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Trends in Pharmacological Sciences

Cellular biomechanics in drug screening and evaluation: Mechanopharmacology

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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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2 **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.

67 **Drug screening and evaluation: the need to consider cellular mechanics**
68
69 The reasons for failure of drug development programmes are the subject of
70 much contemplation. Whilst adverse effects, toxicity, as well as pharmacokinetic
71 features, are often cited as reasons for arrested drug development, several
72 recent studies highlight failure because of a lack of efficacy. A review of Astra-
73 Zeneca's portfolio performance in Phase IIa and IIb studies from 2005 to 2010
74 suggested that 57% and 88%, respectively, of the project closures at this stage
75 were due to a failure of efficacy, whereas attrition due to efficacy in preclinical
76 phase was as low as 6% [1]. There are many reasons to expect that preclinical
77 and clinical pharmacology will differ, including the use of non-human species to
78 support efficacy. However, even when the target is expressed and engaged in
79 human cell types, failure may ensue because the affected pathways are less
80 influential than anticipated from the preclinical studies. When the agent reaches
81 the target in adequate concentration and for a sufficient duration, giving a
82 suitable level of drug exposure, lack of efficacy is likely to result from differences
83 in behavior of the drug target in the assay systems compared with the target
84 behavior *in situ* in the patient-specific context. The screening and preclinical
85 pharmacology for many of these agents is likely to have been established in cell
86 culture, in an oversimplified mechanical micro-environment and/or in non-
87 human models of the targeted disease. We argue that drug screening can be
88 improved with the use of human cells of phenotype most relevant to the
89 condition, ideally being derived from patients (representative of the disease
90 stage being targeted), and then cultured in the most (patho)physiologically
91 relevant conditions. This approach is intended to ensure that the assay emulates
92 the biomechanical environment, in the condition to be treated. Ideally, the assay
93 would also embed cell mechanical measurements of deformability, stiffness,
94 and/or contraction, as in many organs and diseases, these cellular changes often
95 constitute the principal endpoint of therapeutic intent. The use of patient-
96 derived primary cell cultures improves the likelihood of genetic and epigenetic
97 influences being appropriately expressed, with the expectation that the target
98 efficacy would be more accurately predicted. It will be valuable to rigorously

99 test this proposition against comparator preclinical efficacy testing in relevant
100 animal models and in 2 dimensional culture on rigid plastic substrate.
101
102 The impact of biomechanics on cell function has been systematically explored,
103 leading to a broad appreciation of mechano-sensitive processes, with the
104 principal mechanosensors being selected ion channels [2, 3] and less commonly
105 the integrins [4]. Mechanotransduction involves force transmission through
106 bound proteins resulting in conformational changes that entrain functional
107 impacts. For example, conformational changes in vinculin and talin have been
108 shown to subserve the recruitment of the actin cytoskeleton to focal adhesions in
109 the leading edge of migrating cells [5, 6].
110
111 The impact of biomechanics on drug actions is rarely addressed, despite being
112 highlighted as an important consideration repeatedly in the literature, for
113 example, [7, 8]. Recent advances proposed by Donald Ingber and colleagues
114 using "organ-on-a-chip" microfluidics technology involving cell cultures being
115 subjected to cyclical strains (breathing/cardiac cycle/peritoneal
116 peristalsis/renal fluidic shear), raise the prospect of more systematic and
117 relevant drug discovery paradigms using human cell cultures [9] [10]. Similarly,
118 recent advances in cell mechanics have highlighted the suitability of mechanical
119 endpoints as phenotypic targets in high-throughput screening [11]. In this
120 article, we develop selected examples of biomechanical impacts on cell function
121 and drug responsiveness and discuss refined, biomechanically appropriate
122 bioassays, emphasizing those suitable for scaling to medium to high throughput.
123 We exemplify below the selected impacts of different aspects of the
124 biomechanical environment (Box 1.)
125
126
127 **Shear forces**

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

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

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

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

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

176 Shear forces play a key role in determining the pharmacology of anti-platelet
177 agents. Examination of the role of integrins under static and shear stress
178 conditions indicates that shear is able to alter the avidity of integrins not only for
179 their binding partner, but also for some small molecule ligands that inhibit their
180 activation [17]. The impact of inhibitors of the phospho-Inositide -3- Kinases (PI-
181 3K) are also sensitive to whether evaluation takes place under static or shear-
182 stressed conditions, with implications for drug screening approaches [18].
183 Specifically, the role of PI-3Kβ isoform is most evident under the high shear
184 stress where it contributes to the signalling regulating the transmission of force
185 from the actin cytoskeleton of the activated platelet through the $\alpha_2\beta_3$ platelet
186 integrin to the clot fibrin [19].

