



Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

Lessons from (patho)physiological tissue stiffness and their implications for drug screening, drug delivery and regenerative medicine[☆]

Wen Li Kelly Chen^a, Craig A. Simmons^{a,b,c,*}

^a Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON, Canada

^b Department of Mechanical and Industrial Engineering, University of Toronto, Toronto, ON, Canada

^c Faculty of Dentistry, University of Toronto, Toronto, ON, Canada

ARTICLE INFO

Article history:
Received 23 October 2010
Accepted 5 January 2011
Available online xxxx

Keywords:
Substrate stiffness
Elasticity
Biomechanics
Stem cells
Cancer biology
Mechanobiology
Biomaterials

ABSTRACT

Diseased tissues are noted for their compromised mechanical properties, which contribute to organ failure; regeneration entails restoration of tissue structure and thereby functions. Thus, the physical signature of a tissue is closely associated with its biological function. In this review, we consider a mechanics-centric view of disease and regeneration by drawing parallels between *in vivo* tissue-level observations and corroborative cellular evidence *in vitro* to demonstrate the importance of the mechanical stiffness of the extracellular matrix in these processes. This is not intended to devalue the importance of biochemical signaling; in fact, as we discuss, many mechanical stiffness-driven processes not only require cooperation with biochemical cues, but they ultimately converge at common signaling cascades to influence cell and tissue function in an integrative manner. The study of how physical and biochemical signals collectively modulate cell function not only brings forth a more holistic understanding of cell (patho)biology, but it also creates opportunities to control material properties to improve culture platforms for research and drug screening and aid in the rationale design of biomaterials for molecular therapy and tissue engineering applications.

© 2011 Published by Elsevier B.V.

Contents

1. Introduction	0
2. (Patho)physiological tissue stiffness regulates cell (patho)biology <i>in vitro</i>	0
2.1. Tissue-specific elasticity helps maintain the functional phenotype of differentiated cells <i>in vitro</i>	0
2.2. Deviation from normal tissue stiffness undermines normal cell functions and drives pathological transformation <i>in vitro</i>	0
2.3. Matrix stiffness modulates cellular sensitivity to microenvironmental perturbations	0
2.4. Matrix stiffness modulates cellular sensitivity to genetic instabilities and vice versa	0
3. Stiffness regulation of stem cell commitment and pathological differentiation	0
4. Material considerations in the design of drug screening platforms and biomaterials	0
4.1. Modulating substrate stiffness to improve drug screening platforms	0
4.2. Modulating biomaterial stiffness to enhance cellular sensitivity to therapies	0
4.3. Biomaterial considerations for tissue engineering applications	0
5. Conclusions	0
Acknowledgements	0
References	0

Abbreviations: PA, polyacrylamide; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; hBMMSC, human bone marrow mesenchymal stem cell; HCC, hepatocellular carcinoma; kPa, kilopascal; MEC, mammary epithelial cell; MuSC, muscle stem cell; LOX, lysyl oxidase; RGD, arginine–glycine–aspartic acid; ROCK, Rho kinase; SCP, single cell population; TGF- β 1, transforming growth factor- β 1.

[☆] This review is part of the *Advanced Drug Delivery Reviews* theme issue on “From Tissue Engineering to Regenerative Medicine – The Potential and the Pitfalls”.

* Corresponding author. Department of Mechanical and Industrial Engineering, University of Toronto, 5 King’s College Road, Toronto, ON, Canada M5S 3G8. Tel.: +1 416 946 0548; fax: +1 416 978 7753.

E-mail address: c.simmons@utoronto.ca (C.A. Simmons).

0169-409X/\$ – see front matter © 2011 Published by Elsevier B.V.
doi:10.1016/j.addr.2011.01.004

Please cite this article as: W.L.K. Chen, C.A. Simmons, Lessons from (patho)physiological tissue stiffness and their implications for drug screening, drug delivery and regenerative medicine, *Adv. Drug Deliv. Rev.* (2011), doi:10.1016/j.addr.2011.01.004

1. Introduction

Biological tissues display a wide range of mechanical properties, tailored to serve their particular functional and mechanical demands. In particular, tissue elastic modulus (E) – a measure of a tissue's stiffness or more formally, its intrinsic resistance to deform when mechanically stressed – exhibits significant physiological diversity. For example, fat is soft for cushioning vital organs, whereas bone is rigid to protect organs and withstand sizeable mechanical loads. Alterations in tissue stiffness as a result of trauma (scarring), disease or aging have been associated with tissue dysfunction. In particular, soft tissues undergo significant rigidification in many end-stage diseases, including calcific aortic valve disease, atherosclerosis, and liver cirrhosis. It is now recognized that tissue stiffening is not only a phenotypic outcome of many diseases, but may be one of the key contributors to pathological development. It has been postulated that increased tissue rigidity may heighten one's susceptibility to environmental perturbations and/or genetic vulnerabilities [1], in which case the cumulative effect of biochemical, physical and genetic abnormalities reaches a tipping point and instigates disease development. This may explain in part why people with stiffer breast tissue due to inheritance [2], age [3], hormone replacement therapy [3] and/or other causes have a higher incidence of malignancy [2–5], and why the onsets of many diseases are more frequent in the elderly population whose tissues have stiffened with age.

Given the importance of tissue stiffness in maintaining organ function and the strong correlation between tissue rigidity and pathological development, it is notable that polystyrene dishes or cover glass are the predominant culture platforms used in biology. Clearly, these highly rigid substrates ($E \sim 1$ GPa) are poor models for recapitulating the diverse mechanical environment *in vivo*, where most non-mineralized tissue elastic moduli range from roughly 0.01 to 1000 kPa [6,7]. In fact, certain primary cells are known to de-differentiate and lose their functional phenotypes when plated on plastic. This mechanical mismatch between experimental models and the tissue environment of interest had been largely overlooked until recently. In a seminal study, Pelham and Wang pioneered the use of polyacrylamide (PA) hydrogels as flexible substrates for cell growth [8]. The mechanical properties of PA gels can be precisely tuned to recapitulate the wide range of physiological stiffness *in vitro*, thereby enabling the isolation of stiffness as a modulator of cell function under constant biochemical conditions. This technique has since been adapted in a variety of model systems to definitively demonstrate that substrate stiffness alone influences almost every aspect of cell behaviour (e.g., adhesion, spreading, migration, proliferation, and differentiation), in a vast number of cell types, including neurons [9], skeletal myoblasts/smooth muscle cells/cardiomyocytes [10–14], osteoblasts [15,16], chondrocytes [17], hepatocytes [18,19], fibroblasts [20], endothelial cells [21], epithelial cells [1,22,23], and stem/progenitor cells [15,24–31]. Even circulating blood cells such as monocytes and leukocytes, which are anchorage-independent, differentially respond to substrate stiffness during adhesion and migration events [32,33]. For a more comprehensive review of the various model systems used to study substrate/matrix stiffness, readers are referred to the following reviews [7,34,35].

In this review, we draw examples from *in vivo* observations and *in vitro* experimental data to show that knowledge of physiological stiffness¹ and its role in regulating tissue and cell function can: 1) advance fundamental understanding of tissue homeostasis versus

disease; 2) facilitate the identification of therapeutic targets; 3) optimize culture models for research and drug screening applications; and 4) improve biomaterial design for drug delivery and tissue regeneration.

