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Title:
Cellular Biomechanics in Drug Screening and Evaluation: Mechanopharmacology

Date:
2016-02-01

Citation:
Krishnan, R., Park, J. A., Seow, C. Y., Lee, P. V. S. & Stewart, A. G. (2016). Cellular Biomechanics in Drug Screening and Evaluation: Mechanopharmacology. Trends in Pharmacological Sciences, 37 (2), pp.87-100. https://doi.org/10.1016/j.tips.2015.10.005.

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Trends in Pharmacological Sciences

Cellular biomechanics in drug screening and evaluation: Mechanopharmacology

--Manuscript Draft--

Manuscript Number:	TIPS-D-15-00171R1
Article Type:	Review
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Abstract:	The study of mechanobiology is now widespread. The impact of cell and tissue mechanics on cellular responses is well-appreciated. However, knowledge of the impact of cell and tissue mechanics on pharmacological responsiveness, and its application to drug screening and mechanistic investigations have been very limited in scope. We emphasise the need for a heightened awareness of the important bidirectional influence of drugs and biomechanics in all living systems. We propose that the term "Mechanopharmacology" be used to describe a new inter-disciplinary area that uses in vitro systems that are biomechanically appropriate to the relevant (patho)physiology, to identify new drugs and drug targets. Mechanopharmacology as an inter-discipline can be applied more broadly to the understanding of biomechanical influences on drug responses.

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Cellular biomechanics in drug screening and evaluation: Mechanopharmacology

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Keywords: Mechanobiology; Drug screening; stiffness; stress; strain; traction

Trends Box

- **An argument is outlined for a new inter-discipline, mechanopharmacology**
- **Examples of cellular biomechanics influences on drug action are described**
- **The relevance of matrix stiffness, internal and external stresses to drug screening is discussed**
- **Methods for biomechanical perturbation and analysis of single cell, and organoids are reviewed**

Abstract

The study of mechanobiology is now widespread. The impact of cell and tissue mechanics on cellular responses is well-appreciated. However, knowledge of the impact of cell and tissue mechanics on pharmacological responsiveness, and its application to drug screening and mechanistic investigations have been very limited in scope. We emphasise the need for a heightened awareness of the important bidirectional influence of drugs and biomechanics in all living systems. We propose that the term "mechanopharmacology" be applied to approaches that use *in vitro* systems that are biomechanically appropriate to the relevant (patho)physiology, to identify new drugs and drug targets. This article describes models and techniques that are being developed to transform drug screening and evaluation ranging from a 2 dimensional environment to the dynamic 3 dimensional environment of the target expressed in the disease of interest.

Drug screening and evaluation: the need to consider cellular mechanics

The reasons for failure of drug development programmes are the subject of much contemplation. Whilst adverse effects, toxicity, as well as pharmacokinetic features, are often cited as reasons for arrested drug development, several recent studies highlight failure because of a lack of efficacy. A review of Astra-Zeneca's portfolio performance in Phase IIa and IIb studies from 2005 to 2010 suggested that 57% and 88%, respectively, of the project closures at this stage were due to a failure of efficacy, whereas attrition due to efficacy in preclinical phase was as low as 6% [1]. There are many reasons to expect that preclinical and clinical pharmacology will differ, including the use of non-human species to support efficacy. However, even when the target is expressed and engaged in human cell types, failure may ensue because the affected pathways are less influential than anticipated from the preclinical studies. When the agent reaches the target in adequate concentration and for a sufficient duration, giving a suitable level of drug exposure, lack of efficacy is likely to result from differences in behavior of the drug target in the assay systems compared with the target behavior *in situ* in the patient-specific context. The screening and preclinical pharmacology for many of these agents is likely to have been established in cell culture, in an oversimplified mechanical micro-environment and/or in non-human models of the targeted disease. We argue that drug screening can be improved with the use of human cells of phenotype most relevant to the condition, ideally being derived from patients (representative of the disease stage being targeted), and then cultured in the most (patho)physiologically relevant conditions. This approach is intended to ensure that the assay emulates the biomechanical environment, in the condition to be treated. Ideally, the assay would also embed cell mechanical measurements of deformability, stiffness, and/or contraction, as in many organs and diseases, these cellular changes often constitute the principal endpoint of therapeutic intent. The use of patient-derived primary cell cultures improves the likelihood of genetic and epigenetic influences being appropriately expressed, with the expectation that the target efficacy would be more accurately predicted. It will be valuable to rigorously

test this proposition against comparator preclinical efficacy testing in relevant animal models and in 2 dimensional culture on rigid plastic substrate.

The impact of biomechanics on cell function has been systematically explored, leading to a broad appreciation of mechano-sensitive processes, with the principal mechanosensors being selected ion channels [2, 3] and less commonly the integrins [4]. Mechanotransduction involves force transmission through bound proteins resulting in conformational changes that entrain functional impacts. For example, conformational changes in vinculin and talin have been shown to subserve the recruitment of the actin cytoskeleton to focal adhesions in the leading edge of migrating cells [5, 6].

The impact of biomechanics on drug actions is rarely addressed, despite being highlighted as an important consideration repeatedly in the literature, for example, [7, 8]. Recent advances proposed by Donald Ingber and colleagues using "organ-on-a-chip" microfluidics technology involving cell cultures being subjected to cyclical strains (breathing/cardiac cycle/peritoneal peristalsis/renal fluidic shear), raise the prospect of more systematic and relevant drug discovery paradigms using human cell cultures [9] [10]. Similarly, recent advances in cell mechanics have highlighted the suitability of mechanical endpoints as phenotypic targets in high-throughput screening [11]. In this article, we develop selected examples of biomechanical impacts on cell function and drug responsiveness and discuss refined, biomechanically appropriate bioassays, emphasizing those suitable for scaling to medium to high throughput. We exemplify below the selected impacts of different aspects of the biomechanical environment (Box 1.)