187

188 **Force, stress, strain and stiffness**

189 In most organs, smooth muscle functions under an environment of constant
190 fluctuation of stress and strain. Control of the organ function can be achieved by
191 regulating the intrinsic forces generated by the muscle. Conversely, the
192 behaviour of the muscle can be modified by extrinsic forces imposed on the
193 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

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

245

246 **Compressive forces**

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

252

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

258 mechanotransduction signals in airway epithelial cells via the activation of
259 epidermal growth factor receptor. During compressive stress, epithelial cells
260 release mediators that induce proliferation and production of type 1 and 3
261 collagens from co-cultured fibroblasts [37]. In air-liquid interface cultures of
262 airway epithelium, intermittent, repeated compressive stresses cause goblet cell
263 hyperplasia in well-differentiated normal HBE cells [38]. Moreover, compressive
264 stress induces the release of inflammatory and angiogenic mediators, such as
265 tissue factor and YKL-40 from HBE cells via PKC and ERK-dependent pathways
266 [39, 40]. These studies using an *in vitro* compressive system suggest that even in
267 the absence of inflammatory cells, compressive mechanical stress imposed on
268 airway epithelium activates mechanotransduction pathways that participate in
269 airway remodeling of asthma. With an appreciation of previous *in vitro* studies,
270 Grainge *et al*. reported results from a ground-breaking study in mild asthmatics
271 showing that repeated challenges with a bronchoconstricting agent,
272 methacholine, induce two major remodeling events; goblet cell hyperplasia and
273 increased type 3 collagen deposition, in the absence of infiltration of
274 inflammatory cells [41]. Pre-treatment with a bronchodilator, albuterol
275 prevented the remodeling events induced by methacholine, thereby implicating
276 mechanical compression rather than other pharmacological effects of muscarinic
277 receptor activation. Thus, compressive stress may accelerate the progression of
278 remodeling in individuals with pre-existing conditions [42]. Persistent
279 relaxation of the airways by long-acting β_2-adrenoceptor agonists would be
280 expected to offset these stimuli to remodeling, but this has yet to be convincingly
281 demonstrated.

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

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

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

302

303 ***The mechanical environment of the cell***

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

323 cellular mechanics using agents that impact on the cytoskeleton may offer a
324 promising approach to altering the behavior of endogenous cell stem cells to
325 effect repair. Agents that target central structures in the cytoskeleton are usually
326 cytotoxic. However, prospects for selective, safe and tolerable agents are
327 emerging from an improved understanding of the molecular basis of
328 mechanosensing [53]. In addition, pharmacological or genetic manipulation of
329 autologous and allogeneic stem cell phenotype may extend the utility of stem
330 cells as treatments.
331
332 The 2D or 3D environment of the cellular system has profound impacts on
333 cellular behaviour, some of which can be ascribed to changes in the mechanical
334 microenvironment. Thus, the tensile, compression and shear forces experienced
335 by cells within the 3D environment, together with spatial heterogeneity
336 engenders more differentiation of gene expression and cellular phenotype. In cell
337 culture models championed by Mina Bissell, tumour spheroids comprising
338 epithelial cultures are produced in a low adhesion environment, either in a
339 hanging droplet or in low-adhesive plastic culture dishes, usually with some ECM
340 components in solution to facilitate the agglomeration of the cell mass into a
341 sphere. The size of the spheroid is limited to avoid hypoxia due to oxygen
342 diffusion limitation. There are marked differences in epithelial monolayer
343 cultures and 3D spheroids in respect to gene expression [54, 55], activity of
344 chemotherapy [56] and mechanisms of cell migration and invasion. These
345 observations have been taken beyond the tumour environment to virtually all
346 cell types in developing 3D organ-on-a-chip models [57]. Moreover, the use of 3D
347 culture systems allows for identification of more relevant biomarkers [58], an
348 increasingly critical tool in drug discovery and development. The
349 pathophysiological mechanical environment of the tumour can be mimicked in
350 spheroids. Embedding these structures in distinct ECM environments facilitates
351 more relevant investigation of tumour cell invasion and migration [59], whereas
352 the impacts of static and dynamic loadings in this setting are yet to be explored.
353
354 Virtually all lung diseases involve alteration in lung mechanics through disease-
355 related cellular changes. The extent to which these cell-level changes are