2. (Patho)physiological tissue stiffness regulates cell (patho)biology *in vitro*

2.1. Tissue-specific elasticity helps maintain the functional phenotype of differentiated cells *in vitro*

Some differentiated cells are difficult to study in culture because they quickly lose their characteristic phenotypes and functions when plated on rigid surfaces. In contrast, when cells are cultured on substrates with elasticity resembling that of their extracellular matrix *in vivo*, they are better able to retain their functional phenotypes. For instance, neonatal rat ventricular myocytes develop aligned sarcomeres and generate greater contractile forces on myocardium-like stiffness (10 kPa) [13]. Embryonic cardiomyocytes sustain beating at 1 Hz only on substrates with heart-like elasticity (11 kPa) but quickly lose their rhythmically contractile phenotype on rigid substrates with stiffness close to that of an infarcted fibrotic myocardium (30–70 kPa) [12]. Similarly, myotubes mature and striate preferentially on skeletal muscle-like stiffness (12 kPa) [10]. The influence of matrix mechanics is not only important to mechanically-active cell types like cardiomyocytes and muscle cells, but also in less mechanically active cell types, such as neuronal cells. For example, on compliant substrates resembling soft spinal cord and brain tissues, neuronal branching is significantly enhanced [9]. Taken together, these data demonstrate that substrate stiffness is a key modulator of cell behaviour and deviations from tissue-like elasticity can lead to the loss of differentiated phenotypes and functions *in vitro*. Therefore, it has been postulated that cells *in vivo* respond similarly to tissue rigidity, and deviations from physiological tissue stiffness may cause aberrant cell behaviour and contribute to disease development.

2.2. Deviation from normal tissue stiffness undermines normal cell functions and drives pathological transformation *in vitro*

Large scale change in tissue rigidity via fibrosis and/or calcification is the hallmark of many diseases. However, there is growing evidence to suggest that even subtle increases in tissue rigidity can potentiate disease development. For example, a small but significant increase in rat liver tissue stiffness was detected as early as three days after drug-induced liver fibrosis, which correlated with the onset of myofibroblast activation, a key player in tissue fibrosis [37]. Interestingly, early tissue stiffening was mainly attributed to lysyl oxidase (LOX)-mediated matrix crosslinking as opposed to alterations in matrix deposition, and treatment with a LOX inhibitor partially attenuated the initial increase in liver stiffness, suggesting that matrix mechanics and not matrix composition plays a lead role in early disease development. However, *in vivo* observations like these are correlative, and thus *in vitro* experiments on substrates with tunable mechanical properties have been used to clarify the role of alterations in tissue mechanics in disease development. A number of such studies have demonstrated that elevated substrate stiffness can mediate myofibroblastic activation of hepatic stellate cells and portal fibroblasts, which are thought to be the primary mediators of liver fibrosis [38–40]. Hepatic stellate cells and portal fibroblasts transdifferentiate to myofibroblasts on stiff surfaces mimicking the stiffness of diseased liver (>8–22 kPa) [38–40], but remain quiescent on soft PA or Matrigel™ basement membrane matrix with elasticity close to that of normal rat and human liver ($E < 1.5$ kPa) [38]. In multicellular organs, alterations in mechanical stiffness during disease development not only affect normally quiescent cells such as HSCs, but also functional cells, like hepatocytes. For example, growth of primary and embryonic stem cell-derived hepatocytes on compliant substrates that approximate soft liver tissues promotes liver-specific albumin secretion [18,19], while increasing substrate stiffness induces de-differentiation of hepatocytes

¹ There is currently no consensus regarding the length scale at which cells perceive tissue/matrix rigidity signals. *In vitro* evidence suggests that cells can respond to changes in bulk elastic modulus of hydrogels (macro-scale) as well as the intrinsic modulus of collagen fibers (nano-scale) [36]. In this review, most of the examples discussed assume macro-scale rigidity sensing. In reality, however, cells likely perceive an effective modulus encompassing the macro- to nano-scale.

towards a proliferative phenotype and significantly reduced liver-specific albumin secretion [19]. Therefore, small deviations from physiological-level stiffness can lead to altered cell–matrix interactions in multiple cell types in an organ and have a compounding effect in promoting pathological development. However, knowledge of how stiffness simultaneously affects multiple cell types and their crosstalk in tissue-like environments is severely lacking. This is due to the fact that most of our understanding of matrix stiffness effects comes from two-dimensional (2D) model systems, which are not amenable to studying cellular interactions in a physiologically-relevant, organized, three-dimensional (3D) cell–matrix arrangement. This limitation is widely recognized, and the development of new and improved 3D biomaterials with tunable mechanical properties and topographical features for directing multi-cellular organization is an active area of research. For now, 2D systems remain the most accessible models to isolate cellular response to rigidity and despite their limitations, much has been learned with them.

2.3. Matrix stiffness modulates cellular sensitivity to microenvironmental perturbations

Altering substrate stiffness over a wide range *in vitro* yields rapid and dramatic alterations in cellular phenotypes and functions. In contrast, pathological variation in tissue elasticity during disease can be quite subtle, at least in early stages. It has been suggested that substrate stiffness may not only directly influence cell function, but also modulate cellular sensitivity to other microenvironmental signals. This could be a mechanism by which even small variations in tissue stiffness could manifest in disease propagation by magnifying persistent environmental perturbations (such as chronic inflammation). This is intuitive because many diseases evolve from small changes in biochemical and mechanical signaling, and take years to manifest as significantly detectable phenotypic changes. For example, subtle natural variation in tissue stiffness could increase local susceptibility to disease development and eventually lead to distinct pathological phenotypes in focal regions. An intriguing example is calcific aortic valve disease, in which lesions occur predominantly in the fibrosa layer of the valve leaflet, and not the ventricularis layer. In normal valves, the fibrosa is on average stiffer than the ventricularis, with distinct focal regions that are stiffer than any in the ventricularis [41]. It has been proposed that mechanical heterogeneity of valve tissue could differentially modulate local cellular sensitivity to pathological signals, such as transforming growth factor- β 1 (TGF- β 1), which is involved in fibrosis and is expressed in diseased valves. Indeed, under TGF- β 1 stimulation *in vitro*, valve interstitial cells only become activated myofibroblasts when the stiffness threshold falls within the range of tissue modulus of the fibrosa layer [42].

The interplay between substrate stiffness and TGF- β 1 signaling is not unique to valve disease. In fact, matrix stiffness and TGF- β signaling have been implicated in the fibrotic response in many diseases, including cancer [43]. Stiffness can modulate TGF- β 1 signaling by regulating TGF- β 1 receptor expression, latent TGF- β 1 activation and TGF- β 1 downstream crosstalk. Increasing substrate stiffness has been shown to up-regulate TGF- β 1 receptor transcript levels [44]. Elevated cell contractility on stiff substrates can mechanically release matrix-bound TGF- β 1, making it available for signaling [43,45]. In turn, TGF- β 1 intensifies the myofibroblastic response by stimulating secretion of fibrillar collagens, fibronectin, matrix cross-linkers and autocrine TGF- β [46], which further stiffens the matrix and exacerbates fibrosis in a positive feedback manner. Substrate stiffness can also regulate components of the Wnt signaling pathway, which together with TGF- β 1-induced Smad2/3 signaling synergistically promote myofibroblast differentiation [42]. These mechanisms are not mutually exclusive, but likely overlap to fine-tune signal specificity in various contexts, such as in wound healing or different stages of disease. Given the diversity of TGF- β involvement in

development, tissue homeostasis and disease in virtually every tissue in the body, it is not surprising that the intricate specification of TGF- β 1 function requires coordination from local microenvironmental inputs, with physical cues such as substrate stiffness adding a dimension to the complex TGF- β biochemical signaling network. Such systems-level understanding of signaling, incorporating cell–matrix interactions, is invaluable because it may facilitate the identification of novel therapeutic targets. For example, the diverse physiological functions of TGF- β make systemic inhibition a poor therapeutic option for treating fibrotic diseases [47]. Alternatively, since tension transmitted via integrin/cytoskeleton coupling to the latent TGF- β 1 complex is required for TGF- β 1 activation, interruption of this force transduction mechanism could be used to inhibit matrix release of TGF- β 1, thereby providing a more specific treatment strategy for anti-fibrotic therapy. Inhibition of various integrins ($\alpha_v\beta_5$, β_1 , and $\alpha_v\beta_5$) or integrin-binding sequences has been shown to reduce latent TGF- β 1 activation [43] and mouse mutants lacking $\alpha_v\beta_6$ integrin do not develop fibrosis despite aggressive chronic inflammation [48]. Collectively, these data allude to an indispensable involvement of mechanical tension in TGF- β 1 signaling in myofibroblast differentiation and emphasize the existence of complex mechanical and biochemical cross-talk in disease development.