Shear forces

The effects of shear are extensively explored in the cardiovascular system [7], but there are other organs where fluid and gas flows create shear forces that impact on cell and tissue function. Shear represents the frictional force exerted

by flow of gas or liquid over the affected surface and is quantitated in terms of force (Dyne) per unit area (see Box 1).

One of the most instructive exemplars of the interaction between drug action and shear stress is provided by the discovery of excess cardiovascular mortality associated with the use of cyclo-oxygenase-2 (COX-2) selective inhibitors (coxibs). The cyclo-oxygenase enzymes comprise COX-1, which is ubiquitously expressed at significant levels and produces precursor for the formation of prostaglandin E_2, prostacyclin (PGI_2) and thromboxane A_2, to achieve cytoprotective, anti-atherogenic, and hemostatic physiological functions, respectively. COX-2 was discovered in tumour cells and has been shown to be strongly induced by certain cytokines, growth factors and pathogen-associated molecular pattern receptors of the mammalian innate host defence system.

The basis of the anticipated safety profile of selective cyclo-oxygenase-2 (COX-2) inhibitors was in part dependent on a misapprehension of the dependence of vascular endothelial PGI_2 production on COX-1 activity. The anti-thrombotic actions of PGI_2 were well-established a decade before the 1990 discovery of COX-2. The mechanisms of anti-thrombotic actions of low dose aspirin were known to involve preserved endothelial PGI_2 production and diminished production by platelets of the platelet-activating vasoconstrictor, thromboxane A_2. In 1996, two years before the coxibs were approved by the USA FDA, work by Gimbrone and colleagues indicated that under static conditions and with the application of turbulent flow, cultured endothelial cells expressed COX-1, whereas when subject to laminar flow, COX-2 expression was strongly induced and therefore became an important source of enzymatic activity producing precursor for transformation into PGI_2 [12]. This finding is significant for being one of the early observations to draw our attention to the impact of shear forces on gene expression and function. Perhaps more importantly, it highlighted the importance of screening drug actions *in vitro* in settings that are more physiological with regard to flows and forces. The coxibs may compromise endothelial PGI_2 production, particularly in regions of high wall shear stress, which are also known to be sites of higher likelihood for rupture of atherosclerotic lesions [13]. More recent commentary on the basis of the

cardiovascular risk posed by the coxib drug class has suggested that the mechanism may be multifaceted, involving macrophage and other sources of COX-2 activity, in addition to the endothelium [14]. Hypertension, a pro-thrombotic state, and loss of protection from the preconditioning that follows intermittent episodes of ischaemia, are each considered alongside endothelial COX2 expression, as potential contributors to excess cardiovascular morbidity and mortality in patients treated with coxibs [15]. Evaluation of coxibs in endothelium subjected to appropriate shear forces may have alerted developers of this drug class to an otherwise unappreciated risk posed to cardiovascular safety. Moreover, as growth factors and cytokines induce COX-2 [16], the soluble environment of the assay system is also a critical consideration. Regardless of the precise reasons for coxib toxicity, biomechanically accurate screening paradigms are evidently important for safety valuation.

Shear forces play a key role in determining the pharmacology of anti-platelet agents. Examination of the role of integrins under static and shear stress conditions indicates that shear is able to alter the avidity of integrins not only for their binding partner, but also for some small molecule ligands that inhibit their activation [17]. The impact of inhibitors of the phospho-Inositide -3- Kinases (PI-3K) are also sensitive to whether evaluation takes place under static or shear-stressed conditions, with implications for drug screening approaches [18]. Specifically, the role of PI-3Kβ isoform is most evident under the high shear stress where it contributes to the signalling regulating the transmission of force from the actin cytoskeleton of the activated platelet through the $\alpha_2\beta_3$ platelet integrin to the clot fibrin [19].

Force, stress, strain and stiffness

In most organs, smooth muscle functions under an environment of constant fluctuation of stress and strain. Control of the organ function can be achieved by regulating the intrinsic forces generated by the muscle. Conversely, the behaviour of the muscle can be modified by extrinsic forces imposed on the muscle. Both the intrinsic and extrinsic forces lead to changes in the stresses and

194 strains within the smooth muscle, altering its stiffness properties (see Box 1 for a
195 primer on tissue/cell mechanics). Studies of the pharmacology of smooth muscle
196 have conventionally been conducted under static conditions, in which the
197 response to an intervention is usually the isometric force developed by the
198 muscle. The inadequacy of this approach becomes clear when the responses of
199 airway smooth muscle to agonist stimulation under static and dynamic
200 conditions are compared. The dynamic loading experienced by airway smooth
201 muscle in a breathing lung greatly attenuates the contractile responsiveness of
202 the muscle [20, 21]. Similar conclusions can be made for other smooth muscle,
203 such as arterial smooth muscle under pulsatile pressure [22]. More importantly
204 for understanding the pathophysiology of asthma, it appears that the inability of
205 asthmatic airway smooth muscle to relax in response to mechanical perturbation
206 may be the cause of airway hyperresponsiveness [23]. Impairment of airway
207 distensibility may be associated with the lack of bronchodilatation in asthmatic
208 airways under oscillatory strain [24]. Conversely, in healthy individuals
209 prohibition of deep inspiration leads to airway hyperresponsiveness [23], more
210 recent investigations suggest that the maximum response rather than sensitivity
211 is increased [25]. These observations highlight airway stiffness, including
212 airway smooth muscle stiffness, as potential targets for asthma therapy [26, 27].
213 Force and stiffness development in airway smooth muscle appear to be
214 regulated through separate signal pathways [28, 29]. A recent study showed
215 that fibroblast growth factor-2 (FGF-2) reduces the TGF-β–stimulated increase in
216 stiffness of cultured airway smooth muscle cells [30], demonstrating the
217 possibility of developing a new class of anti-asthma drugs that specifically
218 targets smooth muscle stiffness.

