Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Biotechnology

How cells sense extracellular matrix stiffness: a material's perspective

Britta Trappmann and Christopher S Chen

The mechanical properties of the extracellular matrix (ECM) in which cells reside have emerged as an important regulator of cell fate. While materials based on natural ECM have been used to implicate the role of substrate stiffness for cell fate decisions, it is difficult in these matrices to isolate mechanics from other structural parameters. In contrast, fully synthetic hydrogels offer independent control over physical and adhesive properties. New synthetic materials that also recreate the fibrous structural hierarchy of natural matrices are now being designed to study substrate mechanics in more complex ECMs. This perspective examines the ways in which new materials are being used to advance our understanding of how ECM stiffness impacts cell function.

Addresses
Department of Bioengineering, University of Pennsylvania, PA 19104, USA

Corresponding author: Chen, Christopher S (chrischen@seas.upenn.edu)

Current Opinion in Biotechnology 2013, **24**:xx–yy

This review comes from a themed issue on **Tissue, cell and engineering**

Edited by **Jeffrey A Hubbell** and **Kyongbum Lee**

0958-1669/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.copbio.2013.03.020

Introduction

Complex organisms are composed of tissues in which most cells are embedded within a fibrous extracellular matrix (ECM). The mechanical properties of this matrix not only allow such tissues to withstand daily stresses, but also regulate numerous cellular functions such as spreading, migration, proliferation and stem cell differentiation [1], thus impacting many fundamental biological processes including embryonic development, adult tissue homeostasis, and the pathogenesis of diseases such as fibrosis and cancer [2–5].

Although a large body of data loosely suggests that matrix mechanics is an important factor in driving cellular behavior, there are several limitations to each of the approaches taken to study this phenomenon. In particular, because changes in matrix stiffness often occur simultaneously with changes in other material properties, for example, surface chemistry, topography or availability of adhesive ligands, it is difficult to convincingly demonstrate that stiffness alone is responsible for the observed effects. Here, we will attempt to describe the various methods that have been used to modulate matrix mechanics, the biological responses that have been reported, and the potential mechanisms by which these responses occur.