2.4. Matrix stiffness modulates cellular sensitivity to genetic instabilities and vice versa

Cells in tissues with elevated stiffness are not only more vulnerable to microenvironmental disturbances, but also genetic abnormalities. People with hereditary cancers develop tumors locally, despite the fact that every cell carries the same mutation, suggesting that the local tissue environment is a crucial determinant in oncogenic activation [49]. Conversely, many oncogenes are kept in check and dormant by various protective mechanisms, such as a healthy stromal microenvironment, until triggered into activation later in life [49], most frequently in the older population and people with accumulated tissue damage [3,50,51].

It has been suggested that a tissue environment that supports proper tissue organization and function can serve as a protective barrier against tumor formation [52]. On the other hand, elevated tissue stiffness and contractility can distort tissue structures, compromise tissue function and actively drive malignant transformation of premalignant cells [1]. Normal mammary epithelial cell (MEC) function is tightly controlled by matrix stiffness. The expression of β -casein (a milk protein) was enhanced on PA substrates that resemble the soft mammary tissue environment and was down-regulated on stiff substrates [22]. Elevated stiffness not only affects normal cell function but can also instigate malignant transformation. In a soft matrix with tissue-like elasticity (~167 Pa), MECs developed *in vivo*-like acini, whereas progressive increase in stiffness (400–5000 Pa) enhanced MEC tension generation and resulted in disrupted epithelial polarity, destabilized adherens junctions, and increased integrin clustering and focal adhesion kinase (FAK) activity, all characteristics of a premalignant phenotype [1,23]. Similarly, increasing intracellular contractility by constitutively activating RhoA, a key regulator of cytoskeletal tension, produced a similar disturbed tissue phenotype in soft matrices, alluding to the importance of cytoskeletal tension and mechanotransduction in potentiating cancer development. Notably, epidermal growth factor (EGF)-induced extracellular signal-regulated kinase 2 (ERK2) activity is increased and sustained longer in MECs grown in stiff matrices [23]. Since EGF receptor mutations (overexpression) have been found in a number of cancers, especially breast cancers [1], tension-induced hypersensitivity to EGF in a fibrotic environment can aggravate the frequency of tumor progression. Indeed, overexpression of EGF receptor 2 (ErbB2) in MECs induced malignant transformation only in stiff ribose-crosslinked matrices but not in compliant matrices with normal tissue rigidity [1]. Consistent with these *in vitro* findings, artificially-induced LOX-mediated collagen

crosslinking of the stroma *in vivo* also induced invasive behaviour of oncogene-transformed MECs and the injection of a crosslink inhibitor attenuated tumor progression and reduced tumor severity and frequency [1]. Not surprisingly, elevated levels of LOX have been detected in many types of solid tumors and have been shown to facilitate metastasis [53]. Taken together, these data reinforce the notion that genetic and biophysical factors from the microenvironment cooperatively contribute to cancer progression and strategies to temper chronic fibrotic response and alleviate intracellular tension build-up (by lowering matrix stiffness using crosslink breakers or decreasing intracellular tension using contractility inhibitors) may be effective means to prevent oncogenic activation.

While the mechanical environment can magnify genetic instabilities and activate dormant oncogenes, full-blown mutations in turn could manifest in defective mechanotransduction, which desensitizes cells to their physical environment, allowing oncogenic signals to overcome matrix restraints. For instance, although premalignant cells harbouring the pro-oncogene (ErbB2) sense matrix stiffness and remain non-invasive in soft matrices [1], fully transformed cells appear to have altered mechanosensitivity [23,54,55]. Notably, some cancer cells (PAP2 [54] and T4-2 MECs [23]) can generate high traction forces and spread equally well irrespective of substrate stiffness, indicating a malfunction in mechanotransduction. Consequently, tension-mediated mitotic and apoptotic control was lost in these cells [23,54], reminiscent of the uncontrolled cell growth observed in tumors. These abnormal phenotypes are associated with persistent elevated levels of Rho and ROCK (Rho kinase) activity [23]. Rho GTPases are key regulators of cytoskeletal tension, focal adhesion assembly and migration in normal cells, and their aberrant activities have been implicated in cancer progression [56]. Inhibition of Rho or its signaling partners (integrins, ROCK or ERK) have been shown to reduce proliferation and lead to phenotypic reversion *in vitro* [23,56,57], suggesting that components of the mechanotransduction network can serve as potential molecular targets for pharmacological intervention in cancer therapy.

Tumor cells are heterogeneous and only a subpopulation possesses metastatic potential in late stage tumorigenesis [58–60]. Interestingly, although tumor tissues are stiff and elevated Rho/ROCK activity is required to disrupt cell–cell contacts and facilitate tumor cell invasion to local tissue [56,61], highly metastatic cells are extremely deformable [56,62,63]. This may be because reduced cytoskeletal stiffness is required to enable metastatic cell passage through small capillaries to invade distant tissues and form secondary tumors. However the mechanism by which they acquire such phenotype is unclear. Normal cells are known to remodel their cytoskeleton and adjust their internal stiffness to match the rigidity of the underlying substrate [20]; the fact that metastatic cells develop a highly deformable cytoskeleton despite the rigid tumor environment suggests faulty mechanotransduction, where intracellular tension is decoupled from extracellular matrix stiffness. This is supported by observations that in metastatic colon cancer cells subjected to force application at integrin receptors, strain-induced stiffening of the cytoskeleton was significantly reduced compared to non-metastatic tumor cells, suggestive of compromised integrin–cytoskeleton coupling [64]. Interestingly, although inhibition of the constitutive FAK activity in metastatic cells restored force-induced integrin–cytoskeleton strengthening, defects in spreading and migration were not rescued. It was postulated that the continuous turnover of focal adhesion proteins rather than their absolute expression levels is critical to active mechanotransduction [64]. This dynamic aspect of focal adhesion assembly in normal cell function, especially during spreading and migration, may explain the discrepant findings *in vivo* where both elevated and reduced FAK activities have been associated with cancer progression and metastasis [65–68].

While some cancer cells appear to be irresponsive to substrate stiffness [23,54,64], others exhibit altered mechanosensitivity and differential responses to matrix rigidity, which may relate to their

preferential metastatic targets [55]. For instance, several single cell populations (SCPs) of a metastatic breast cancer cell line (MDA-MB231) with specific affinities for lung (soft) or bone (stiff) tissue exhibited preferential growth on the substrates with elasticity mimicking that of the target organ [55]. Specifically, SCPs with high metastatic potential to the lung had increased proliferation and migration in a compliant environment [55], whereas bone-targeting SCPs had increased proliferation on stiff substrates, while motility was not affected by matrix rigidity. However, SCPs with metastatic potential to both the lung and the bone grew better on soft substrates and showed no stiffness-dependent motility. While the latter seemingly discrepant results are difficult to reconcile, selective invasion and homing of metastatic cells to distant organs seem to rely in part on the microenvironmental conditions (both mechanical and biochemical) of the target tissues which provide motility and growth advantages for invading cells to take root and proliferate. Together, an improved understanding of how the microenvironment couples with genetic factors that are implicated in tissue-specific metastasis [69] may allow for prediction of potential sites of secondary tumor formation and facilitate preventive intervention.