219

220 **Tensile forces**

221 Tensile stiffness of a muscle does not manifest itself until the muscle is stretched
222 via tensile forces. To study any drug effect on muscle stiffness it is therefore
223 necessary to apply the mechano-pharmacological approach. A recent study
224 examining the combined effect of oscillatory strain and isoprenaline in dilating
225 carbachol-constricted bronchial segments has found a synergy between the

mechanical and pharmacological bronchodilators [31]. A conventional pharmacological or purely static mechanical approach would have yielded smaller bronchodilator effects, suggesting that neither stimulus was effective. With increasing understanding of how smooth muscle contracts, we are now able to separate different phases of smooth muscle contraction regulated by different signalling pathways. In airway smooth muscle, force maintenance during the sustained phase of contraction is particularly sensitive to rho-kinase inhibitors. Synergy in reducing the ability of the muscle to maintain force becomes apparent when oscillatory strain is applied to the muscle in the presence of a rho-kinase inhibitor [32]. These examples underline the importance of applying mechanopharmacological approaches in identifying new drugs to target the range of contributions to asthma. In the context of fixed airway obstruction in asthmatics treated with optimal doses of inhaled glucocorticoids to treat inflammation and β_2-adrenoceptor agonists to relieve the bronchospasm, there may be a limitation on the efficacy of the β_2 adrenoceptor agonist in airway smooth muscle that has assumed a more rigid state. Alternative targets to the β_2-adrenoceptor may need to be evaluated in airways that are appropriately mechanically conditioned to reflect this particular unmet need in severe asthma.

Compressive forces

The mechanotransduction paradigm of asthma was proposed following observations in an *in vitro* compressive system that mimicks the estimated maximal compressive force exerted on airway epithelial cells during bronchoconstriction. This system has been used to show the recapitulation of various remodeling events as detected in the asthmatic airways [33-36].

While normal breathing imposes cyclic compressive stress at 3 cm H_2O of pressure on airway epithelium, bronchospasm in asthma imposes static compressive stress at 25-30 cm H_2O pressure. Application of compressive stress reduces the lateral interstitial volume, thereby recapitulating the folded and compressed epithelium in narrowed airways [34]. Compressive stress cues

mechanotransduction signals in airway epithelial cells via the activation of epidermal growth factor receptor. During compressive stress, epithelial cells release mediators that induce proliferation and production of type 1 and 3 collagens from co-cultured fibroblasts [37]. In air-liquid interface cultures of airway epithelium, intermittent, repeated compressive stresses cause goblet cell hyperplasia in well-differentiated normal HBE cells [38]. Moreover, compressive stress induces the release of inflammatory and angiogenic mediators, such as tissue factor and YKL-40 from HBE cells via PKC and ERK-dependent pathways [39, 40]. These studies using an *in vitro* compressive system suggest that even in the absence of inflammatory cells, compressive mechanical stress imposed on airway epithelium activates mechanotransduction pathways that participate in airway remodeling of asthma. With an appreciation of previous *in vitro* studies, Grainge *et al*. reported results from a ground-breaking study in mild asthmatics showing that repeated challenges with a bronchoconstricting agent, methacholine, induce two major remodeling events; goblet cell hyperplasia and increased type 3 collagen deposition, in the absence of infiltration of inflammatory cells [41]. Pre-treatment with a bronchodilator, albuterol prevented the remodeling events induced by methacholine, thereby implicating mechanical compression rather than other pharmacological effects of muscarinic receptor activation. Thus, compressive stress may accelerate the progression of remodeling in individuals with pre-existing conditions [42]. Persistent relaxation of the airways by long-acting β_2-adrenoceptor agonists would be expected to offset these stimuli to remodeling, but this has yet to be convincingly demonstrated.

In connection with physical behaviors of cells, the asthmatic airway epithelium exhibits difference in the jamming transition. Like coffee beans in a chute, cells can become jammed or cells can flow [43, 44]. When primary HBE cells are cultured from normal donors, they are initially unjammed, but when cells are differentiated and mature, they eventually become jammed [36]. However, when cells are cultured from asthmatic donors, the transition from the unjammed state to the jammed state is significantly delayed. Furthermore, the jammed state or the normal transition toward the jammed state can be disturbed by external stimuli or disease conditions. Compressive stress provokes the

transition from the jammed state back to the unjammed one. These findings suggest cell jamming can be a novel concept in understanding the role of the airway epithelium in asthma [45].

Although the impacts of mechanotransduction on the inflammatory response have not been extensively explored, a recent fascinating study indicates that elasticity influences the innate host defense functions of the macrophages and that, conversely, both pathogen-associated molecular patterns (PAMPs e.g. LPS) and cytokines, including interferon γ (INFγ) modulate macrophage elasticity [46]. It is notable that a number of the extracellular factors elaborated in response to strain and compression, including type 1 collagen, INFγ and TGFβ are able to induce glucocorticoid-resistance [47-49].