356 influenced by intrinsic (age, sex, and genetic background) and/or extrinsic
357 (exposures to environmental stimuli) factors is a subject of much recent
358 investigation. The example of adult respiratory distress syndrome (ARDS), a
359 lethal disease endemic to intensive care units worldwide, is instructive.
360 Mechanical ventilation was established as a treatment soon after ARDS was first
361 described [60]. Subsequently ventilation volume guidelines were refined in
362 response to the finding that low levels of ventilator-imposed stretch are life-
363 saving, whereas higher tidal volumes associated with greater stretch exacerbate
364 lung injury [61]. In common with other causes of ARDS, increased plasma
365 leakage via the compromised microvascular endothelial cell (EC) barrier is the
366 major pathological outcome with ventilator-associated lung injury (VALI).
367 Increased plasma leak is largely attributable to the impact of the large amplitude
368 stretch on EC intercellular forces, signaling pathways, cytoskeletal components,
369 and cell-cell and cell-substrate adhesions [62, 63]. Stretch-induced injury may
370 be elicited mechanically or be secondary to inflammatory processes and/or
371 mediators acting on the EC cytoskeleton. Regardless, targeting
372 mechanotransduction pathways within the EC has emerged as a dominant
373 therapeutic strategy in ARDS [64, 65]. Key amongst these targets are myosin
374 light chain kinase, small GTPase RhoA, sphingosine-1-phosphate, Rho-specific
375 guanosine nucleotide exchange factor, and protein kinases, amongst others [62,
376 65]. In this connection, Grigoryev *et al*. examined EC specific gene-expression in
377 human pulmonary microvascular endothelial cells exposed to high levels of
378 cyclic mechanical stretch [66]. They identified several gene variants that confer
379 risk for VALI, including nicotinamide phosphoribosyltransferase (NAMPT) [67]
380 and growth arrest DNA damage inducible alpha (GADD45a) [68], each of which
381 is currently being pursued as a novel therapeutic target. Taken together, these
382 studies highlight the importance of using cell mechanics to probe gene-
383 environmental interactions for drug discovery.

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

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

398

399 **Selected Bio-assays for use in mechanopharmacology**

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

404

405 *Substrate stiffness*

406 Substrate stiffness has a pervasive influence on cell biology, as it modulates cell
407 adhesion, spreading, deformation, and migration, as well as contraction, growth,
408 differentiation, and apoptosis [50, 70-73]. For example, Engler *et al.* [72]
409 demonstrated that lineage specification in naïve mesenchymal stem cells is
410 intimately linked to stiffness of the substrate upon which the cells are adherent;
411 soft substrates that mimic brain-like stiffness promote neurogenesis, while
412 stiffer substrates that mimic muscle-like and bone-like stiffness promote
413 myogenesis and osteogenesis, respectively. Substrate stiffness is also of great
414 relevance to the pathogenesis of a wide range of diseases including diabetes,
415 cancer, pulmonary fibrosis, hypertension, and acute lung injury. Yet, standard
416 cell-culture platforms in high-throughput biology have lacked the ability to
417 incorporate soft-substrates in multi-well preparations. To address that gap, Mih
418 *et al.* developed a method to miniaturize polyacrylamide substrates in 96-well or
419 384-well plates [74]. The stiffness of the substrate is tunable from ~0.5kPa to 90
420 kPa, spanning the known (patho)physiological range. The platform is compatible
421 with standard imaging systems and opens new opportunities to investigate cell