3. Stiffness regulation of stem cell commitment and pathological differentiation

Similar to differentiated cells, stem cell function is tightly regulated by physiological levels of substrate stiffness. Human bone marrow-derived mesenchymal stem cells (hBMMSCs) have been shown to remain quiescent and maintain their multipotency on compliant substrates with marrow-like elasticity [31]. Remarkably, hBMMSCs preferentially differentiate toward neurogenic, myogenic and osteogenic lineages on substrates with elasticity mimicking the tissue modulus of the brain, muscle and precalcified bone, respectively [25]. Inhibition of non-muscle myosin II (a key player in cell contractility) abolished stiffness-dependent differentiation, suggesting a causal link between mechanical tension and stem cell lineage commitment [25]. Similarly, muscle stem cells (MuSCs) have been shown to self-renew and retain their differentiation potential on substrates with muscle-like stiffness (12 kPa) [27]. More importantly, MuSCs expanded on these compliant substrates demonstrated superior homing efficiency to their native satellite cell niche and contributed to myofiber formation when implanted *in vivo* [27]. This study was the first to demonstrate that cells conditioned by the appropriate matrix mechanical cues can result in significant functional improvement in an *in vivo* and clinically relevant context.

It is now recognized that postnatal stem/progenitor cells exist in many adult tissues and they are responsible for tissue homeostasis and tissue repair during injury. However, changes in tissue modulus during aging or disease could contribute to the pathological differentiation of tissue-resident progenitors and/or circulating BMMSCs, thereby perpetuating disease progression. For example, a multipotent progenitor population exists in the aortic heart valve and is thought to participate in tissue calcification and fibrosis in calcific aortic valve disease [70]. *In vitro*, these valve interstitial cells can acquire a calcifying phenotype via osteogenic differentiation, and this process is regulated by substrate stiffness [44]. Similarly, circulating BMMSCs could home to various tissues and contribute to tissue fibrosis via myofibroblast activation. Tail vein injection of BMMSCs into mice with induced liver cirrhosis resulted in enhanced liver fibrosis [71]. Strikingly, 70% of the myofibroblast population and 68% of the hepatic stellate cells (which can differentiate into myofibroblasts) were derived from the gender-mismatch bone marrow transplanted cells [71]. Knowing that liver stiffening occurs early in disease [37] and mechanics is a major driving force in myofibroblastic differentiation [43], it is not surprising that BMMSCs engrafted to a fibrotic liver could actively contribute to added fibrosis. Interestingly, liver cirrhosis is highly correlated with the incidence of hepatocellular

carcinoma (HCC) [51], and 40% of HCC is clonal, suggestive of a stem/progenitor cell origin [72]. Moreover, several studies have demonstrated that MSCs can home to sites of inflammation and tumorigenesis [73]. Is it possible that chronic exposure to a stiff fibrotic environment could induce malignant transformation of tissue-resident or circulating stem cells to become cancer stem cells? These speculations await further exploration.

The understanding of how local mechanical inputs influence stem cell pathological differentiation is not only crucial to understanding disease development but is of practical relevance to regenerative medicine. For instance, direct injection of stem cells into a diseased environment with pathological tissue stiffness could be counter-productive and even dangerous. Injection of BMMSCs into an infarcted heart, with tissue stiffness similar to pre-calcified bone, has been shown to induce MSC osteogenic differentiation and resulted in calcification of the host myocardium [25,74]. Therefore, stem cells by themselves have limited regenerative capacity if the correct microenvironment is not provided. Similarly, tissue engineering constructs for stem cell delivery must have appropriate mechanical and biochemical properties to create a conducive microenvironment that guides stem cell differentiation and at the same time, protects against pathological transformation.

4. Material considerations in the design of drug screening platforms and biomaterials

The understanding of how cells interact with their mechanical environment has practical relevance to the development of drug screening substrates, macromolecule delivery vehicles and tissue engineering scaffolds. The ability to direct cellular response by manipulating the mechanical properties of materials may be more cost-effective than biochemical modifications because physical features are more reproducible and they are arguably longer-lasting, while bioactive components could be quickly degraded or masked by endogenous proteins.

4.1. Modulating substrate stiffness to improve drug screening platforms

Currently, large-scale, high-throughput cell-based screening and validation of therapeutic compounds are carried out almost exclusively on cells cultured on rigid multiple-well plates. As alluded to earlier, this unnaturally rigid substrate environment is not representative of the physiological or pathological tissue environment. In fact, many studies have shown that cellular sensitivity to soluble factors are differentially regulated depending on substrate stiffness, and hypersensitivity on stiff surfaces [75] could contribute to false positives in cell-based screens. However, currently available hydrogel-based elastic substrates are not amenable to scale-up operations due to high cost, laborious fabrication procedures, and mechanical instability and variability (due to swelling). Recently, an alternative technique, originally developed for measuring cell traction forces [76], has been exploited to manipulate substrate elasticity in a new way, which is potentially more suitable for industrial production. In this approach, ECM protein-coated flexible silicone micropillars of varying heights are used to confer substrate elasticity [77,78]. Upon cell adhesion, short pillars that deform less represent a more rigid substrate, while longer pillars, which have more freedom to bend, represent a more elastic substrate. Indeed, hBMMSCs preferentially differentiated into osteoblasts on short pillars (i.e., stiff substrate) and into adipocytes on long pillars (i.e., soft substrate) in constant bipotential media conditions [77]. This result confirmed previous findings suggesting that a soft environment resembling fat tissue is conducive to stem cell adipogenic differentiation, while a stiff environment mimicking precalcified bone could drive osteogenic commitment [28]. The ability to manipulate substrate stiffness by simply varying the geometry of micro-topographical features allows the use of materials which have greater mechanical stability and reproduc-

ibility, could simplify the manufacturing procedure and facilitate industrial adoption. Additionally, it has been suggested that these micropost-laden surfaces can be used as a direct and rapid force readout for screening of drugs that affect cell contractility [79]. Indeed, it is conceivable that the screening of, for example, cardiostimulatory drugs could be performed on multi-well substrates fabricated with arrays of microposts that convey the effective modulus of diseased cardiac tissues and by tracking the deformation of the microposts, a functional output of drug efficacy can be obtained. Although substrates with physiological-relevant elasticity still do not fully capture the complex cellular microenvironment, they could incrementally improve drug screening efficiency by narrowing the list of potential drug candidates for subsequent and more rigorous *in vitro* and *in vivo* validations.

4.2. Modulating biomaterial stiffness to enhance cellular sensitivity to therapies

The effectiveness of therapeutic treatment (e.g., with chemical compounds, proteins, antibodies, DNA or small interfering RNA) is not only dependent on the bioactivity of the molecules but also on the microenvironmental conditions under which the drug is delivered [80]. Biomaterials (used alone or with cells) are an excellent vehicle for molecular therapy, because they provide the opportunity to enhance the drug response of encapsulated or infiltrated cells by modulating local matrix conditions. Of particular interest to this discussion, substrate stiffness has been shown to influence *in vitro* cellular sensitivity to various chemical compounds, antibodies, growth factors and even uptake of plasmid DNA [42,75,81]. Though the clinical significance of these findings remains to be tested, enhancing therapeutic responsiveness via modulating the local cellular environment could alleviate the need for supraphysiological dosing, thereby reducing unwanted side-effects.

4.3. Biomaterial considerations for tissue engineering applications

The ability to engineer specialized biochemical and biophysical niches that predictably control cell function is critical to regenerative medicine and provides insight into fundamental biological processes. Tissue engineering strategies have the potential to incorporate and optimize these niches to predictively guide stem cell function and ultimately generate functional tissues.