The mechanical environment of the cell

A large number of studies have shown that mechanical interactions between cells and extracellular matrix (ECM) play a fundamental role in biological processes such as migration, growth, and morphogenesis. Cellular responses to mechanical forces are highly complex. The mechanical environment may be viewed as a combination of 'outside-in' and 'inside-out' forces. When external forces are applied to tissue, these forces are transferred from the ECM to the cell, propagating from the outside into the cell ('outside-in'). However, the cell also generates forces within itself, affecting its overall deformability and stiffness. These 'inside-out' forces are also often referred to as the cellular traction force. The forces applied to the cell and the traction forces generated by the cell are closely linked via a feedback loop. Growing cells on hard or soft substrates could increase or decrease cellular stiffness and traction force, respectively, with the cell responding constantly to its external microenvironment [50]. This feedback loop has generated wide interest in the research community, most strikingly in the area of stem cell biology. Pivotal advances in stem cell differentiation technology have been built on the finding that the biomechanical environment of stem cells contributes critically to the nature of the niche that determines the fate of cellular differentiation [51]. Researchers are now able to tune substrate or ECM stiffness to guide differentiation [52]. Pharmacological manipulation of

cellular mechanics using agents that impact on the cytoskeleton may offer a promising approach to altering the behavior of endogenous cell stem cells to effect repair. Agents that target central structures in the cytoskeleton are usually cytotoxic. However, prospects for selective, safe and tolerable agents are emerging from an improved understanding of the molecular basis of mechanosensing [53]. In addition, pharmacological or genetic manipulation of autologous and allogeneic stem cell phenotype may extend the utility of stem cells as treatments.

The 2D or 3D environment of the cellular system has profound impacts on cellular behaviour, some of which can be ascribed to changes in the mechanical microenvironment. Thus, the tensile, compression and shear forces experienced by cells within the 3D environment, together with spatial heterogeneity engenders more differentiation of gene expression and cellular phenotype. In cell culture models championed by Mina Bissell, tumour spheroids comprising epithelial cultures are produced in a low adhesion environment, either in a hanging droplet or in low-adhesive plastic culture dishes, usually with some ECM components in solution to facilitate the agglomeration of the cell mass into a sphere. The size of the spheroid is limited to avoid hypoxia due to oxygen diffusion limitation. There are marked differences in epithelial monolayer cultures and 3D spheroids in respect to gene expression [54, 55], activity of chemotherapy [56] and mechanisms of cell migration and invasion. These observations have been taken beyond the tumour environment to virtually all cell types in developing 3D organ-on-a-chip models [57]. Moreover, the use of 3D culture systems allows for identification of more relevant biomarkers [58], an increasingly critical tool in drug discovery and development. The pathophysiological mechanical environment of the tumour can be mimicked in spheroids. Embedding these structures in distinct ECM environments facilitates more relevant investigation of tumour cell invasion and migration [59], whereas the impacts of static and dynamic loadings in this setting are yet to be explored.

Virtually all lung diseases involve alteration in lung mechanics through disease-related cellular changes. The extent to which these cell-level changes are

influenced by intrinsic (age, sex, and genetic background) and/or extrinsic (exposures to environmental stimuli) factors is a subject of much recent investigation. The example of adult respiratory distress syndrome (ARDS), a lethal disease endemic to intensive care units worldwide, is instructive. Mechanical ventilation was established as a treatment soon after ARDS was first described [60]. Subsequently ventilation volume guidelines were refined in response to the finding that low levels of ventilator-imposed stretch are life-saving, whereas higher tidal volumes associated with greater stretch exacerbate lung injury [61]. In common with other causes of ARDS, increased plasma leakage via the compromised microvascular endothelial cell (EC) barrier is the major pathological outcome with ventilator-associated lung injury (VALI). Increased plasma leak is largely attributable to the impact of the large amplitude stretch on EC intercellular forces, signaling pathways, cytoskeletal components, and cell-cell and cell-substrate adhesions [62, 63]. Stretch-induced injury may be elicited mechanically or be secondary to inflammatory processes and/or mediators acting on the EC cytoskeleton. Regardless, targeting mechanotransduction pathways within the EC has emerged as a dominant therapeutic strategy in ARDS [64, 65]. Key amongst these targets are myosin light chain kinase, small GTPase RhoA, sphingosine-1-phosphate, Rho-specific guanosine nucleotide exchange factor, and protein kinases, amongst others [62, 65]. In this connection, Grigoryev *et al*. examined EC specific gene-expression in human pulmonary microvascular endothelial cells exposed to high levels of cyclic mechanical stretch [66]. They identified several gene variants that confer risk for VALI, including nicotinamide phosphoribosyltransferase (NAMPT) [67] and growth arrest DNA damage inducible alpha (GADD45a) [68], each of which is currently being pursued as a novel therapeutic target. Taken together, these studies highlight the importance of using cell mechanics to probe gene-environmental interactions for drug discovery.

This emerging approach to gene-environmental interactions is ideally exemplified by the use of epithelial organoids of intestinal origin to assess cystic fibrosis transmembrane conductance regulator (CFTR) phenotypes in patients with cystic fibrosis to ascertain their sensitivity to newly developed CFTR enhancers [69]. Misfolding or deficiency of expression of CFTR results in

thickened mucus on epithelial surfaces causing nutritional and respiratory problems. The biopsy-derived epithelia from CF patients are formed into a spheroid that is suspended in cell culture media. The structure forms tight junctions that serve to seal the internal extracellular compartment on the basolateral aspect of the epithelial from the external bathing fluid. As the CFTR channel activity increases, the spheroid swells, producing a very simple optical endpoint in an assay that personalizes drug screening. This latter model can be considered to be mechanically active. Its output is not dependent on mechanosensing, but simply to swelling caused by fluid transport.

Selected Bio-assays for use in mechanopharmacology

Two main principles for mechanopharmacological bio-assays are that the *input* dynamic environment is biomechanically similar to that of the cells in the target organ and that there is a quantitative *output* measure of the biomechanical activity of the target structure.

