Chen and coworkers demonstrated that cell spreading area is a critical determinant between cell proliferation and apoptosis,[53] and directs differentiation of constrained mesenchymal stem cells between adipogenic and osteogenic lineages[3] (Sidebar 1). These studies were made possible through the ability to restrict cell attachment to specific regions of a 2D substrate (reviewed elsewhere[54,55]). Briefly, adhesive proteins can be micropatterned on a substrate and the remaining areas are rendered non-adhesive to cell attachment using a suitable chemical or physical method. One of the most commonly used techniques to micropattern protein features on a surface is microcontact printing,[56,57] in which a PDMS stamp with microfabricated features is used to transfer patterns of proteins onto the desired substrate. Alternatively, a PDMS stencil can be fabricated with through-holes at the regions to be patterned. Adhesive proteins deposited on top of the stencil come in contact with the underlying substrate only at specific regions. Removal of the stencil results in the formation of a pattern of adhesive proteins or cells.[58] This method can also be used to selectively activate the surface by plasma treatment, before subsequent deposition of the adhesive proteins and blocking agents.[59,60] Alternatively, removable microfluidic channels can also be used to deliver adhesive molecules to specific regions on a substrate.[61]

As cells adhere and increase spread area on a rigid 2D substrate, internal cytoskeletal tension increases. Differentiation of stem cells has been shown to be related to cytoskeletal tension, as demonstrated by Ruiz *et al.*, in which MSCs under increased tension at the edge of patterned multicellular islands underwent osteogenic differentiation, while those in the center became adipocytes.[62] Similarly epithelial clusters undergo a TGF-β1 induced epithelial-to-mesenchymal transition preferentially at the edges of micropatterns in regions of tension.[63] More recently, pattern *shape* has also been identified as having a substantial impact on how cells function, suggesting that oriented internal tension plays a role in cellular response.

 

Kilian *et al.* demonstrated that high-aspect ratio rectangular patterns promoted osteogenic MSC differentiation, whereas more rounded patterns resulted in differentiation towards an adipocyte lineage[64] (Sidebar 1). Cells patterned on square and rectangular islands have been shown to develop increased traction forces,[64,65] demonstrating that cytoskeletal tension is intrinsically linked to cell shape, as well as spread area. Pattern orientation also directs other mechanical behaviours, including lamellipodia extension[66] and migration,[67] as cells migrate towards the blunt end of a tear-drop shaped pattern.[68]

Micropatterning techniques can also be coupled with other fabrication paradigms to create more complex microenvironments, including substrate-bound protein gradients[69] and designing temporally-manipulated matrix environments[70,71] with electrically,[72,73] or photo-controllable[74] adhesive surfaces. Such approaches are promising in their ability to determine the temporal aspect of mechanobiology, and are beginning to be used to explore cooperative behaviour in collective migration of epithelial sheets.[73]

The development of sub-cellular micropatterning techniques has enabled precise control over mechanical constraints applied to cells, and has resulted in an improved understanding of how cells integrate environmental information through the cytoskeleton in 2D culture. Extending this technology to three-dimensions remains to be attained with sub-cellular control of cell adhesion and spreading within a homogenous material, but shaped microwells have been successfully used to manipulate cell geometries.[75] Nelson and co-workers have also developed patterned multicellular epithelial constructs within a three-dimensional collagen matrix and studied branching morphogenesis in regions of shape-induced stress.[76] Understanding the relationship between spreading morphology and cell function in 3D remains to be explored.

**3.1.2 Surface topographies.** Cells are able to sense physical topography at a number of scales: curvatures in the underlying substrate,[77] micro-scaled ridges and grooves,[78] nanoscale topographies,[79] and anisotropic gradients in topography.[80] The effects of physical topography have been shown to be more influential on cell alignment and function than patterned chemical cues,[81] and have been shown to better recapitulate *in vivo* cell behaviour.[82,83] These substantial mechano-biological effects on cell adhesion, alignment and migration[84] have been well-established over the past 20 years,[78] particularly on substrates consisting of micropatterned grooves of varying heights and widths. Thus, the standard techniques and methods will not be reviewed in detail here. Interested readers are referred to a number of relevant reviews on the subject.[37,85,86] Technological development in this area remains active however. Recently, in order to understand the temporal effects of topographical stimulation, a PDMS platform to produce a substrate with reconfigurable microtopographies has been developed. Compression of a PDMS substrate results in 550–800 nm high features, spaced ~6 μm apart, and cells repeatably switched orientations in response to the applied topographical cues.[87] Other recent studies have shown that nanoscale topographies have a profound influence on cell function. MSCs differentiate to osteoblasts under the influence of nanopatterned substrates, without osteogenic components in the nutrient media.[88] Cell geometry, action potential conduction velocity and cell-to-cell coupling in nanopatterned cardiac tissue constructs are extraordinarily sensitive to the underlying patterns.[89] Likewise, neurons are able to sense nanometre scaled roughness.[90] Thus, both micro- and nano-topographies can play important roles in cellular response to the mechanical microenvironment, and can be eventually used to manipulate migration and matrix production in tissue engineering applications.

Though the effects of sub-cellular micro and nanotopographical features are well documented, the underlying integrative mechanosensory mechanisms remain undetermined.

**Sidebar 1: Probing mechanically-influenced stem cell differentiation**

Understanding the effects of mechanical forces and constraints on single cells cannot be achieved by conventional techniques. Micropatterning surfaces with adhesive and non-adhesive regions to limit and direct cell spreading has proven to be a powerful tool in understanding how the physical architecture of the cell and cytoskeletal tension drive stem cell differentiation. Chen and co-workers[3] demonstrated that manipulating cell spreading area directs a mesenchymal stem cell (MSC) commitment switch between the osteogenic and adipogenic lineages, independent of exogenous soluble cues (Figure s1A). This switch was modulated by RhoA-ROCK signaling and required actomyosin contraction-generated tension. More recently, Mrksich and co-workers[64] investigated the more subtle effects of geometric shape on regulating this switch, and confirmed that increasing actomyosin contractility by increasing aspect ratio or defining convex shapes (while maintaining adhesive area) promotes osteogenic differentiation. Contraction-induced MSC osteogenic differentiation was mediated through mitogen activated protein kinase and wingless-type (Wnt) signaling, pathways that have also been implicated in biochemically-induced osteogenesis. Together these experiments demonstrate the mechanosensitivity of MSCs and the pivotal impact of mechanical cues on cell fate, and suggest biophysical means to enhance and rationally guide stem cell differentiation.

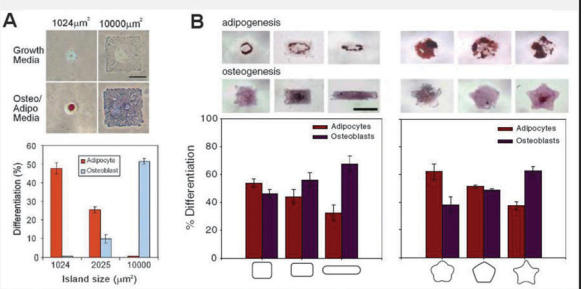


***Figure s1.*** *Geometric cues regulate adipogenic versus osteogenic differentiation of mesenchymal stem cells. Cytoskeletal tension regulates the switch between adipogenesis and osteogenesis, and is manipulated by (A) controlling spreading area (source: reprinted from ref. 3, with permission from Elsevier), and (B) controlling aspect ratio and pattern shape (source: Kilian et al. (ref. 64) Copyright 2010, National Academy of Sciences, USA).*

As topographical features may be considered as an intermediate between a planar 2D environment and a 3D environment, such studiesmayhelpdeterminehowcellsintegratemechanicalresponses differentlyin3Denvironments.