Among the extrinsic factors that influence stem cell functions, extracellular matrix signals are especially important because of their potential translation to the rationale design of biomaterials. Matrix stiffness and matrix protein effects are intrinsically linked because cells respond to rigidity by the formation and reinforcement of the ECM-integrin-cytoskeleton association. Binding of different integrins can modulate the strength of this extracellular-to-intracellular coupling and influence force transmission [82,83], thereby imparting control over cell functions. Expectedly, ECM protein identities and densities modulate cellular response to substrate stiffness [29,30]. Early changes (24 h) in spatial and structural organization of the cytoskeleton are predictive of stem cell osteogenic specification [84]. Therefore, by fine-tuning ECM composition and stiffness, it is possible to control cytoskeletal tension generation and optimally drive lineage commitment. The ability to promote proper force transmission is emerging as a new criterion for biomaterial design [6].

Moreover, mechanical conditioning of tissue engineered constructs not only improves mass transport, but may directly affect cell/tissue functions by activating force-sensitive proteins and mechanotransduction pathways. Matrix stiffness can modulate how dynamic forces are transduced at the cellular level [85]. The optimal scaffold stiffness and surface biochemistry in static culture is unlikely the same in dynamic conditions. Therefore, it will be necessary to adopt an empirical approach and systematically screen for large permutations of design parameters, including mechanical, matrix, and biochemical inputs, to

optimize the conditions for regeneration. The need for high-throughput combinatorial screening studies is reflected in the ongoing effort to develop microfabricated platforms for probing cell–microenvironment interactions. With the advances in microfluidic and microelectromechanical technologies, more and more aspects of the cellular microenvironment can be assayed simultaneously [85–94]. The knowledge obtained from these microscale studies may help to illuminate the complexity in microenvironmental control of cell function and facilitate the development of better engineered tissues.

The ultimate goal of tissue engineering is to produce tissue replacements; however, a more immediate contribution of tissue engineering perhaps is the generation of 3D *in vitro* models for research and drug testing. It is well accepted that most cells naturally exist in a 3D environment, and that cellular behaviour differs in 3D versus 2D conditions [95,96]. However, the investigation of matrix stiffness in 3D is limited [14,24,28,97]. This is primarily due to the difficulty of independently changing stiffness over a wide range without concurrently altering other matrix properties, such as ligand density, fiber diameter, or pore size, which may affect cell function directly and/or indirectly by influencing mass transport. Nonetheless, 3D systems now exist, such as RGD-modified alginate hydrogels [28], which allow for modulation of elastic modulus over a wide range (2.5–110 kPa) independent of confounding influences (e.g., ligand density and pore size), thereby enabling the isolation of mechanical effects from biochemical contributions. Interestingly, stiffness-dependent human BMMSC differentiation in 3D was similar to previous 2D findings, with soft matrices promoting adipogenesis and intermediate stiffness favouring osteogenesis, despite altered integrin engagement. It was found that cell–RGD interactions in 3D involved both α_v and α_5 integrin binding, whereas 2D matrix interactions engaged primarily α_v integrins [28]. The surprising consistency in matrix stiffness-regulation of MSC differentiation outputs between 2D and 3D, in spite of differential integrin signaling, suggests that perhaps cells have the ability to compensate for suboptimal matrix environment via redundant pathways. The generation of clinically-relevant *in vitro* models, therefore, may not necessarily require the complete recreation of the physiological niche; rather, the identification of subsets of key microenvironmental cues capable of initiating and complementing the endogenous adaptive cellular program may be sufficient. The search for these key factors will greatly benefit from the development of micro-technologies, quantitative assays and mathematical modeling techniques that allow efficient screening and evaluation of the relative contributions from various culture inputs.

5. Conclusions

Tissue and matrix mechanics plays an important role in pathological development; understanding how altered mechanics affects disease and vice versa is critical to the ultimate goal of regeneration. *In vivo* and *in vitro* data demonstrate that matrix stiffness not only impacts cell functions, but also modulates cellular sensitivity to other microenvironmental and genetic cues to impact common signaling pathways in an integrative fashion. Improved understanding of how matrix mechanics influences cell/tissue function will have direct translational impact in material design for drug screening, macromolecular delivery, and tissue engineering applications.

Acknowledgements

We acknowledge the support of the Canadian Institutes of Health Research (MOP-102721), the Natural Science and Engineering Research Council of Canada (NSERC) (RGPIN 327627-06), and the Heart and Stroke Foundation of Ontario (NA6654). WLK is supported by an NSERC Postgraduate Scholarship (Doctoral) and CAS is supported by the Canada Research Chair in Mechanobiology.