Substrate stiffness

Substrate stiffness has a pervasive influence on cell biology, as it modulates cell adhesion, spreading, deformation, and migration, as well as contraction, growth, differentiation, and apoptosis [50, 70-73]. For example, Engler *et al*. [72] demonstrated that lineage specification in naïve mesenchymal stem cells is intimately linked to stiffness of the substrate upon which the cells are adherent; soft substrates that mimic brain-like stiffness promote neurogenesis, while stiffer substrates that mimic muscle-like and bone-like stiffness promote myogenesis and osteogenesis, respectively. Substrate stiffness is also of great relevance to the pathogenesis of a wide range of diseases including diabetes, cancer, pulmonary fibrosis, hypertension, and acute lung injury. Yet, standard cell-culture platforms in high-throughput biology have lacked the ability to incorporate soft-substrates in multi-well preparations. To address that gap, Mih *et al*. developed a method to miniaturize polyacrylamide substrates in 96-well or 384-well plates [74]. The stiffness of the substrate is tunable from ~0.5kPa to 90 kPa, spanning the known (patho)physiological range. The platform is compatible with standard imaging systems and opens new opportunities to investigate cell

spreading, division, growth, migration, and apoptosis in a biomechanically relevant microenvironment, using high-throughput platforms. This higher throughput system is currently limited to a 2D environment.

Substrate stretch

Mechano-screening is at an advanced stage of development for shear and stretch. Lung-on-a-chip technology incorporating an air liquid interface with epithelium apposed to vascular endothelial cells has been used to emulate the stretching caused by breathing, coupled with shear caused by fluid flow [75]. These chips were used to establish the potential of TRPV4 inhibitors in treating vascular injury leading to pulmonary oedema of interleukin-2 [76], an immuno-stimulant used to treat some solid tumours. This work has been translated, with successful preclinical animal studies showing the orally active TRV4 channel inhibitors prevent pulmonary oedema associated with heart failure [77]. Interest in the potential to modulate TRPV4 in respiratory disease is growing with its importance in mechanosensing being more widely appreciated [78], and recent evidence suggesting a role in pulmonary fibrosis [79].

Commercial application of devices that emulate (patho)physiological shear forces characteristically experienced by the vascular endothelium has now been achieved, although further evaluation is required to fully assess any improvements in predictive value. Devices enabling cyclical stretch such as the Flexcell™ have been available since the 90s. Recent advances in micro-scaled devices that enable physiological frequency and amplitudes of stretch to be applied to organoids, so-called organ-on-a-chip devices, are at the threshold of widespread commercial use. These developments have prompted interest from the US National Centre on Advancing Translational Sciences (NCATS) to fund development of models and to initiate conversations between academics, Pharma/Biotech and regulators, including a programme funding "Tissue chip projects" (https://ncats.nih.gov/tissuechip), predicating "disease-on-a-chip".

Cellular properties in suspended cells

A better understanding of the relationship between cell mechanical changes and disease outcomes has also allowed us to exploit new methods for drug mechano-screening. For example, the micropipette aspiration technique [80] has been used to characterize changes in whole cell mechanical properties due to drugs or diseases. A controlled suction pressure is applied through to the surface of a single cell using a micropipette. The pressure is finely regulated and real-time images of the aspirated cell or cell elongation into the micropipette are recorded as suction pressure increases. Assuming the cell as a homogeneous, isotropic, linear elastic and incompressible half-space medium, the whole cell stiffness and the viscoelastic properties can be calculated from the applied pressure and the corresponding cell elongation [80]. Airway smooth muscle (ASM) cell exposure to TGFβ increases ASM cell stiffness, whereas bFGF prevents this increase in stiffness [30], as measured by micropipette aspiration (Figure 1). The micropipette aspiration technique has been used to evaluate the effects of antimitotic microtubule-targetting agents on cancer cells; correlating increased whole cell stiffness and viscoelastic properties to microtubule/microfilament content in the cell cytoskeletal network [81]. Treatment of tumour cells with anti-mitotic agents reduced the elevated cell stiffness. Each of these studies highlights the potential of the intrinsic cellular mechanical properties, such as stiffness, to serve as a biomarker of disease activity and of therapeutic activity of interventions. The potential for high-throughput screening could also be seen in some lab-on-chip devices combining micropipette aspiration technique with microfluidics for mechano-phenotyping applications. Lee *et al.* described the combined polydimethylsiloxane (PDMS) chip fabricated using soft lithography technique [82]. The chip consisted of a filtering unit allowing only single cells (as opposed to cell clumps) to enter 16 micro-channels or arrays. Each array incorporated four aspiration chambers on each side, allowing single cells to be aspirated in a high-throughput manner. These studies confirmed earlier observations suggesting that metastatic tumour cells have a lower stiffness enabling greater deformability to transmigrate vascular barriers to metastasis. Another high-throughput approach in suspended cells involves applying hydrodynamic stretching on single cells. Dudani *et al.* described a process called 'hydropipetting', whereby a cell in suspension is moved at high speed in

microfluidic channels and is deformed by hydrodynamic forces generated when the cell flows past perpendicular branching channels that generate a cross flow, causing a pinching effect on the cell [83]. Unlike the devices based on micropipette aspiration, the cells are not in contact with the channel walls. Speeds of up to 65000 cells/second are possible with 'hydropipetting'. Other approaches in suspended cells include varied methods for screening cell deformability in the context of malaria [84], cancer [85], and malignant pleural effusions [86]. Analyses of cells in suspension provides access to fluidics technologies, but if the tissue of origin is a solid organ then the disruption of isolation and lack of ECM attachment comprises a perturbation of the normal cell physiology, thereby potentially limiting to the applicability of the data.