### 3.2 Precise control of mechanical environments

As discussed earlier in this review, independently manipulating specific parameters in the mechanical microenvironment is challenging. Microfabricated technologies are able to address some of these issues, by decoupling correlated phenomena in cell-environment interactions and by decoupling mechanical stimuli that occur simultaneously under an applied load. Devices demonstrated or suggested to address these concerns are reviewed in this section, specifically in the areas of supposedly 'passive' mechanical interactions, in which cells interact with the stiffness of the surrounding environment, and in technologies to externally apply mechanical deformation to the cellular milieu.

**3.2.1 Passive mechanical interactions.** The stiffness of the mechanical environment is a critical parameter in cell fate and function. Polyacrylamide (PA) hydrogel systems are perhaps the best established biomaterial substrate for this purpose, and were first used in the 1990s to study cell locomotion and focal adhesion formation as a function of substrate stiffness.[91] In seminal work, Engler *et al*. used PA gels to show that substrate stiffness directs stem cell lineage differentiation. MSCs differentially displayed neurogenic, myogenic and osteogenic differentiation on substrates with modulus increasing from 0.1 to 40 kPa.[92] Although PA gels are most widely used, a variety of other biomaterial systems have been utilized for substrate stiffness studies, including poly(ethylene) glycol (PEG),[93] collagen-poloxamine[94] and gelatin methacrylate.[95] Cells cultured on soft substrates tend to remain rounded up, as the environment does not provide a strong reaction force to cell-generated traction forces required for the cell to spread out. Conversely, cells on stiff substrates spread well. Hence, internal cytoskeletal tension, cell spreading area, and substrate stiffness are tightly coupled parameters in 2D culture systems.

Substrate (two-dimensional) and matrix (three-dimensional) stiffness is generally manipulated by means of differentially crosslinked polymer substrates. By increasing cross-link density, or by decreasing the spacing between polymer bonds, hydrogel substrates can be made more rigid. However, this also alters the number of binding sites available to cells cultured on or in the gel, and changes gel permeability, which may influence cell function. Though some chemical approaches have been employed to address these issues,[96] microfabricated approaches may provide alternative decoupling techniques. When working on the length scale of tens of microns, spacing between hydrogel surfaces and adhesive structures can be used to modulate effective stiffness experienced by cells.[97,98] For example, though not ostentatiously a "microfabricated device", Arora *et al*. reported a culture technique by which cells cultured on thick ($\sim$1 mm) collagen gels experience lower stiffness than thin ($\sim$10 μm) gels firmly attached to a glass coverslip.[99] As is the case with the fictional princess who is able to feel a hard pea beneath several mattresses,[98] cells 'feel' the stiffer substrate through the thin, compliant hydrogel (Fig. 3A).

A conceptually similar approach has also been applied to cells cultured on vertical microposts of different dimensions, in which cells respond to the stiffness of the underlying cantilevers (Fig. 3B);[44] and in 3D in which microfabricated cantilevered support structures anchor a cell-laden hydrogel (Fig. 3C).[100] Contraction and remodeling of the collagen hydrogels caused deflection in the cantilevered posts, which was monitored to determine the forces generated by the gel. Interestingly, different contraction forces were generated for cantilevers of different dimensions. Hence, the altered stiffness of the supporting cantilevers influences the effective stiffness experienced by the cells in the hydrogel, and subsequently, cellular contraction and remodeling forces. This approach to manipulating mechanical stiffness avoids complicating factors in changing concentrations of crosslinking agents.

Substrate stiffness studies are hampered by an inability to distinguish internal cytoskeletal tension and cell spread area. A novel system was recently developed by Mitrossilis *et al*.[101] to decouple substrate stiffness from cell-generated forces. In this system, a real-time feedback mechanism dynamically and independently manipulates the reactive force available to the cell and the deformation of the cell (Fig. 3D). By independently controlling these parameters, environments of different stiffnesses can be created on demand. Their findings demonstrate that early response of cells is triggered by stiffness, and not by force. This finding has been recently mirrored in three-dimensional biomaterial culture, in which Mooney and co-workers found that cellular differentiation in response to 3D matrix stiffness is independent of cell spread area.[102] This is in contrast to studies on 2D surfaces, and the mechanism underlying these differences remains an open question.

**3.2.2 Externally applied deformation.** Mechanical cues presented to cells by way of deformation of the external environment can also be critical factors in cell regulation. Depending on the stiffness of the surrounding matrix, cell-generated traction forces produce large or small deflections, which dictate cell fate and function.[103] Similarly, cells sense deformations in their surroundings caused by externally applied deformations, and respond accordingly.[25,39] Although *in vivo* mechanical strain modes are quite complex, the effects and underlying mechanisms can be studied using simplified *in vitro* models. Strains can be applied to cells cultured on substrates by uniaxial, biaxial, equibiaxial, compressive and tensile loading, in both two- and three-dimensional materials.[104] Cells are sensitive to strain magnitude,[105] applied strain field[106] and stimulation frequency;[107] and responses to these mechanical parameters are also modulated by other features of the microenvironment.

Commercial platforms to apply dynamic, cyclic strain to cells cultured on a 2D surface exhibit significant strain reduction over multiple loading cycles.[108] This is likely due to material fatigue, and engineering microfabricated substrates may address this issue. Moraes *et al*.[105] developed a microfabricated array-based bioreactor system, in which an array of circular loading posts are vertically actuated to distend a culture diaphragm, producing an equibiaxial uniform strain in the culture membrane (Fig. 4A–D). This loading scheme is similar to that of commercial platforms,