References

- [1] K.R. Levental, H. Yu, L. Kass, J.N. Lakin, M. Egeblad, J.T. Erler, S.F. Fong, K. Csizsar, A. Giaccia, W. Weninger, M. Yamauchi, D.L. Gasser, V.M. Weaver, Matrix crosslinking forces tumor progression by enhancing integrin signaling, *Cell* 139 (2009) 891–906. 621
- [2] N.F. Boyd, G.S. Dite, J. Stone, A. Gunasekara, D.R. English, M.R. McCredie, G.G. Giles, D. Tritchler, A. Chiarelli, M.J. Yaffe, J.L. Hopper, Heritability of mammographic density, a risk factor for breast cancer, *N. Engl. J. Med.* 347 (2002) 886–894. 625
- [3] C.M. Rutter, M.T. Mandelson, M.B. Laya, D.J. Seger, S. Taplin, Changes in breast density associated with initiation, discontinuation, and continuing use of hormone replacement therapy, *Jama* 285 (2001) 171–176. 628
- [4] N. Boyd, L. Martin, J. Stone, C. Greenberg, S. Minkin, M. Yaffe, Mammographic densities as a marker of human breast cancer risk and their use in chemoprevention, *Current Oncology Reports, Current Medicine Group LLC*, 2001, pp. 314–321. 631
- [5] D.T. Butcher, T. Alliston, V.M. Weaver, A tense situation: forcing tumour progression, *Nat. Rev. Cancer* 9 (2009) 108–122. 632
- [6] G.C. Reilly, A.J. Engler, Intrinsic extracellular matrix properties regulate stem cell differentiation, *J. Biomech.* 43 (2009) 55–62. 634
- [7] J.Y. Wong, J.B. Leach, X.Q. Brown, Balance of chemistry, topography, and mechanics at the cell–biomaterial interface: issues and challenges for assessing the role of substrate mechanics on cell response, *Surf. Sci. Biosurf. V Funct. Polym. Surf. Biotechnol.* 570 (2004) 119–130. 639
- [8] R.J. Pelham Jr., Y. Wang, Cell locomotion and focal adhesions are regulated by substrate flexibility, *Proc. Natl. Acad. Sci. USA* 94 (1997) 13661–13665. 641
- [9] L.A. Flanagan, Y.E. Ju, B. Marg, M. Osterfield, P.A. Janmey, Neurite branching on deformable substrates, *NeuroReport* 13 (2002) 2411–2415. 643
- [10] A.J. Engler, M.A. Griffin, S. Sen, C.G. Bonnemann, H.L. Sweeney, D.E. Discher, Myotubes differentiate optimally on substrates with tissue-like stiffness: pathological implications for soft or stiff microenvironments, *J. Cell Biol.* 166 (2004) 877–887. 647
- [11] A. Engler, L. Bacakova, C. Newman, A. Hategan, M. Griffin, D. Discher, Substrate compliance versus ligand density in cell on gel responses, *Biophys. J.* 86 (2004) 617–628. 649
- [12] A.J. Engler, C. Carag-Krieger, C.P. Johnson, M. Raab, H.Y. Tang, D.W. Speicher, J.W. Sanger, J.M. Sanger, D.E. Discher, Embryonic cardiomyocytes beat best on a matrix with heart-like elasticity: scar-like rigidity inhibits beating, *J. Cell Sci.* 121 (2008) 3794–3802. 654
- [13] J.G. Jacot, A.D. McCulloch, J.H. Omens, Substrate stiffness affects the functional maturation of neonatal rat ventricular myocytes, *Biophys. J.* 95 (2008) 3479–3487. 656
- [14] K. Shapira-Schweitzer, D. Seliktar, Matrix stiffness affects spontaneous contraction of cardiomyocytes cultured within a PEGylated fibrinogen biomaterial, *Acta Biomater.* 3 (2007) 33–41. 659
- [15] S.X. Hsiong, P. Carampin, H.J. Kong, K.Y. Lee, D.J. Mooney, Differentiation stage alters matrix control of stem cells, *J. Biomed. Mater. Res. A* 85 (2008) 145–156. 661
- [16] C.B. Khatiwala, S.R. Peyton, M. Metzke, A.J. Putnam, The regulation of osteogenesis by ECM rigidity in MC3T3-E1 cells requires MAPK activation, *J. Cell. Physiol.* 211 (2007) 661–672. 664
- [17] N.G. Genes, J.A. Rowley, D.J. Mooney, L.J. Bonassar, Effect of substrate mechanics on chondrocyte adhesion to modified alginate surfaces, *Arch. Biochem. Biophys.* 422 (2004) 161–167. 667
- [18] L. Li, N. Sharma, U. Chippada, X. Jiang, R. Schloss, M.L. Yarmush, N.A. Langrana, Functional modulation of ES-derived hepatocyte lineage cells via substrate compliance alteration, *Ann. Biomed. Eng.* 36 (2008) 865–876. 670
- [19] E.J. Semler, P.A. Lancin, A. Dasgupta, P.V. Moghe, Engineering hepatocellular morphogenesis and function via ligand-presenting hydrogels with graded mechanical compliance, *Biotechnol. Bioeng.* 89 (2005) 296–307. 673
- [20] J. Solon, I. Levental, K. Sengupta, P.C. Georges, P.A. Janmey, Fibroblast adaptation and stiffness matching to soft elastic substrates, *Biophys. J.* 93 (2007) 4453–4461. 675
- [21] T. Yeung, P.C. Georges, L.A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, P.A. Janmey, Effects of substrate stiffness on cell morphology, cytoskeletal structure, and adhesion, *Cell Motil. Cytoskeleton* 60 (2005) 24–34. 678
- [22] J. Alcaraz, R. Xu, H. Mori, C.M. Nelson, R. Mroue, V.A. Spencer, D. Brownfield, D.C. Radisky, C. Bustamante, M.J. Bissell, Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia, *EMBO J.* 27 (2008) 2829–2838. 682
- [23] M.J. Paszek, N. Zahir, K.R. Johnson, J.N. Lakin, G.I. Rozenberg, A. Gefen, C.A. Reinhart-King, S.S. Margulies, M. Dembo, D. Boettiger, D.A. Hammer, V.M. Weaver, Tensional homeostasis and the malignant phenotype, *Cancer Cell* 8 (2005) 241–254. 686
- [24] A. Banerjee, M. Arha, S. Choudhary, R.S. Ashton, S.R. Bhatia, D.V. Schaffer, R.S. Kane, The influence of hydrogel modulus on the proliferation and differentiation of encapsulated neural stem cells, *Biomaterials* 30 (2009) 4695–4699. 689
- [25] A.J. Engler, S. Sen, H.L. Sweeney, D.E. Discher, Matrix elasticity directs stem cell lineage specification, *Cell* 126 (2006) 677–689. 691
- [26] N.D. Evans, C. Minelli, E. Gentleman, V. LaPointe, S.N. Patankar, M. Kallivretaki, X. Chen, C.J. Roberts, M.M. Stevens, Substrate stiffness affects early differentiation events in embryonic stem cells, *Eur. Cell Mater.* 18 (2009) 1–13, discussion 13–14. 694
- [27] P.M. Gilbert, K.L. Havenstrite, K.E. Magnusson, A. Sacco, N.A. Leonard, P. Kraft, N.K. Nguyen, S. Thrun, M.P. Lutolf, H.M. Blau, Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture, *Science* 329 (2010) 1078–1081. 697
- [28] N. Huebsch, P.R. Arany, A.S. Mao, D. Shvartsman, O.A. Ali, S.A. Bencherif, J. Rivera-Feliciano, D.J. Mooney, Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate, *Nat. Mater.* 9 (2010) 518–526. 700
- [29] A.S. Rowlands, P.A. George, J.J. Cooper-White, Directing osteogenic and myogenic differentiation of MSCs: interplay of stiffness and adhesive ligand presentation, *Am. J. Physiol. Cell Physiol.* 295 (2008) C1037–C1044. 703