Cellular properties in adherent cells

Limited information is provided by cell stiffness measurements in the suspended cells, as this setting does not provide quantitative data on the interactions between the cell and its microenvironment or ECM. The main cellular structural element is the cytoskeleton, which comprises an array of protein filaments that provide the mechanical connections from the cell membrane to the nucleus. The cytoskeleton is anchored to the ECM and less commonly to adjacent cells via discrete points known as focal adhesions (FA) that serve as the conduit for mechanosignaling. The FA sense external mechanical forces, as well acting to balance the internal forces produced with the cytoskeleton [87]. A common approach to measuring forces transmitted at the FA is the technique of traction force microscopy. One embodiment of traction force microscopy uses culture of cells on micro-pillars [88]. PDMS micro-pillars are fabricated using a silicon mold etched by standard micromachining techniques (Figure 2). The micro-pillar array is then calibrated using a micromanipulator to obtain its spring constant. The cells placed on top of the pillars attach to the individual pillars via the FA. The cell traction is calculated by the amount of deflection of the individual pillars. Another embodiment of traction force microscopy uses culture of cells upon deformable substrates. Impregnated in the substrates are fluorescent microbeads; by tracking microbead displacements cell-exerted substrate deformations are calculated.

Based on substrate deformation and with knowledge of substrate stiffness, the cell traction forces can be obtained [70-72, 89-91].

The aforementioned technologies are largely limited to low-throughput settings and are therefore impractical for use in screening for agents targeted to mechanisms subserving cell traction. Instead, available drug screening technologies use varied biochemical (e.g. cytoplasmic calcium levels) or structural surrogates (e.g. expression levels of filamentous actin) for investigation of traction. These approaches do not adequately deal with false positives or false negatives. To address this gap, Park *et al*. recently miniaturized the setting of traction force microscopy to 96-well plates and developed a new medium to high-throughput method called contractile force screening (CFS) [11]. CFS is likely to facilitate drug discovery and drug repurposing in circumstances in which modulation of cell traction is the logical therapeutic target, including but not limited to asthma, COPD, vascular and cardiac disease, pulmonary arterial hypertension, glaucoma, kidney disease, and cancer.

Another biomechanical screening approach in the adherent cell is the magnetic twisting cytometry, a commonly used technique to screen for impacts on cell stiffness [92-94]. During magnetic twisting cytometry measurements, ferromagnetic beads (4.5 μm in diameter) are tightly anchored to the cell cytoskeleton and oscillated using a known magnetic field (5 to 75 Gauss). From the ratio of the imposed bead torque to the resulting bead motion, the cell stiffness can be experimentally determined.

Cellular properties in 3D

Pseudo 3D techniques have been attempted by growing cells in-between micro pillars [95], or by hanging cells using bent cantilever beams [96]. However, these methods do not fully mimic the physiological nature of cells embedded in 3D, particularly because the ensemble responses of multicellular structures are not accounted for in these assays. To overcome this limitation, cell aggregates or cell spheroid preparations have been proposed. Spheroid stiffness has been measured using the micropipette aspiration techniques [97]. Nevertheless, significant challenges remain in handling spheroids under high-throughput conditions, due to their size and the potential to disintegrate. Traction force

553 measurements in spheroids have also been attempted using large aspect ratio
554 micro-pillars [98]. Nonetheless, high-throughput systems based on micro-pillar
555 or cantilever principles have been extremely difficult to construct. Instead, the
556 current state-of-the-art in 3D traction force microscopy is to culture a population
557 of cells in a 3D collagen gel matrix embedded with fluorescent beads. The forces
558 generated by the cells create a pre-stress condition on the gel. Following the
559 experiment, the cell cytoskeleton is destroyed using drugs causing the cell to
560 detach from the gel matrix, releasing the overall pre-stress on the gel, which is
561 tracked by measuring the fluorescent bead displacements [99]. However,
562 challenges remain in relating the overall pre-stress on the gel to the traction
563 force contributions of individual cells. Computational techniques, such as finite
564 element (FE) analysis, have been used to predict the individual cell traction force
565 using information gathered from the overall displacement of beads within the gel
566 [100]. 2D traction force microscopy offers significant potential for high
567 throughput drug screening, but further development is needed for 3D screening
568 with significant throughput. The lung slice may be subjected to traction force
569 microscopy through similar approaches to those used in cell monolayer
570 experiments and has the advantage of maintaining spatial geometry and cell
571 heterogeneity [101]. Particle tracking microrheology may offer an approach to
572 these challenges [102].
573
574 **Concluding Remarks**
575
576 The emergence of methodologies to culture cells in microenvironments that
577 better reflect the mechanical, soluble and insoluble (ECM) environment of the
578 diseased tissue bearing the drug target creates an impetus to use such models for
579 drug screening. Efforts to increase the throughput of such methodologies,
580 whilst maintaining cells in a 3D setting with appropriate mechanical loading
581 characteristics need to be complemented by improvements in the patterns of
582 drug and mediator exposure that are also aligned to the patterns prevailing at
583 the drug target in the diseased tissue. A focus on these
584 pharmacodynamic/pharmacokinetic relationships using human cell–derived 3D
585 organoids appears likely to improve their predictive value for target

identification and drug screening (see Outstanding questions). We have not covered exciting developments in what might be regarded as mechano-pharmaceutics, in which nanotechnology is being applied to pharmaceutical formulation to exploit mechanical forces that cause localized or selective liberation of drugs from their nanoparticle delivery systems [103].

Our view is that there is a need for a sub-discipline of mechanopharmacology, which will serve as a focus for activities that are highly interdisciplinary. The closest term to our proposal is biomechanopharmacology [7], but this descriptor has not achieved widespread acceptance, perhaps because it is not aligned with similar terms, including mechanomedicine, mechanotherapy [104], mechanotherapeutics [103] and mechanobiology. Mechanopharmacology has been used previously to describe the study of the mechanics of individual proteins [6, 105]; our proposed usage covers a wider conceptual field. The discussion of best-practice in models, scope of studies, state-of–the-art, and the development of a repository for accepted principles needs to be concentrated rather than distributed amongst a large number of diverse professional/learned societies, as is presently the case. The drive to establish this inter-discipline derives from the desire to improve the relevance of target discovery and screening models to tissues *in situ* and to human disease, with the ultimate hope of better prediction of efficacy in the clinic.