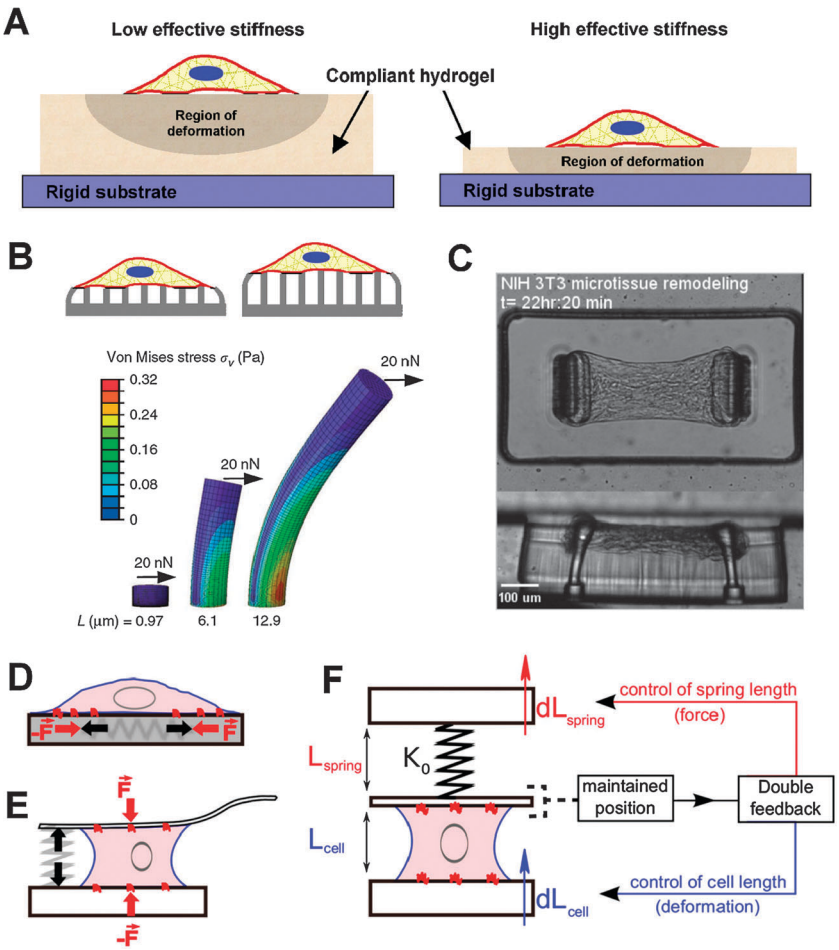


**Fig. 3** Use of microfabricated systems to manipulate environmental stiffness. (A) Thickness of a compliant hydrogel attached to a rigid substrate regulates effective stiffness of the substrate. (B) Similarly, cells cultured on an array of microfabricated posts experience a range of substrate stiffness by modulating the height of the posts (source: reprinted by permission from Macmillan Publishers Ltd: (ref. 44), Copyright 2010), and cells cultured in a collagen gel respond differently to anchoring posts of different stiffness. Measurement of post deflection (B, C) also enables characterization of mechanical forces exerted by cells in the system (source: Legant *et al.* (ref. 100) Copyright 2009, National Academy of Sciences, USA). Rather than modulate underlying substrate stiffness (D), environmental stiffness can be altered by allowing cells to attach to (E) a flexible cantilever arm, such as on an AFM. (F) Using a dual feedback control system to maintain position of the attached plate, the reactive forces generated by the environment and the deformation of the cell can be manipulated (source: Mitrossilis *et al.* (ref. 101) Copyright 2010, National Academy of Sciences, USA).

except for a significant reduction in the thickness and diameter of the flexible culture substrates. Strains were characterized over 100 000 loading cycles, and showed no significant material fatigue, suggesting that microfabricated polymer membranes ($<15$ μm thick) may have better fatigue resistant properties than macroscale polymers.

A second important limitation of macroscale substrate deformation systems is that the volumetric displacements of fluid caused by deforming the substrate with a large loading post causes transient reactive normal forces on cell cultures, which may influence cell function.[40] In the microfabricated substrate strain system discussed above, small displacements are required to produce similar surface strain, due to the miniaturized dimensions of the system. Hence, by virtue of minimized system perturbation, undesirable mechanical effects caused by fluid-structure interactions can be substantially minimized.

Moraes *et al.* also extended their technology to apply compressive stimuli to cells in three-dimensional biomaterials, under unconfined[109] or semi-confined[110] conditions (Fig. 4E and F). Cell-laden biomaterial hydrogels were photopatterned between vertically actuated loading posts and a rigid glass substrate. Raising the posts applied compressive strains to the micropatterned hydrogel constructs. Biphasic materials such as hydrogels exhibit complex deformation behaviour on the macroscale. Deformation of the solid component of the matrix causes a transient increase in hydrostatic pressure, which equilibrates as the liquid is forced out of the small pores in the gel. This transient pressure wave can have a significant impact on cellular function. On the microscale however, the increased surface area-to-volume ratio should enable rapid normalization of hydrostatic pressure waves, suggesting that designing microscale compression systems can address some of the mechanical coupling problems associated with macroscale compression systems.

### 3.3 Screening platforms

The inherent variability in biological systems, coupled with the ability of the cell to integrate multiple mechanobiological cues necessitates higher throughput screening platforms to

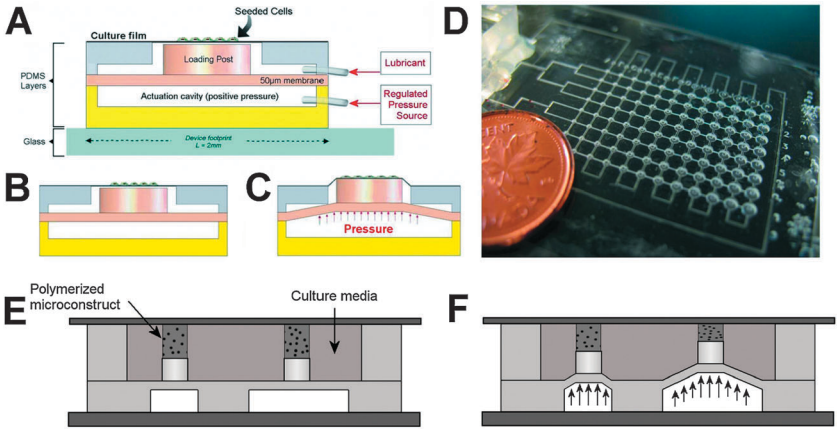


**Fig. 4** Microfabricated systems to apply physical deformation to cells on an (A–D) two-dimensional substrate, and (E–F) in a three-dimensional matrix. (A) Schematic outline of device construction and operation under (B) rest and (C) actuated conditions. (D) Increased throughput screening of the effects of substrate deformation magnitude on a chip (source: Moraes *et al.* (ref. 105), reproduced by permission of the Royal Society of Chemistry). (E, F) Moraes *et al.* extended their technology to apply mechanical deformation to cells in a three-dimensional matrix by (E) photopatterning cell-laden hydrogels into the device, and (F) applying compressive forces to the constructs (source: reprinted from ref. 109, Copyright 2010 with permission from Elsevier).



identify combinations of parameters ideally suited for a specific biological application. The ability to increase experimental throughput is a common motivation for microdevice development. This section highlights technological development designed to increase the ability to screen mechanobiological response against a screened parameter or combination of parameters.

**3.3.1 Fluid stresses.** Fluid-related forces are a defining component of the *in vivo* mechanical environment, either through hydrostatic compression, or through shear forces generated by interstitial fluid flow, or pulsatile or continuous blood flow. Microfluidic devices are ideally suited to manipulate these parameters, and various technologies have been developed to rapidly screen for the effects of mechanobiological forces.

Hydrostatic pressures are known to play a role in cell biology, particularly in cartilage[25] and ocular tissues.[28] However, classifying hydrostatic pressure as "mechanical" stimulation is somewhat contentious, as increases in pressure external to the cell causes a corresponding increase in internal pressure, presumably resulting in no net cell deformation. Changes in cell function may instead be due to differences in gas solubility at different pressures, if gas concentrations are not controlled independently of the applied pressure. The only reported microfabricated system designed to apply hydrostatic pressures to cells was developed by Sim *et al.*, who used a single pressure source to create a range of deflections in suspended PDMS membranes of various diameters. The differing deflections cause different pressures in isolated culture chambers, enabling the high-throughput evaluation of MSC response to a range of hydrostatic pressures.[111]

Fluid shear stress plays a critical role in development and differentiation, and a large number of temporal and spatial shear stress patterns and magnitudes exist *in vivo*.[29,112,113] The use of artificial microfabricated channels is a suitable approach to mimic many such environments as the reduction in scale enables well-controlled laminar flow in the channels. Simple PDMS channels can be used in combination with passive pumping,[114] pressure-driven flows or syringe pumps to apply shear to cultured cells. For example, Higgins *et al.* used a single microfluidic channel to study the behaviour of sickle-type red blood cells in a physiologically relevant environment.[115] Pressure (gravity)-driven flow was used to drive the defective red blood cells through a channel, to study the effects of geometric, physical, and biological factors in vascular occlusion and rescue.