422 spreading, division, growth, migration, and apoptosis in a biomechanically
423 relevant microenvironment, using high-throughput platforms. This higher
424 throughput system is currently limited to a 2D environment.
425
426 *Substrate stretch*
427 Mechano-screening is at an advanced stage of development for shear and stretch.
428 Lung-on-a-chip technology incorporating an air liquid interface with epithelium
429 apposed to vascular endothelial cells has been used to emulate the stretching
430 caused by breathing, coupled with shear caused by fluid flow [75]. These chips
431 were used to establish the potential of TRPV4 inhibitors in treating vascular
432 injury leading to pulmonary oedema of interleukin-2 [76], an immuno-stimulant
433 used to treat some solid tumours. This work has been translated, with successful
434 preclinical animal studies showing the orally active TRV4 channel inhibitors
435 prevent pulmonary oedema associated with heart failure [77]. Interest in the
436 potential to modulate TRPV4 in respiratory disease is growing with its
437 importance in mechanosensing being more widely appreciated [78], and recent
438 evidence suggesting a role in pulmonary fibrosis [79].
439
440 Commercial application of devices that emulate (patho)physiological shear
441 forces characteristically experienced by the vascular endothelium has now been
442 achieved, although further evaluation is required to fully assess any
443 improvements in predictive value. Devices enabling cyclical stretch such as the
444 Flexcell™ have been available since the 90s. Recent advances in micro-scaled
445 devices that enable physiological frequency and amplitudes of stretch to be
446 applied to organoids, so-called organ-on-a-chip devices, are at the threshold of
447 widespread commercial use. These developments have prompted interest from
448 the US National Centre on Advancing Translational Sciences (NCATS) to fund
449 development of models and to initiate conversations between academics,
450 Pharma/Biotech and regulators, including a programme funding "Tissue chip
451 projects" (https://ncats.nih.gov/tissuechip), predicating "disease-on-a-chip".
452
453 *Cellular properties in suspended cells*

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

487 microfluidic channels and is deformed by hydrodynamic forces generated when
488 the cell flows past perpendicular branching channels that generate a cross flow,
489 causing a pinching effect on the cell [83]. Unlike the devices based on
490 micropipette aspiration, the cells are not in contact with the channel walls.
491 Speeds of up to 65000 cells/second are possible with 'hydropipetting'. Other
492 approaches in suspended cells include varied methods for screening cell
493 deformability in the context of malaria [84], cancer [85], and malignant pleural
494 effusions [86]. Analyses of cells in suspension provides access to fluidics
495 technologies, but if the tissue of origin is a solid organ then the disruption of
496 isolation and lack of ECM attachment comprises a perturbation of the normal cell
497 physiology, thereby potentially limiting to the applicability of the data.
498
499 *Cellular properties in adherent cells*
500 Limited information is provided by cell stiffness measurements in the
501 suspended cells, as this setting does not provide quantitative data on the
502 interactions between the cell and its microenvironment or ECM. The main
503 cellular structural element is the cytoskeleton, which comprises an array of
504 protein filaments that provide the mechanical connections from the cell
505 membrane to the nucleus. The cytoskeleton is anchored to the ECM and less
506 commonly to adjacent cells via discrete points known as focal adhesions (FA)
507 that serve as the conduit for mechanosignaling. The FA sense external
508 mechanical forces, as well acting to balance the internal forces produced with the
509 cytoskeleton [87]. A common approach to measuring forces transmitted at the
510 FA is the technique of traction force microscopy. One embodiment of traction
511 force microscopy uses culture of cells on micro-pillars [88]. PDMS micro-pillars
512 are fabricated using a silicon mold etched by standard micromachining
513 techniques (Figure 2). The micro-pillar array is then calibrated using a
514 micromanipulator to obtain its spring constant. The cells placed on top of the
515 pillars attach to the individual pillars via the FA. The cell traction is calculated by
516 the amount of deflection of the individual pillars. Another embodiment of
517 traction force microscopy uses culture of cells upon deformable substrates.
518 Impregnated in the substrates are fluorescent microbeads; by tracking
519 microbead displacements cell-exerted substrate deformations are calculated.

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

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

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

542

543 ***Cellular properties in 3D***

544 Pseudo 3D techniques have been attempted by growing cells in-between micro
545 pillars [95], or by hanging cells using bent cantilever beams [96]. However, these
546 methods do not fully mimic the physiological nature of cells embedded in 3D,
547 particularly because the ensemble responses of multicellular structures are not
548 accounted for in these assays. To overcome this limitation, cell aggregates or cell
549 spheroid preparations have been proposed. Spheroid stiffness has been
550 measured using the micropipette aspiration techniques [97]. Nevertheless,
551 significant challenges remain in handling spheroids under high-throughput
552 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.