- 704 [30] W.L. Chen, M. Likhitanichkul, A. Ho, C.A. Simmons, Integration of statistical
705 modeling and high-content microscopy to systematically investigate cell-
706 substrate interactions, *Biomaterials* 31 (2010) 2489–2497.
- 707 [31] J.P. Winer, P.A. Janmey, M.E. McCormick, M. Funaki, Bone marrow-derived human
708 mesenchymal stem cells become quiescent on soft substrates but remain
709 responsive to chemical or mechanical stimuli, *Tissue Eng. A* 15 (2009) 147–154.
- 710 [32] K.M. Stroka, H. Aranda-Espinoza, Neutrophils display biphasic relationship
711 between migration and substrate stiffness, *Cell Motil. Cytoskeleton* 66 (2009)
712 328–341.
- 713 [33] E.F. Irwin, K. Saha, M. Rosenbluth, L.J. Gamble, D.G. Castner, K.E. Healy, Modulus-
714 dependent macrophage adhesion and behavior, *J. Biomater. Sci. Polym. Ed.* 19
715 (2008) 1363–1382.
- 716 [34] S. Nemir, J.L. West, Synthetic materials in the study of cell response to substrate
717 rigidity, *Ann. Biomed. Eng.* 38 (2010) 2–20.
- 718 [35] C.Y. Yip, J.H. Chen, C.A. Simmons, Engineering substrate mechanics to regulate cell
719 response, in: A. Khademhosseini (Ed.), *Micro- and Nanoengineering of the Cell*
720 *Microenvironment: Technologies and Applications*, Artech House, Boston, London,
721 2008, pp. 161–178.
- 722 [36] P.C. McDaniel, G.A. Shaw, J.T. Elliott, K. Bhadriraju, C. Meuse, K.-H. Chung, A.L.
723 Plant, The stiffness of collagen fibrils influences vascular smooth muscle cell
724 phenotype, *Biophys. J.* 92 (2007) 1759–1769.
- 725 [37] P.C. Georges, J.J. Hui, Z. Gombos, M.E. McCormick, A.Y. Wang, M. Uemura, R. Mick,
726 P.A. Janmey, E.E. Furth, R.G. Wells, Increased stiffness of the rat liver precedes
727 matrix deposition: implications for fibrosis, *Am. J. Physiol. Gastrointest. Liver*
728 *Physiol.* 293 (2007) G1147–G1154.
- 729 [38] R.G. Wells, The role of matrix stiffness in hepatic stellate cell activation and liver
730 fibrosis, *J. Clin. Gastroenterol.* 39 (2005) S158–S161.
- 731 [39] R.G. Wells, The role of matrix stiffness in regulating cell behavior, *Hepatology* 47
732 (2008) 1394–1400.
- 733 [40] Z. Li, J.A. Dranoff, E.P. Chan, M. Uemura, J. Sevigny, R.G. Wells, Transforming
734 growth factor-beta and substrate stiffness regulate portal fibroblast activation in
735 culture, *Hepatology* 46 (2007) 1246–1256.
- 736 [41] R. Zhao, K.L. Sider, C.A. Simmons, Measurement of layer-specific mechanical
737 properties in multilayered biomaterials by micropipette aspiration, *Acta Biomater.*
738 (in press), Corrected Proof.
- 739 [42] J.-H. Chen, W.L.K. Chen, K.L. Sider, C.Y.Y. Yip, C.A. Simmons, {beta}-catenin
740 mediates mechanically regulated, transforming growth factor- β 1-induced
741 myofibroblast differentiation of aortic valve interstitial cells, *Arterioscler. Thromb.*
742 *Vasc. Biol.* (2010), doi:10.1161/ATVBAHA.110.220061, ATVBAHA.110.220061.
- 743 [43] B. Hinz, Tissue stiffness, latent TGF- β 1 activation, and mechanical signal
744 transduction: implications for the pathogenesis and treatment of fibrosis, *Curr.*
745 *Rheumatol. Rep.* 11 (2009) 120–126.
- 746 [44] C.Y. Yip, J.H. Chen, R. Zhao, C.A. Simmons, Calcification by valve interstitial cells is
747 regulated by the stiffness of the extracellular matrix, *Arterioscler. Thromb. Vasc.*
748 *Biol.* (2009).
- 749 [45] P.J. Wipff, D.B. Rifkin, J.J. Meister, B. Hinz, Myofibroblast contraction activates
750 latent TGF- β 1 from the extracellular matrix, *J. Cell Biol.* 179 (2007) 1311–1323.
- 751 [46] M.J. Paszek, V.M. Weaver, The tension mounts: mechanics meets morphogenesis
752 and malignancy, *J. Mammary Gland Biol. Neoplasia* 9 (2004) 325–342.
- 753 [47] J. Varga, B. Pasche, Antitumor growth factor-beta therapy in fibrosis: recent
754 progress and implications for systemic sclerosis, *Curr. Opin. Rheumatol.* 20 (2008)
755 720–728.
- 756 [48] J.S. Munger, X. Huang, H. Kawakatsu, M.J.D. Griffiths, S.L. Dalton, J. Wu, J.-F. Pittet,
757 N. Kaminski, C. Garat, M.A. Matthay, D.B. Rifkin, D. Sheppard, A mechanism for
758 regulating pulmonary inflammation and fibrosis: the integrin α 5 β 1 binds and
759 activates latent TGF β 1, *Cell* 96 (1999) 319–328.
- 760 [49] M.J. Bissell, M.A. Labarge, Context, tissue plasticity, and cancer: are tumor stem
761 cells also regulated by the microenvironment? *Cancer Cell* 7 (2005) 17–23.
- 762 [50] T.W. Jacobs, C. Byrne, G. Colditz, J.L. Connolly, S.J. Schnitt, Radial scars in benign
763 breast-biopsy specimens and the risk of breast cancer, *N. Engl. J. Med.* 340 (1999) 430–436.
- 764 [51] G. Zhao, J. Cui, Q. Qin, J. Zhang, L. Liu, S. Deng, C. Wu, M. Yang, S. Li, C. Wang,
765 Mechanical stiffness of liver tissues in relation to integrin β 1 expression may
766 influence the development of hepatic cirrhosis and hepatocellular carcinoma,
767 *J. Surg. Oncol.* (2010), n/a.
- 768 [52] E. Cukierman, D.E. Bassi, Physico-mechanical aspects of extracellular matrix
769 influences on tumorigenic behaviors, *Semin. Cancer Biol.* 20 (2010) 139–145.
- 770 [53] J.T. Ertler, K.L. Bennewith, T.R. Cox, G. Lang, D. Bird, A. Koong, Q.-T. Le, A.J. Giaccia,
771 Hypoxia-induced lysyl oxidase is a critical mediator of bone marrow cell
772 recruitment to form the premetastatic niche, *Cancer Cell* 15 (2009) 35–44.
- 773 [54] H.B. Wang, M. Dembo, Y.L. Wang, Substrate flexibility regulates growth and
774 apoptosis of normal but not transformed cells, *Am. J. Physiol. Cell Physiol.* 279
775 (2000) C1345–C1350.
- 776 [55] A. Kostic, C.D. Lynch, M.P. Sheetz, Differential matrix rigidity response in breast
777 cancer cell lines correlates with the tissue tropism, *PLoS ONE* 4 (2009) e6361.
- 778 [56] D.E. Jaalouk, J. Lammerding, Mechanotransduction gone awry, *Nat. Rev. Mol. Cell*
779 *Biol.* 10 (2009) 63–73.
- 780 [57] V.M. Weaver, O.W. Petersen, F. Wang, C.A. Larabell, P. Briand, C. Damsky, M.J. Bissell,
781 Reversion of the malignant phenotype of human breast cells in three-dimensional
782 culture and *in vivo* by integrin blocking antibodies, *J. Cell Biol.* 137 (1997) 231–245.
- 783 [58] A.-M. Chioni, R. Grose, Organotypic modelling as a means of investigating
784 epithelial–stromal interactions during tumourigenesis, *Fibrogenesis Tissue Repair*
785 1 (2008) 8.
- 786 [59] I.J. Fidler, The pathogenesis of cancer metastasis: the ‘seed and soil’ hypothesis
787 revisited, *3 (2003) 453–458.*
- 788 [60] C. Wittekind, M. Neid, Cancer invasion and metastasis, *Oncology* 69 (Suppl 1)
789 (2005) 14–16.
- [61] E. Sahai, C.J. Marshall, RHO-GTPases and cancer, *Nat. Rev. Cancer* 2 (2002) 790
133–142. 791
- [62] S.E. Cross, Y.S. Jin, J. Rao, J.K. Gimzewski, Nanomechanical analysis of cells from 792
cancer patients, *Nat. Nanotechnol.* 2 (2007) 780–783. 793
- [63] S. Suresh, Biomechanics and biophysics of cancer cells, *Acta Biomater.* 3 (2007) 794
413–438. 795
- [64] G. von Wichert, D. Krdijia, H. Schmid, G. Haerter, G. Adler, T. Seufferlein, M.P. Sheetz,
796 Focal adhesion kinase mediates defects in the force-dependent reinforcement of
797 initial integrin–cytoskeleton linkages in metastatic colon cancer cell lines, *Eur. J. Cell*
798 *Biol.* 87 (2008) 1–16. 799
- [65] M. Ayaki, K. Komatsu, M. Mukai, K. Murata, M. Kameyama, S. Ishiguro, J. Miyoshi,
800 M. Tatsuta, H. Nakamura, Reduced expression of focal adhesion kinase in liver
801 metastases compared with matched primary human colorectal adenocarcinomas,
802 *Clin. Cancer Res.* 7 (2001) 3106–3112. 803
- [66] L.J. Kornberg, Focal adhesion kinase and its potential involvement in tumor
804 invasion and metastasis, *Head Neck* 20 (1998) 745–752. 805
- [67] B. Gabriel, A. zur Hausen, E. Stickeler, C. Dietz, G. Gitsch, D.C. Fischer, J. Bouda, C.
806 Tempfer, A. Hasenburger, Weak expression of focal adhesion kinase (pp 125FAK) in
807 patients with cervical cancer is associated with poor disease outcome, *Clin. Cancer*
808 *Res.* 12 (2006) 2476–2483. 809
- [68] M. Luo, J.L. Guan, Focal adhesion kinase: a prominent determinant in breast cancer
810 initiation, progression and metastasis, *Cancer Lett.* 289 127–139. 811
- [69] A. Klein, C. Olendrowitz, R. Schmutzler, J. Hampl, P.M. Schlag, N. Maass, N. Arnold,
812 R. Wessel, J. Ramser, A. Meindl, S. Scherneck, S. Seitz, Identification of brain- and
813 bone-specific breast cancer metastasis genes, *Cancer Lett.* 276 (2009) 212–220. 814
- [70] J.H. Chen, C.Y. Yip, E.D. Sone, C.A. Simmons, Identification and characterization of
815 aortic valve mesenchymal progenitor cells with robust osteogenic calcification
816 potential, *Am. J. Pathol.* 174 (2009) 1109–1119. 817
- [71] F.P. Russo, M.R. Alison, B.W. Bigger, E. Amofah, A. Florou, F. Amin, G. Bou-Gharios,
818 R. Jeffery, J.P. Iredale, S.J. Forbes, The bone marrow functionally contributes to liver
819 fibrosis, *Gastroenterology* 130 (2006) 1807–1821. 820
- [72] Z. Yao, L. Mishra, Cancer stem cells and hepatocellular carcinoma, *Cancer Biol.*
821 *Ther.* 8 (2009) 1691–1698. 822
- [73] G. Lazennec, C. Jorgensen, Concise review: adult multipotent stromal cells and
823 cancer: risk or benefit? *Stem Cells* 26 (2008) 1387–1394. 824
- [74] M. Breitbach, T. Bostani, W. Roell, Y. Xia, O. Dewald, J.M. Nygren, J.W.U. Fries, K.
825 Tiemann, H. Bohlen, J. Hescheler, A. Welz, W. Bloch, S.E.W. Jacobsen, B.K.
826 Fleischmann, Potential risks of bone marrow cell transplantation into infarcted
827 hearts, *Blood* 110 (2007) 1362–1369, doi:10.1182/blood-2006-12-063412. 828
- [75] F. Rehfeldt, A.J. Engler, A. Eckhardt, F. Ahmed, D.E. Discher, Cell responses to the
829 mechanochemical microenvironment—implications for regenerative medicine and
830 drug delivery, *Adv. Drug Deliv. Rev.* 59 (2007) 1329–1339. 831
- [76] C.A. Lemmon, N.J. Sniadecki, S.A. Ruiz, J.L. Tan, L.H. Romer, C.S. Chen, Shear force at
832 the cell–matrix interface: enhanced analysis for microfabricated post array
833 detectors, *Mech. Chem. Biosyst.* 2 (2005) 1–16. 834
- [77] J. Fu, Y.K. Wang, M.T. Yang, R.A. Desai, X. Yu, Z. Liu, C.S. Chen, Mechanical
835 regulation of cell function with geometrically modulated elastomeric substrates,
836 *Nat. Methods* 7 733–736. 837
- [78] J.L. Tan, J. Tien, D.M. Pironne, D.S. Gray, K. Bhadriraju, C.S. Chen, Cells lying on a bed
838 of microneedles: an approach to isolate mechanical force, *Proc. Natl. Acad. Sci.*
839 *USA* 100 (2003) 1484–1489. 840
- [79] A. Buxboim, D.E. Discher, Stem cells feel the difference, *7 (2010) 695–697.* 841
- [80] H.J. Kong, D.J. Mooney, Microenvironmental regulation of biomacromolecular
842 therapies, *Nat. Rev. Drug Discov.* 6 (2007) 455–463. 843
- [81] H.J. Kong, J. Liu, K. Riddle, T. Matsumoto, K. Leach, D.J. Mooney, Non-viral gene
844 delivery regulated by stiffness of cell adhesion substrates, *Nat. Mater.* 4 (2005)
845 460–464. 846
- [82] D.E. Ingber, Mechanical signaling and the cellular response to extracellular matrix
847 in angiogenesis and cardiovascular physiology, *Circ. Res.* 91 (2002) 877–887. 848
- [83] N. Wang, J.P. Butler, D.E. Ingber, Mechanotransduction across the cell surface and
849 through the cytoskeleton, *Science* 260 (1993) 1124–1127. 850
- [84] M.D. Treiser, E.H. Yang, S. Gordonov, D.M. Cohen, I.P. Androulakis, J. Kohn, C.S.
851 Chen, P.V. Moghe, Cytoskeleton-based forecasting of stem cell lineage fates, *Proc.*
852 *Natl. Acad. Sci. USA* 107 (2010) 610–615. 853
- [85] C. Moraes, G. Wang, Y. Sun, C.A. Simmons, A microfabricated platform for high-
854 throughput unconfined compression of micropatterned biomaterial arrays, *Biomater-*
855 *ials* 31 (2010) 577–584. 856
- [86] S. Neuss, C. Apel, P. Buttler, B. Denecke, A. Dhanasingh, X. Ding, D. Grafahrend, A.
857 Groger, K. Hemmrich, A. Herr, W. Jahnen-Dechent, S. Mastitskaya, A. Perez-Bouza, S.
858 Rosewick, J. Salber, M. Wolte, M. Zenke, Assessment of stem cell/biomaterial
859 combinations for stem cell-based tissue engineering, *Biomaterials* 29 (2008) 302–313. 860
- [87] Y. Mei, K. Saha, S.R. Bogatyrev, J. Yang, A.L. Hook, Z.I. Kalciglu, S.W. Cho, M.
861 Mitalipova, N. Pyzocha, F. Rojas, K.J. Van Vliet, M.C. Davies, M.R. Alexander, R.
862 Langer, R. Jaenisch, D.G. Anderson, Combinatorial development of biomaterials for
863 clonal growth of human pluripotent stem cells, *Nat. Mater.* 9 (2010) 768–778. 864
- [88] N. Zaari, P. Rajagopalan, S. Kim, A. Engler, J. Wong, Photopolymerization in
865 microfluidic gradient generators: microscale control of substrate compliance to
866 manipulate cell response, *Adv. Mater.* 16 (2004) 2133–2137. 867
- [89] D.G. Anderson, S. Levenberg, R. Langer, Nanoliter-scale synthesis of arrayed
868 biomaterials and application to human embryonic stem cells, *Nat. Biotechnol.* 22
869 (2004) 863–866. 870
- [90] D.G. Anderson, D. Putnam, E.B. Lavik, T.A. Mahmood, R. Langer, Biomaterial
871 microarrays: rapid, microscale screening of polymer–cell interaction, *Biomaterials*
872 26 (2005) 4892–4897. 873
- [91] D.R. Albrecht, V.L. Tsang, R.L. Sah, S.N. Bhatia, Photo- and electropatterning of
874 hydrogel-encapsulated living cell arrays, *Lab Chip* 5 (2005) 111–118. 875