Various design considerations need to be factored into scaling up such simple channel systems for higher-throughput studies, and one of the key criteria is the method for driving fluid flow through the system. External connections to devices can often hinder scalability, and passive pumping is one technique that does not require these external connectors. In passive pumping, surface tension differences between droplets of different sizes at either end of a microfluidic channel drive fluid flow.[114] Although passive pumping has not yet been used to apply physiologically relevant shear stresses to cells, the authors suggest that this is one possible application of the technique,[116] and may be relevant in simulating relatively slow interstitial flow.[117] Beebe and coworkers used this principle to demonstrate an automated high-throughput microfluidic system, in which a robotic system deposits and removes droplets across an array of microfluidic channels.[116] Syringe pump and pressure-driven flows are harder to implement in high-throughput systems, but serve adequately for devices designed for relatively lower-throughput experiments. Careful design of the microfluidic channels can be used to maintain increased throughput while minimizing the world-to-chip interface connection issues (connections are typical sources of device failure). Channels with varying widths connected to a single fluid delivery source can be used to generate a range of fluid velocities, and hence apply shear stresses across a single device.[118] Carefully designed channels of increasing width can be used to apply linearly increasing shear stresses across

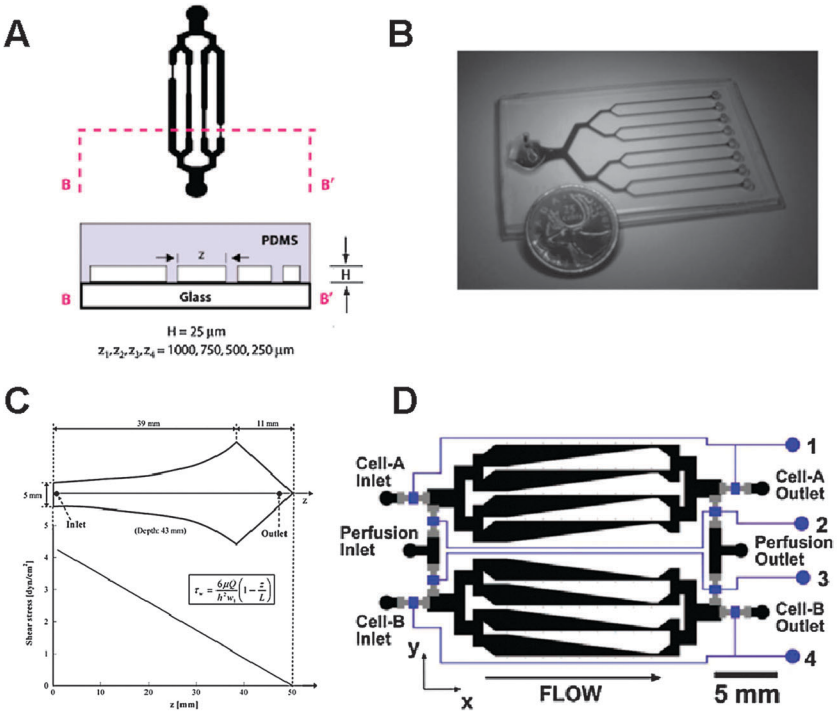


**Fig. 5** Microfluidic devices designed to apply shear stress to cultured cells. (A) Varying channel dimensions enables multiple shear forces to be applied simultaneously on a single chip (source: reprinted with permission from ref. 118, Copyright 2004 American Chemical Society). (B) Use of multiple flow channels enables higher-throughput testing of adhesion on multiple matrix protein coatings (source: Young *et al.* (ref. 144), reproduced by permission of the Royal Society of Chemistry). (C) Logarithmic design used to apply a linearly increasing shear stress along the microchannel length (source: reprinted with permission from ref. 145. Copyright 2007 American Chemical Society); and (D) Channels with varying shear stress profiles along each channel (source: reprinted with permission from ref. 146. Copyright 2007 American Chemical Society).

a single channel[119] Channel bends and curves can also be used to apply spatially distinctive shear stresses[120] (Fig. 5).

More complex technologies have also been developed to further miniaturize such systems. The development of the microfluidic valve by the Quake[121] and Mathies[122] groups enabled the large-scale integration of multiplexed microfluidic valves on a single chip.[123] Using a multi-layered PDMS microfluidic system, the valves consist of a pressure control channel which deforms thin PDMS films to block flow in fluidic channels. The valves can be used to direct fluid flow or drive it by operating as a peristaltic pump. Using these valves, an automated, high-throughput microfluidic cell culture system was developed, in which 96 culture chambers can be individually addressed.[124] Such systems have not as yet been used to explicitly explore the effects of shear stress on cultured cells, but can be used for this purpose.

Using Quake valves still requires an undesirably large number of world-to-chip interfaces. Takayama *et al.* mitigated this limitation by using commercially available Braille displays to deform the base of a flexible microfluidic channel.[125] Pin actuation can be independently and automatically controlled to manipulate fluid within the microchannels. Although it is not as scalable, it is simpler to implement and has been used to apply shear stress to endothelial cells in culture.[126]

Microengineered technologies for fluid shear can also improve functionality in several ways. *In situ* measurements of shear stresses can be made using MEMS-based 'hair' sensors, incorporated directly into the shear channels.[127] Direct readouts can also be integrated into the microfluidic devices. For example, Tolan *et al.* developed an integrated luminescence detection system in which fluorescent reagents react with erythrocyte lysates to simultaneously monitor various biochemicals produced by erythrocytes under shear in underlying channels.[128]

**3.3.2 Combinatorial and increased-throughput studies.** Microdevices enable the application of multiple modes of mechanical stimuli to cultured cells, in order to elucidate how combinations of factors influence cell function. A few microfabricated systems have been developed to study the combined effects of topographical patterning and substrate deformation. Kurpinski *et al.*[129] and Wang *et al.*[130] seeded MSCs and fibroblasts along topographically patterned stretchable substrates, before applying uniaxial strains in a macroscale bioreactor. Leduc and coworkers developed a system to apply compressive strains to topographically patterned surfaces.[131] They each demonstrated that cells align to the topographically patterned ridges, and that mechanical stimulation influences gene expression, protein expression and proliferation differentially dependent on the direction of strain to the aligned patterns. Their results suggest that preconditioning the cytoskeleton influences cellular response to mechanical stretch. Tan *et al.* used a similar

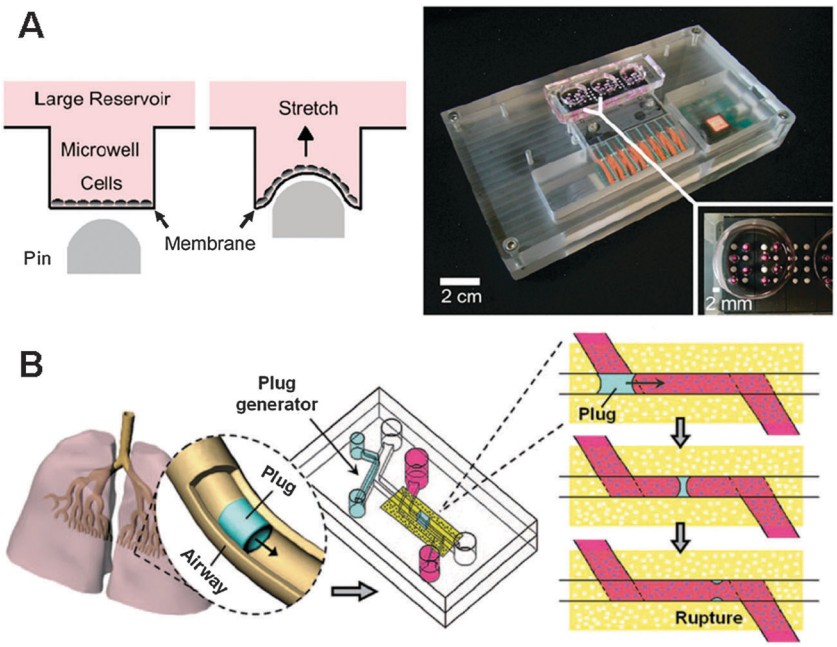


**Fig. 6** Microfabricated devices designed to increase throughput or apply combinations of mechanical stimuli to cultured cells. (A) Braille display unit to screen for the effects of deformation frequency on cells cultured in fabricated microwells (source: reprinted from ref. 107, Copyright 2008, with permission from Elsevier). (B) Plug generator system designed to simulate acoustic crackling in the lungs by rupturing air liquid plugs in a microchannel, thereby applying physiological shear and pressure to a cultured epithelial sheet (source: Huh *et al.* (ref. 147) Copyright 2007, National Academy of Sciences, USA).