Outstanding questions

Will improvements in the cellular mechanics of evaluation and screening paradigms result in better prediction of efficacy?

Will disease-on-a-chip technology be widely adopted?

Will personalized drug screening becoming commonplace through the use of organoids from patient-derived cells (possibly reprogrammed through an induced pluripotent stem cell pathway to the relevant phenotype)?

Will the relationship between pharmacokinetics and pharmacodynamics be explored in sub-acute time frames of days to weeks in lower throughput settings to better predict drug efficacy in chronic disease?

Can a new interdiscipline of mechanopharmacology serve to accelerate progress in applying new insights in cellular mechanics to drug screening and evaluation?

638 **Fig. 1**. Micropipette aspiration of a single cell [30] or cell aggregate (shown)
639 causes the cellular mass to be drawn into the lumen of the micropipette and
640 elongated in a pressure-dependent manner. These aspiration experiments
641 provide the data required for calculation of cell stiffness, which is related to the
642 slope of the relationship between applied pressure and corresponding
643 elongation.

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653 **Fig 2.** Single cell traction force measurements on micropillars. The region of
654 interest marked by the grey outline identifies an area corresponding to a portion
655 of a single cell, which is exerting traction on the underlying pillars. The direction
656 and the extent of the deflection of the pillar are used to calculate a traction vector
657 (blue arrow).
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Box 1

Cell mechanics made easy

Tension, compression, bending and shear: There are four major loads that a cell, a tissue segment, and/or an organ must withstand. These loads are illustrated diagrammatically in Figure I. The loads can arise external to the cell due to fluid flow, stretch and/or constriction, or arise internally within the cell due to active force generation through the cellular contractile apparatus. An external force acting over an area may be applied as a tensile, compressive, bending, or shear stress. Such stress causes a local deformation (strain). The material properties of the tissue can be probed by establishing the stress/strain relationship.

Cell mechanics: Cell mechanics refers to any cellular process during which mechanical forces are generated, imparted, or sensed (Figure I). The underlying mechanical-biochemical interactions not only mediate physical and structural changes [90, 106, 107], but also promote cell adhesion [108], polarization [70], stem cell differentiation[72], locomotion[109-111], wound healing [112], gene expression [113], angiogenesis [114], and apoptosis [115]. Consequently, defects in cell or extracellular (ECM) mechanics, often caused by maladaptation or malfunction of cell and ECM proteins, have been implicated in the development of numerous diseases (see Table 1 for selected examples).

Traction: The local force per unit area that is imposed on the microenvironment by an adherent cell is called traction. Tractions arise due to complex interactions between molecular motors, cytoskeletal filaments, and adhesion molecules within the cell, together with the microenvironment. Ensuing interactions are powered by three intracellular processes: adenosine triphosphate (ATP) hydrolysis; actin polymerization; and, cell-cell and cell-substrate adhesion assembly/disassembly.

Cell Stiffness: In response to an external force, the extent to which a cell resists deformation is called stiffness. To measure this property the external force can

be imposed locally via a magnetic bead, a tweezer, a cantilever beam, an intracellular tracer particle, or a microneedle pulling on receptors (e.g. integrins) on the cell surface [116, 117], or can be imposed globally via whole-cell aspiration[118] or stretch[119] [90, 106].

Figure I. The potential biomechanical impacts of cell and matrix are depicted and the terms are discussed in detail in the text. The cross sectional area (A) of the cell is the usual orientation for the forces (compression, contraction, load) to be applied, especially in smooth muscle bundles. The term F/A denotes the stress applied to or exerted by the cell when being compressed or contracting, respectively.

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Acknowledgements. We thank Fernando Guzman for the image used in Figure 1. The authors' work discussed in this manuscript was supported grants from NHMRC (#105966; #1045372), Dyason Fellowship and Global Mobility Strategic Enhancement grant (University of Melbourne).

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Trends Box

- **An argument is outlined for a new inter-discipline, mechanopharmacology**
- **Examples of cellular biomechanics influences on drug action are described**
- **The relevance of matrix stiffness, internal and external stresses to drug screening is discussed**
- **Methods for biomechanical perturbation and analysis of single cell, and organoids are reviewed**