612 **Outstanding questions**

613

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

616

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

618

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

622

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

626

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

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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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664 **Box 1**

665 **Cell mechanics made easy**

666

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

676

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

686

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

694

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

697 be imposed locally via a magnetic bead, a tweezer, a cantilever beam, an
698 intracellular tracer particle, or a microneedle pulling on receptors (e.g. integrins)
699 on the cell surface [116, 117], or can be imposed globally via whole-cell
700 aspiration[118] or stretch[119] [90, 106].
701
702 **Figure I.** The potential biomechanical impacts of cell and matrix are depicted
703 and the terms are discussed in detail in the text. The cross sectional area (A) of
704 the cell is the usual orientation for the forces (compression, contraction, load) to
705 be applied, especially in smooth muscle bundles. The term F/A denotes the
706 stress applied to or exerted by the cell when being compressed or contracting,
707 respectively.
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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

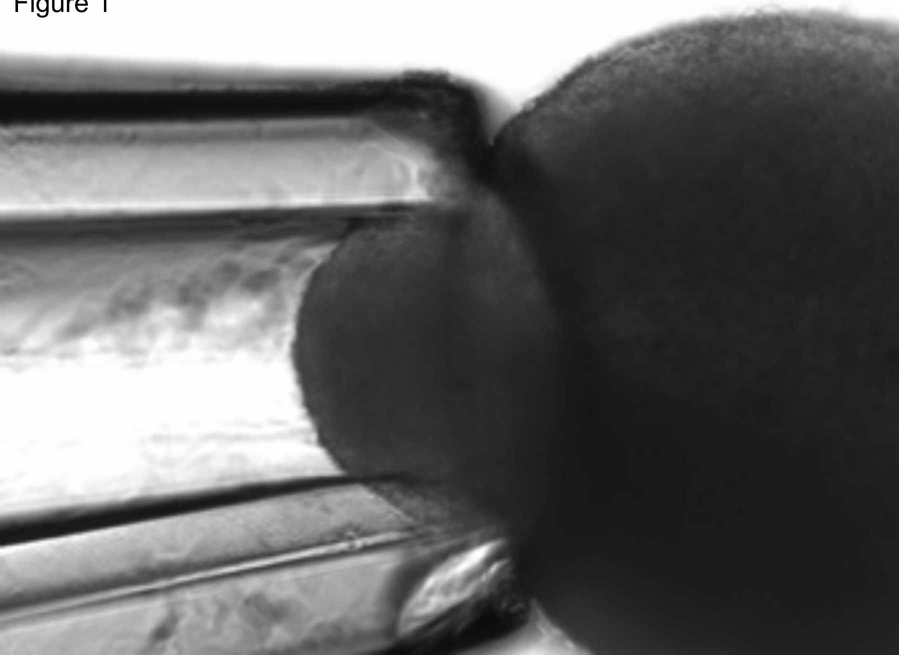


Figure 2

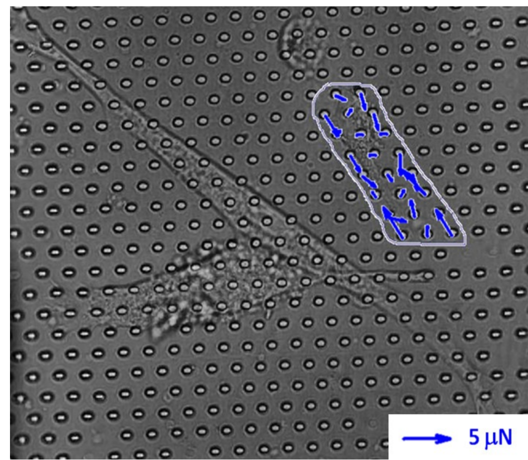
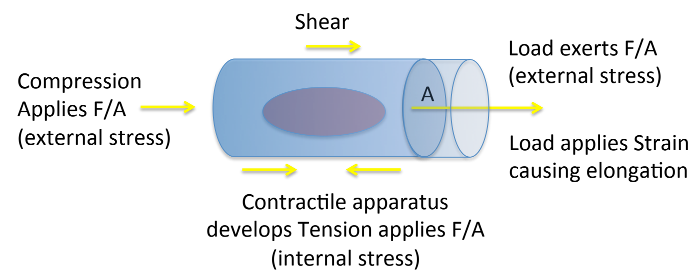


Figure 3



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