topographical patterning approach to align cells along specific orientations, but applied a pressure differential across circular patterned diaphragms.[132] The pressure differential caused the diaphragm to bulge, creating non-uniform anisotropic biaxial strains in different regions of the device. Gopalan *et al.* followed a similar approach, except the diaphragms were distended by a loading post in a manually actuated screw-type system.[133]

However, each of the systems described is relatively limited in throughput, particularly for the mechanical stretch component of the system. To address this, Takayama and co-workers used their Braille system to apply non-uniform substrate deformations to cells cultured on thin films. The films were distended by the hemispherical-headed pin, applying non-uniform strains. The automated Braille displays enabled screening for various cyclic loading frequencies ranging from 0.2 to 5 Hz, and differences were found in degree of alignment of various cell types in response to frequency and stimulation duration (Fig. 6A).[107] Rather than screen for the effects of frequency, Moraes *et al.* developed an approach to screen for the effects of strain magnitude, by developing an actuation scheme in which an array of pneumatically driven microposts can be simultaneously actuated to a range of heights, using a single pressure source.[134] This actuation scheme was then used in their cell stretching device to simultaneously generate cyclic equibiaxial strains ranging from 2 to 15%, where they identified a novel time- and strain-magnitude dependent response of for translocation of the protein β-catenin into the nucleus of MSCs.[105] Though neither of these groups demonstrated the inclusion of topographical patterns on the deforming substrates, both fabrication processes can be readily modified to include this parameter.

Integrating fluid shear stresses and substrate deformation has also been demonstrated by Leduc and coworkers, who developed a system to utilize a pressure differential to deform an elastomeric slab containing a microfluidic channel network.[135] By manipulating the boundary conditions around the clamped elastomeric slab, they were able to generate a variety of biaxial strain fields. This group more recently developed a system to apply uniaxial substrate strains, in combination with fluid shear.[136] Combining substrate deformation and fluid shear stress can be a powerful tool in mimicking the cellular microenvironment in model organ systems, as was recently demonstrated by Huh *et al.*[137] They developed a system to apply fluid shear and biaxial substrate deformation to a porous membrane. By tissue engineering an epithelial and endothelial cell layer on either side of the membrane, they were able to mimic the lung air-blood barrier under conditions simulating breathing (Sidebar 2).

Mimicking other mechanical aspects of the respiratory system requires other combinations of mechanical forces, which can be produced using microfabricated systems. Simulating surfactant disorders in the small airways of the lung was achieved by developing an air-liquid plug generator, which was used then to study the effects of plug propagation and rupture on small airway epithelial cells cultured on a porous membrane (Fig. 6B).[138] Using this system, Takayama *et al.* demonstrated a potential link between acoustic crackling heard during breathing, and epithelial damage caused by rupture of the liquid plugs. A platform to study the combined effects of pressure and shear in the moving air–liquid interface in combination with a mechanically deforming substrates has also been developed by Douville *et al.*, to

 

study epithelial damage caused by surfactant disorders during breathing.[139] Each of these examples demonstrates an ability to better simulate mechanical factors *in vivo*, and has resulted in an improved understanding of cell function within that system.

**Sidebar 2: Recreating mechanically dynamic *in vivo* environments**

High-throughput cell-based assays have been developed to accelerate drug discovery in the pharmaceutical industry, prior to conducting expensive and time-consuming studies with animal models. Such techniques aim to study cultured cell response to large libraries of chemical stimulants, to identify therapeutic candidates and to test toxicity. However, this approach has had only limited success in translating potential 'hits' to animal models and the clinic. One factor that could account for these low success rates is the inability of current screening platforms to incorporate physiologically relevant mechanical forces that are critical for normal organ function.

Recently, Ingber and co-workers[138] used a novel microfabricated platform to investigate nanoparticle toxicity in the lung. Their platform simulated the mechanical and architectural microenvironment of an alveolar air-blood barrier by culturing alveolar epithelial and vascular endothelial cells on either side of a membrane that was cyclically stretched to mimic physiological breathing (Figure s2A). Treatment of the epithelium with ultrafine silica nanoparticles (mimicking aerosol inhalation) increased reactive oxygen species generation in both the epithelium and the underlying endothelium, increased endothelial expression of ICAM-1, and promoted capture and transmigration of circulating neutrophils. Importantly, the inflammatory response to the nanoparticles was accentuated by physiological cyclic stretch, and in some cases was observed only with stretch (Figure s2B). These results would not have been observed in conventional static cultures, but were replicated in an *in vivo* mouse model. These experiments demonstrate that incorporating physiological mechanical cues into culture systems significantly improves their relevance and utility as low-cost alternatives to current *in vitro* and preclinical models for high-throughput screening applications.

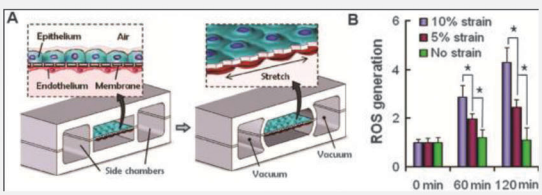


***Figure s2.*** *Reconstituting organ function on a chip. (A) Microenvironmental cues including co-culture models, mechanical stretch and fluid flow were incorporated in a microfluidic model. (B) The model predicted the mechanical strain-enhanced inflammatory responses (e.g., reactive oxygen species (ROS) generation) of an in vivo mouse model on exposure to inhaled nanoparticles (source: from ref. 138. Reprinted with permission from AAAS).*

## 4. Conclusions

The development and use of microfabricated tools for experiments in biomechanics and mechanobiology can have a profound impact on understanding the relationship between mechanics and cellular form and function. Increases in experimental throughput and experimental simplicity can substantially improve our understanding of rare cell populations, and how these cells respond to varied parameters. Microfabrication also allows designers to combine multiple stimulation and measurement techniques. Sniadecki *et al*. linked biomechanics and mechanobiology in developing a system designed to measure traction forces in response to an externally applied deformation.[140] Combinatorial stimulation with a variety of mechanical cues is also possible, and has been demonstrated to better simulate *in vivo* systems.[137]

However, recent findings have suggested that the use of certain microfabricated systems in studying biological cells may have under-appreciated and substantial side effects. Beebe and coworkers determined that PDMS, used widely in microfabricated devices, sequesters small bioactive chain polymers into the surrounding media, which are then incorporated into the cell membrane.[141,142] This suggests that alternative techniques, such as hot embossing, to create microfluidic channels using generally accepted materials for cell culture, such as polystyrene, would be a more appropriate approach. Alternatively, others have investigated using coating films of polyurethane on PDMS materials, to provide cell adhesion sites and improve biological compatibility.[143] In general, better characterization of the effects of microdevice materials and cell culture techniques on biological function is needed before such techniques can be broadly adopted into mainstream wetlabs.