Figure 1

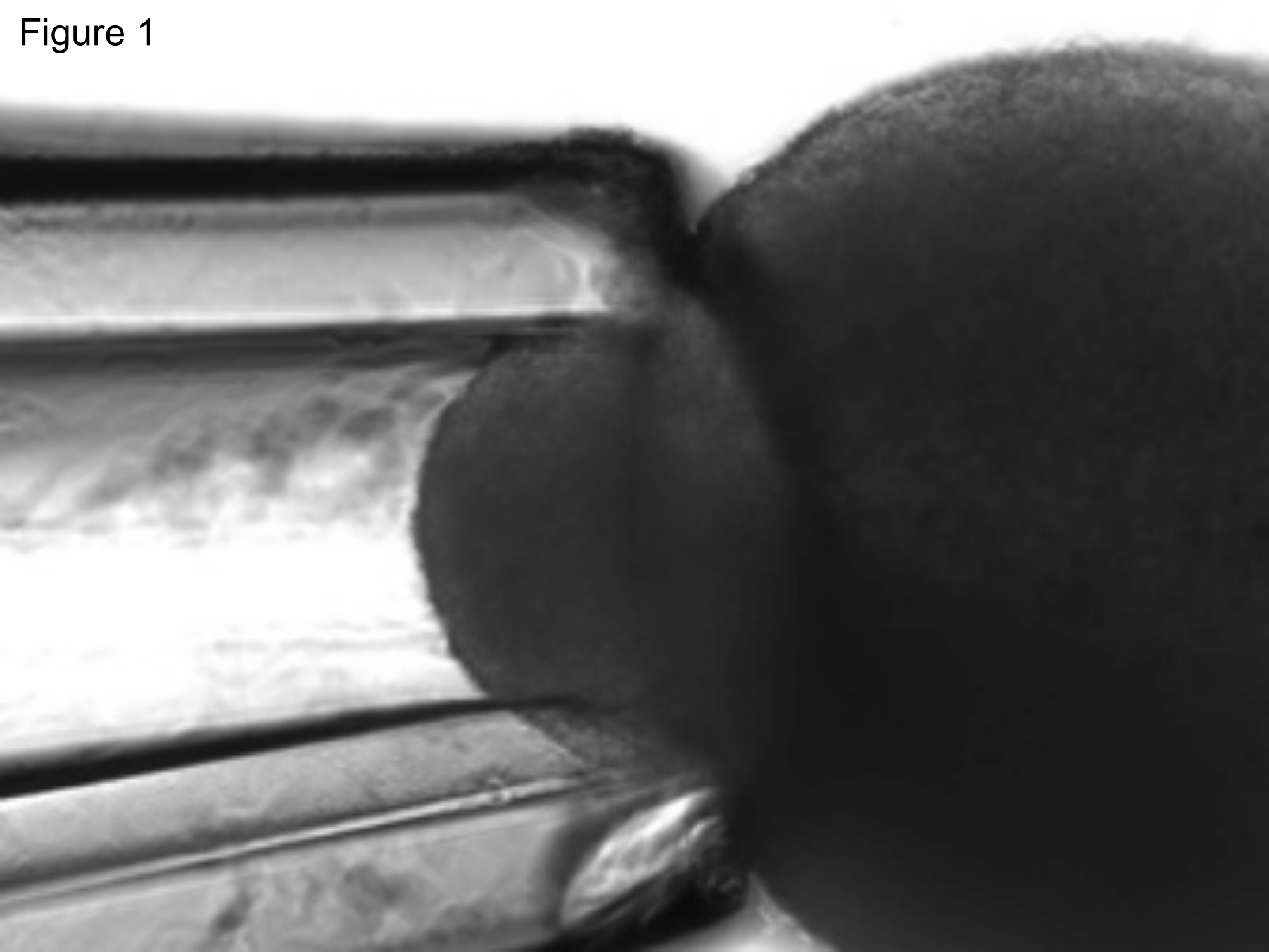


Figure 2

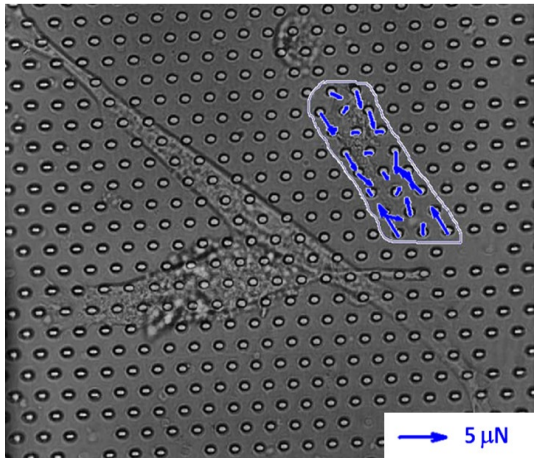
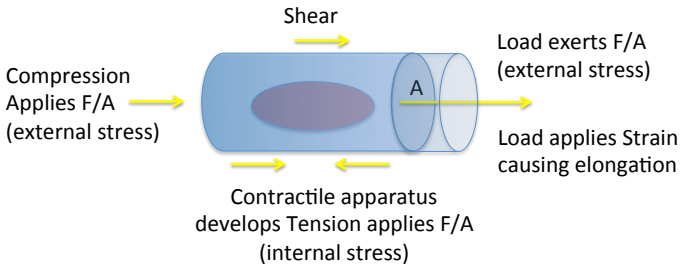


Figure 3


Shear
Compression
Applies F/A
(external stress)
A
Load exerts F/A
(external stress)
Load applies Strain
causing elongation
Contractile apparatus
develops Tension applies F/A
(internal stress)

Outstanding questions

Will improvements in the cellular mechanics of evaluation and screening paradigms result in better prediction of efficacy?

Will disease-on-a-chip technology be widely adopted?

Will personalized drug screening becoming commonplace through the use of organoids from patient-derived cells (possibly reprogrammed through an induced pluripotent stem cell pathway to the relevant phenotype)?

Will the relationship between pharmacokinetics and pharmacodynamics be explored in sub-acute time frames of days to weeks in lower throughput settings to better predict drug efficacy in chronic disease?

Can a new interdiscipline of mechanopharmacology serve to accelerate progress in applying new insights in cellular mechanics to drug screening and evaluation?

Table

Table 1. Defects in cell mechanics (C) and ECM mechanics (E) are at the forefront of a wide range of diseases. Adapted from Ingber. [104]

Bladder	Detrusor underactivity	C
Eye	Glaucoma Macular degeneration	C, E C, E
Vascular	Hypertension	C,E
Lung	Asthma, COPD Fibrosis, emphysema	C, E E
Brain	Traumatic brain injury	C,E
Oncology	Cancer Metastasis	C, E C
Reproductive	Pre-eclampsia	C, E
Skin	Scleroderma	E
Aging		C,E