- 876 [92] C.J. Flaim, S. Chien, S.N. Bhatia, An extracellular matrix microarray for probing cellular differentiation, *Nat. Methods* 2 (2005) 119–125. 884
877 885
878 [93] R. Gomez-Sjoberg, A.A. Leyrat, D.M. Pirone, C.S. Chen, S.R. Quake, Versatile, fully automated, microfluidic cell culture system, *Anal. Chem.* 79 (2007) 8557–8563, doi:10.1021/ac071311w. 886
879 887
880 [94] C. Moraes, J.H. Chen, Y. Sun, C.A. Simmons, Microfabricated arrays for high-throughput screening of cellular response to cyclic substrate deformation, *Lab Chip* 10 (2010) 227–234. 888
881 889
882 [95] E. Cukierman, R. Pankov, D.R. Stevens, K.M. Yamada, Taking cell–matrix adhesions to the third dimension, *Science* 294 (2001) 1708–1712. 890
883 890
884 [96] C. Fischbach, R. Chen, T. Matsumoto, T. Schmelzle, J.S. Brugge, P.J. Polverini, D.J. Mooney, Engineering tumors with 3D scaffolds, *Nat. Methods* 4 (2007) 855–860. 887
885 888
886 [97] S.R. Peyton, P.D. Kim, C.M. Ghajar, D. Seliktar, A.J. Putnam, The effects of matrix stiffness and RhoA on the phenotypic plasticity of smooth muscle cells in a 3-D biosynthetic hydrogel system, *Biomaterials* 29 (2008) 2597–2607. 889
887 890

UNCORRECTED PROOF