There is also a substantial divide to cross in terms of expertise. Device design, fabrication and validation require specific and detailed skill sets, and it can often be difficult for experts in microdevice design to thoroughly understand the relevant biological issues, and *vice versa*. Devices can often be complicated to operate in practice, and are frequently unable to provide a reliable platform to study biological systems. Simplifying device designs may aid in solving these issues of usability, but more generally, bridging the gap between microdevice engineers and cell biologists will require close interdisciplinary collaborations, or integrative thinkers in both areas developing tools to understand and answer specific biological questions. The promise of such techniques is powerful, and successful research programs integrating these disciplines will produce new insights and advances in both.

## References

1 D. A. Fletcher and R. D. Mullins, *Nature*, 2010, **463**, 485–492.
2 M. H. Hsieh and H. T. Nguyen, *Int. Rev. Cytol.*, 2005, **245**, 45–90.
3 R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju and C. S. Chen, *Dev. Cell*, 2004, **6**, 483–495.
4 A. J. Ridley, M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons and A. R. Horwitz, *Science*, 2003, **302**, 1704–1709.
5 K. Kaibuchi, S. Kuroda and M. Amano, *Annu. Rev. Biochem.*, 1999, **68**, 459–486.
6 H. J. Kong, J. Liu, K. Riddle, T. Matsumoto, K. Leach and D. J. Mooney, *Nat. Mater.*, 2005, **4**, 460–464.
7 D. E. Ingber, *FASEB J.*, 2006, **20**, 811–827.

8 A. W. Orr, B. P. Helmke, B. R. Blackman and M. A. Schwartz, *Dev. Cell*, 2006, **10**, 11–20.
9 E. M. Darling, M. Topel, S. Zauscher, T. P. Vail and F. Guilak, *J. Biomech.*, 2008, **41**, 454–464.
10 H. D. Huang, R. D. Kamm and R. T. Lee, *Am. J. Physiol.: Cell Physiol.*, 2004, **287**, C1–C11.
11 I. Titushkin and M. Cho, *Biophys. J.*, 2007, **93**, 3693–3702.
12 M. D. Treiser, E. H. Yang, S. Gordonov, D. M. Cohen, I. P. Androulakis, J. Kohn, C. S. Chen and P. V. Moghe, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **107**, 610–615.
13 D. E. Ingber, *Ann. Med.*, 2003, **35**, 564–577.
14 P. A. Janmey and R. T. Miller, *J. Cell Sci.*, 2010, **124**, 9–18.
15 W. L. Chen and C. A. Simmons, *Adv. Drug Delivery. Rev.*, 2011, **63**, 269–276.
16 D. E. Ingber, *Int. J. Dev. Biol.*, 2006, **50**, 255–266.
17 M. A. Wozniak and C. S. Chen, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 34–43.
18 N. Gjorevski and C. M. Nelson, *Birth Defects Res., Part C*, 2010, **90**, 193–202.
19 D. O. Freytes, L. Q. Wan and G. Vunjak-Novakovic, *J. Cell. Biochem.*, 2009, **108**, 1047–1058.
20 W. D. Merryman and A. J. Engler, *J. Biomech.*, 2010, **43**, 1–1.
21 J. F. Stoltz and X. Wang, *Biorheology*, 2002, **39**, 5–10.
22 J. Guck, S. Schinkinger, B. Lincoln, F. Wottawah, S. Ebert, M. Romeyke, D. Lenz, H. M. Erickson, R. Ananthakrishnan, D. Mitchell, J. Kas, S. Ulvick and C. Bilby, *Biophys. J.*, 2005, **88**, 3689–3698.
23 R. A. Desai, L. Gao, S. Raghavan, W. F. Liu and C. S. Chen, *J. Cell Sci.*, 2009, **122**, 905–911.
24 M. C. van der Meulen and R. Huiskes, *J. Biomech.*, 2002, **35**, 401–414.
25 J. H. Wang and B. P. Thampatty, *Biomech. Model. Mechanobiol.*, 2006, **5**, 1–16.
26 G. Bao and S. Suresh, *Nat. Mater.*, 2003, **2**, 715–725.
27 M. Makale, *Birth Defects Res., Part C*, 2007, **81**, 329–343.
28 D. E. Jaalouk and J. Lammerding, *Nat. Rev. Mol. Cell Biol.*, 2009, **10**, 63–73.
29 J. H. Chen, C. Liu, L. You and C. A. Simmons, *J. Biomech.*, 2010, **43**, 108–118.
30 J. H. Wang, *J. Biomech.*, 2006, **39**, 1563–1582.
31 J. T. Butcher, C. A. Simmons and J. N. Warnock, *J. Heart Valve Dis.*, 2008, **17**, 62–73.
32 L. A. Setton and J. Chen, *Spine*, 2004, **29**, 2710–2723.
33 M. J. Lammi, *Biorheology*, 2004, **41**, 593–596.
34 C. Huselstein, P. Netter, N. de Isla, Y. Wang, P. Gillet, V. Decot, S. Muller, D. Bensoussan and J. F. Stoltz, *Biomed. Mater. Eng.*, 2008, **18**, 213–220.
35 Y. Zheng and Y. Sun, *Micro Nano Lett.*, 2011, **6**, 327–331.
36 O. Loh, A. Vaziri and H. D. Espinosa, *Exp. Mech.*, 2007, **49**, 105–124.
37 D. H. Kim, P. K. Wong, J. Park, A. Levchenko and Y. Sun, *Annu. Rev. Biomed. Eng.*, 2009, **11**, 203–233.
38 S. A. Vanapalli, M. H. Duits and F. Mugele, *Biomicrofluidics*, 2009, **3**, 12006.
39 T. D. Brown, *J. Biomech.*, 2000, **33**, 3–14.
40 T. D. Brown, M. Bottlang, D. R. Pedersen and A. J. Banes, *Am. J. Med. Sci.*, 1998, **316**, 162–168.
41 F. Guilak and V. C. Mow, *J. Biomech.*, 2000, **33**, 1663–1673.
42 D. Kirchenbuchler, S. Born, N. Kirchgessner, S. Houben, B. Hoffmann and R. Merkel, *J. Phys.: Condens. Matter*, 2010, **22**, DOI: 10.1088/0953-8984/22/19/194109.
43 A. K. Harris, P. Wild and D. Stopak, *Science*, 1980, **208**, 177–179.
44 J. Fu, Y. K. Wang, M. T. Yang, R. A. Desai, X. Yu, Z. Liu and C. S. Chen, *Nat. Methods*, 2010, **7**, 733–736.
45 B. Hinz, *Curr. Rheumatol. Rep.*, 2009, **11**, 120–126.
46 Y. W. Lin, C. M. Cheng, P. R. Leduc and C. C. Chen, *PLoS One*, 2009, **4**, e4293.
47 J. P. Winer, S. Oake and P. A. Janmey, *PLoS One*, 2009, **4**, e6382.
48 C. A. Reinhart-King, M. Dembo and D. A. Hammer, *Biophys. J.*, 2008, **95**, 6044–6051.
49 S. M. Brierley, *Auton. Neurosci.*, 2010, **153**, 58–68.
50 C. Moraes, Y. Sun and C. A. Simmons, in *Cellular and Biomolecular Mechanics and Mechanobiology*, ed. A. Gefen, Springer, 2011, vol. 4.
51 M. Thery, *J. Cell Sci.*, 2010, **123**, 4201–4213.
52 J. Nakanishi, T. Takarada, K. Yamaguchi and M. Maeda, *Anal. Sci.*, 2008, **24**, 67–72.
53 C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides and D. E. Ingber, *Science*, 1997, **276**, 1425–1428.
54 D. Falconnet, G. Csucs, H. M. Grandin and M. Textor, *Biomaterials*, 2006, **27**, 3044–3063.
55 I. Barbulovic-Nad, M. Lucente, Y. Sun, M. Zhang, A. R. Wheeler and M. Bussmann, *Crit. Rev. Biotechnol.*, 2006, **26**, 237–259.
56 K. E. Schmalenberg, H. M. Buettner and K. E. Uhrich, *Biomaterials*, 2004, **25**, 1851–1857.
57 A. Bernard, J. P. Renault, B. Michel, H. R. Bosshard and E. Delamarche, *Adv. Mater.*, 2000, **12**, 1067–1070.
58 E. Ostuni, R. Kane, C. S. Chen, D. E. Ingber and G. M. Whitesides, *Langmuir*, 2000, **16**, 7811–7819.
59 B. A. Langowski and K. E. Uhrich, *Langmuir*, 2005, **21**, 10509–10514.
60 S. W. Rhee, A. M. Taylor, C. H. Tu, D. H. Cribbs, C. W. Cotman and N. L. Jeon, *Lab Chip*, 2005, **5**, 102–107.
61 S. Takayama, J. C. McDonald, E. Ostuni, M. N. Liang, P. J. Kenis, R. F. Ismagilov and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 1999, **96**, 5545–5548.
62 S. A. Ruiz and C. S. Chen, *Stem Cells*, 2008, **26**, 2921–2927.
63 E. W. Gomez, Q. K. Chen, N. Gjorevski and C. M. Nelson, *J. Cell. Biochem.*, 2010, **110**, 44–51.
64 K. A. Kilian, B. Bugarija, B. T. Lahn and M. Mrksich, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 4872–4877.
65 N. Wang, E. Ostuni, G. M. Whitesides and D. E. Ingber, *Cell Motil. Cytoskeleton*, 2002, **52**, 97–106.
66 K. K. Parker, A. L. Brock, C. Brangwynne, R. J. Mannix, N. Wang, E. Ostuni, N. A. Geisse, J. C. Adams, G. M. Whitesides and D. E. Ingber, *FASEB J.*, 2002, **16**, 1195–1204.
67 A. Brock, E. Chang, C. C. Ho, P. LeDuc, X. Jiang, G. M. Whitesides and D. E. Ingber, *Langmuir*, 2003, **19**, 1611–1617.
68 X. Jiang, D. A. Bruzewicz, A. P. Wong, M. Piel and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 975–978.
69 X. Jiang, Q. Xu, S. K. Dertinger, A. D. Stroock, T. M. Fu and G. M. Whitesides, *Anal. Chem.*, 2005, **77**, 2338–2347.
70 W. S. Yeo, M. N. Yousaf and M. Mrksich, *J. Am. Chem. Soc.*, 2003, **125**, 14994–14995.
71 W. S. Yeo and M. Mrksich, *Langmuir*, 2006, **22**, 10816–10820.
72 C. Y. Fan, Y. C. Tung, S. Takayama, E. Meyhofer and K. Kurabayashi, *Adv. Mater.*, 2008, **20**, 1418.
73 S. Raghavan, R. A. Desai, Y. Kwon, M. Mrksich and C. S. Chen, *Langmuir*, 2010, **26**, 17733–17738.
74 J. Nakanishi, Y. Kikuchi, T. Takarada, H. Nakayama, K. Yamaguchi and M. Maeda, *Anal. Chim. Acta*, 2006, **578**, 100–104.
75 M. Ochsner, M. R. Dusseiller, H. M. Grandin, S. Luna-Morris, M. Textor, V. Vogel and M. L. Smith, *Lab Chip*, 2007, **7**, 1074–1077.
76 N. Gjorevski and C. M. Nelson, *Integr. Biol.*, 2010, **2**, 424–434.
77 J. Y. Park, D. H. Lee, E. J. Lee and S. H. Lee, *Lab Chip*, 2009, **9**, 2043–2049.
78 A. Curtis and C. Wilkinson, *Biomaterials*, 1997, **18**, 1573–1583.
79 D. H. Kim, H. J. Lee, Y. K. Lee, J. M. Nam and A. Levchenko, *Adv. Mater.*, 2010, **22**, 4551–4566.
80 D. H. Kim, K. Han, K. Gupta, K. W. Kwon, K. Y. Suh and A. Levchenko, *Biomaterials*, 2009, **30**, 5433–5444.
81 J. L. Charest, M. T. Eliason, A. J. Garcia and W. P. King, *Biomaterials*, 2006, **27**, 2487–2494.
82 D. Motlagh, T. J. Hartman, T. A. Desai and B. Russell, *J. Biomed. Mater. Res.*, 2003, **67A**, 148–157.
83 L. Wang, S. K. Murthy, W. H. Fowle, G. A. Barabino and R. L. Carrier, *Biomaterials*, 2009, **30**, 6825–6834.
84 J. Y. Mai, C. Sun, S. Li and X. Zhang, *Biomed. Microdevices*, 2007, **9**, 523–531.
85 J. Y. Wong, J. B. Leach and X. Q. Brown, *Surf. Sci.*, 2004, **570**, 119–133.
86 J. Y. Lim and H. J. Donahue, *Tissue Eng.*, 2007, **13**, 1879–1891.
87 M. T. Lam, W. C. Clem and S. Takayama, *Biomaterials*, 2008, **29**, 1705–1712.


 

88 M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. W. Wilkinson and R. O. C. Oreffo, *Nat. Mater.*, 2007, **6**, 997–1003.
89 D. H. Kim, E. A. Lipke, P. Kim, R. Cheong, S. Thompson, M. Delannoy, K. Y. Suh, L. Tung and A. Levchenko, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **107**, 565–570.
90 V. Brunetti, G. Maiorano, L. Rizzello, B. Sorce, S. Sabella, R. Cingolani and P. P. Pompa, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 6264–6269.
91 R. J. Pelham and Y. L. Wang, *Proc. Natl. Acad. Sci. U. S. A.*, 1997, **94**, 13661–13665.
92 A. J. Engler, S. Sen, H. L. Sweeney and D. E. Discher, *Cell*, 2006, **126**, 677–689.
93 S. R. Peyton, C. B. Raub, V. P. Keschrumrus and A. J. Putnam, *Biomaterials*, 2006, **27**, 4881–4893.
94 A. Sosnik and M. V. Sefton, *Biomaterials*, 2005, **26**, 7425–7435.
95 J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar and A. Khademhosseini, *Biomaterials*, 2010, **31**, 5536–5544.
96 C. Cha, S. Y. Kim, L. Cao and H. Kong, *Biomaterials*, 2010, **31**, 4864–4871.
97 Y. C. Lin, D. T. Tambe, C. Y. Park, M. R. Wasserman, X. Trepat, R. Krishnan, G. Lenormand, J. J. Fredberg and J. P. Butler, *Phys. Rev. E*, 2010, **82**, DOI: 10.1103/PhysRevE.82.041918.
98 A. Buxboim, K. Rajagopal, A. E. X. Brown and D. E. Discher, *J. Phys.: Condens. Matter*, 2010, **22**, 194116.
99 P. D. Arora, N. Narani and C. A. McCulloch, *Am. J. Pathol.*, 1999, **154**, 871–882.
100 W. R. Legant, A. Pathak, M. T. Yang, V. S. Deshpande, R. M. McMeeking and C. S. Chen, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 10097–10102.
101 D. Mitrossilis, J. Fouchard, D. Pereira, F. Postic, A. Richert, M. Saint-Jean and A. Asnacios, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 16518–16523.
102 N. Huebsch, P. R. Arany, A. S. Mao, D. Shvartsman, O. A. Ali, S. A. Bencherif, J. Rivera-Feliciano and D. J. Mooney, *Nat. Mater.*, 2010, **9**, 518–526.
103 D. E. Discher, P. Janmey and Y. L. Wang, *Science*, 2005, **310**, 1139–1143.
104 K. Bilodeau and D. Mantovani, *Tissue Eng.*, 2006, **12**, 2367–2383.
105 C. Moraes, J. H. Chen, Y. Sun and C. A. Simmons, *Lab Chip*, 2010, **10**, 227–234.
106 D. M. Geddes-Klein, K. B. Schiffman and D. F. Meaney, *J. Neurotrauma*, 2006, **23**, 193–204.
107 Y. Kamotani, T. Bersano-Begey, N. Kato, Y. C. Tung, D. Huh, J. W. Song and S. Takayama, *Biomaterials*, 2008, **29**, 2646–2655.
108 F. H. Bieler, C. E. Ott, M. S. Thompson, R. Seidel, S. Ahrens, D. R. Epari, U. Wilkening, K. D. Schaser, S. Mundlos and G. N. Duda, *J. Biomech.*, 2009, **42**, 1692–1696.
109 C. Moraes, G. Wang, Y. Sun and C. A. Simmons, *Biomaterials*, 2010, **31**, 577–584.
110 C. Moraes, R. Zhao, M. Likhitpanichkul, C. A. Simmons and Y. Sun, *J. Micromech. Microeng.*, 2011, **21**, 054014.
111 W. Y. Sim, S. W. Park, S. H. Park, B. H. Min, S. R. Park and S. S. Yang, *Lab Chip*, 2007, **7**, 1775–1782.
112 A. D. van der Meer, A. A. Poot, M. H. Duits, J. Feijen and I. Vermes, *J. Biomed. Biotechnol.*, 2009, **2009**, 823148.
113 E. W. Young and C. A. Simmons, *Lab Chip*, 2010, **10**, 143–160.
114 G. M. Walker and D. J. Beebe, *Lab Chip*, 2002, **2**, 131–134.
115 J. M. Higgins, D. T. Eddington, S. N. Bhatia and L. Mahadevan, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 20496–20500.
116 I. Meyvantsson, J. W. Warrick, S. Hayes, A. Skoien and D. J. Beebe, *Lab Chip*, 2008, **8**, 717–724.
117 J. Y. Park, S. J. Yoo, L. Patel, S. H. Lee and S. H. Lee, *Biorheology*, 2010, **47**, 165–178.
118 H. Lu, L. Y. Koo, W. C. M. Wang, D. A. Lauffenburger, L. G. Griffith and K. F. Jensen, *Anal. Chem.*, 2004, **76**, 5257–5264.
119 S. Usami, H. H. Chen, Y. H. Zhao, S. Chien and R. Skalak, *Ann. Biomed. Eng.*, 1993, **21**, 77–83.
120 J. V. Green, T. Kniazeva, M. Abedi, D. S. Sokhey, M. E. Taslim and S. K. Murthy, *Lab Chip*, 2009, **9**, 677–685.
121 M. A. Unger, H. P. Chou, T. Thorsen, A. Scherer and S. R. Quake, *Science*, 2000, **288**, 113–116.
122 W. H. Grover, A. M. Skelley, C. N. Liu, E. T. Lagally and R. A. Mathies, *Sens. Actuators, B*, 2003, **89**, 315–323.
123 J. Melin and S. R. Quake, *Annu. Rev. Biophys. Biomol. Struct.*, 2007, **36**, 213–231.
124 R. Gomez-Sjoberg, A. A. Leyrat, D. M. Pirone, C. S. Chen and S. R. Quake, *Anal. Chem.*, 2007, **79**, 8557–8563.
125 W. Gu, X. Y. Zhu, N. Futai, B. S. Cho and S. Takayama, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 15861–15866.
126 J. W. Song, W. Gu, N. Futai, K. A. Warner, J. E. Nor and S. Takayama, *Anal. Chem.*, 2005, **77**, 3993–3999.
127 C. Liu, *Bioinspir. Biomimetics*, 2007, **2**, S162–169.
128 N. V. Tolan, L. I. Genes, W. Subasinghe, M. Raththagala and D. M. Spence, *Anal. Chem.*, 2009, **81**, 3102–3108.
129 K. Kurpinski, J. Chu, C. Hashi and S. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 16095–16100.
130 J. H. Wang, G. Yang and Z. Li, *Ann. Biomed. Eng.*, 2005, **33**, 337–342.
131 C. M. Cheng, R. L. Steward and P. R. Leduc, *J. Biomech.*, 2009, **42**, 187–192.
132 W. Tan, D. Scott, D. Belchenko, H. J. Qi and L. Xiao, *Biomed. Microdevices*, 2008, **10**, 869–882.
133 S. M. Gopalan, C. Flaim, S. N. Bhatia, M. Hoshijima, R. Knoell, K. R. Chien, J. H. Omens and A. D. McCulloch, *Biotechnol. Bioeng.*, 2003, **81**, 578–587.
134 C. Moraes, Y. Sun and C. A. Simmons, *J. Micromech. Microeng.*, 2009, **19**, 065015.
135 J. D. Kubicek, S. Brelsford, P. Ahluwalia and P. R. Leduc, *Langmuir*, 2004, **20**, 11552–11556.
136 R. L. Steward, Jr., C. M. Cheng, D. L. Wang and P. R. LeDuc, *Cell Biochem. Biophys.*, 2009, **56**, 115–124.
137 D. Huh, B. D. Matthews, A. Mammoto, M. Montoya-Zavala, H. Y. Hsin and D. E. Ingber, *Science*, 2010, **328**, 1662–1668.
138 D. Huh, H. Fujioka, Y. C. Tung, N. Futai, R. Paine, 3rd, J. B. Grotberg and S. Takayama, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 18886–18891.
139 N. J. Douville, P. Zamankhan, Y.-C. Tung, R. Li, B. L. Vaughan, C.-F. Tai, J. White, P. J. Christensen, J. B. Grotberg and S. Takayama, *Lab Chip*, 2011, **11**, 609–619.
140 N. J. Sniadecki, A. Anguelouch, M. T. Yang, C. M. Lamb, Z. Liu, S. B. Kirschner, Y. Liu, D. H. Reich and C. S. Chen, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 14553–14558.
141 M. W. Toepke and D. J. Beebe, *Lab Chip*, 2006, **6**, 1484–1486.
142 K. J. Regehr, M. Domenech, J. T. Koepsel, K. C. Carver, S. J. Ellison-Zelski, W. L. Murphy, L. A. Schuler, E. T. Alarid and D. J. Beebe, *Lab Chip*, 2009, **9**, 2132–2139.
143 C. Moraes, Y. K. Kagoma, B. M. Beca, R. L. Tonelli-Zasarsky, Y. Sun and C. A. Simmons, *Biomaterials*, 2009, **30**, 5241–5250.
144 E. W. K. Young, A. R. Wheeler and C. A. Simmons, *Lab Chip*, 2007, **7**, 1759–1766.
145 B. D. Plouffe, D. N. Njoka, J. Harris, J. H. Liao, N. K. Horick, M. Radisic and S. K. Murthy, *Langmuir*, 2007, **23**, 5050–5055.
146 E. Gutierrez and A. Groisman, *Anal. Chem.*, 2007, **79**, 2249–2258.
147 E. E. Hui and S. N. Bhatia, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 5722–5726.