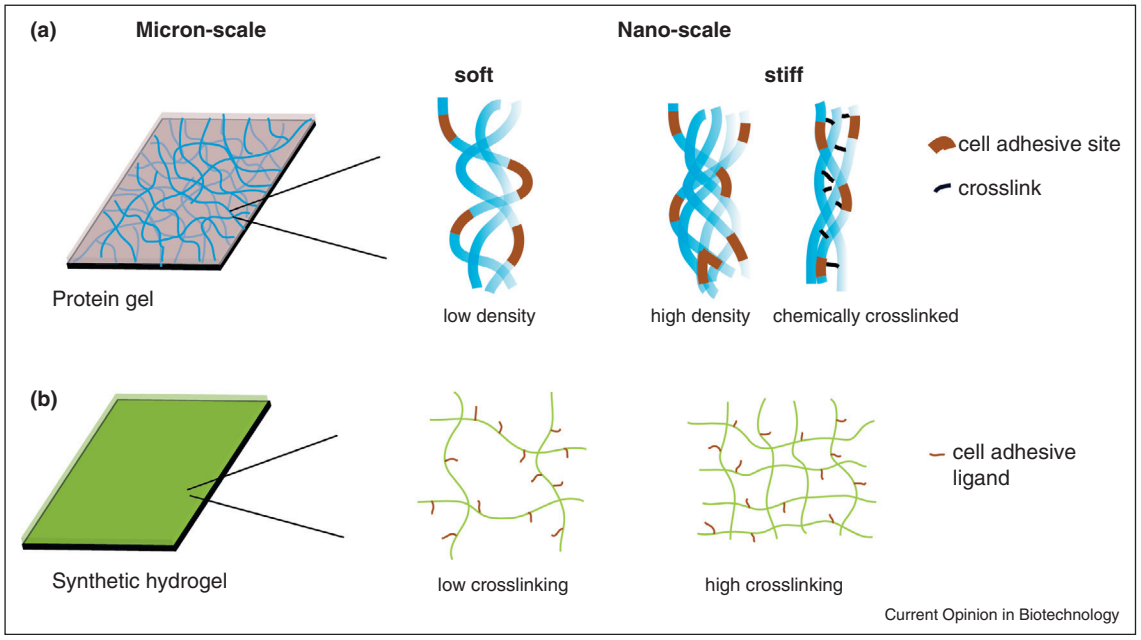
Protein gels as mimics of natural ECM

Gels based on natural ECMs, such as type I collagen, Matrigel and fibrin are composed of proteins that self-assemble *in vitro* into higher order nanofibrous structures, which reasonably mimic many *in vivo* settings, and were the first materials used to suggest an impact of stiffness on cell fate. Studies in collagen and fibrin gels demonstrated that increasing crosslinking of matrix, which modulates matrix stiffness, impacts integrin signaling and actomyosin-mediated cellular tension, important parameters in tumor growth [6,7,8•]. Differentiation and proliferation of normal cells are also regulated by these ECM protein gels. For example, mammary epithelial cells cultured on soft, as opposed to stiff, gels mimicking normal tissue stiffness maintained expression of β-casein, a milk protein used as functional differentiation marker for mammary epithelial cells [9].

Despite the clear evidence that matrix manipulations appear to impact cell function, it is difficult in these systems to unequivocally isolate the contribution of substrate stiffness from other important structural changes in the matrix (Figure 1a). In most studies, gel stiffness is varied by changing protein weight percentage in the gel precursor solution, making orthogonal control over mechanics and ligand density impossible. Importantly, ligand density alone (which can be varied simply by coating ECM onto rigid surfaces) is known to influence integrin dependent signaling [10]. Hence, if we are to understand the contribution of stiffness per se, it is necessary to decouple the two parameters. Recent approaches to control natural ECM stiffness have used non-enzymatic collagen glycation to obtain scaffolds with compressive moduli ranging from 175 to 730 Pa, without changing overall collagen density [11]. Although this is a promising approach, the obtainable stiffness range is inadequate compared to the relevant *in vivo* range spanning hundreds of kPa, and the chemical composition of the material changes for different stiffnesses as proteins are crosslinked by varying concentrations of ribose. Other chemical crosslinking methods including glutaraldehyde, carbodiimide or hexamethylene diisocyanate

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020

Figure 1



Structural features of protein gels **(a)** and synthetic hydrogels **(b)** at the micron-scale and nano-scale. Substrate mechanics influence matrix density, ligand availability (a) and hydrogel pore size (b).

treatment of collagen [12] and more recently, photochemically induced fibrin crosslinking [13], also yield limited stiffness ranges. Furthermore, many of these crosslinking agents target primary amines and carboxylates on the matrix backbone, which also contain specific adhesion receptor binding sequences. Thus, such crosslinking agents may also directly alter adhesion ligand availability.

Fully synthetic hydrogels

To address these limitations in natural ECMs, fully synthetic, covalently crosslinked hydrogels with tunable stiffness and orthogonal control over adhesive ligand density have been developed as new model ECMs. Unlike the majority of natural fibrous materials, synthetic gel stiffness is generally not affected by deformation magnitude or rate, and the gels are therefore considered linear elastic and defined by a single bulk modulus. Studies of cells cultured on the surface of matrix-coated polyacrylamide hydrogels have revealed numerous effects of substrate stiffness on cells. Stiffness modulates the speed of migration of cultured cells, with lower motility on stiff substrates correlating with increased focal adhesion formation [14]. Substrates patterned with stiffness gradients revealed preferential movement of cells from soft toward stiff regions, a process called durotaxis [15]. Finally, Engler *et al.* demonstrated that substrate mechanics can direct lineage fate in human mesenchymal stem cells (hMSCs) [16]. The stiffness that optimally drove specific lineages (e.g. myoblasts) corresponded with the stiffness of the relevant target tissue (e.g. skeletal muscle).

To study multiple parameters governing cell–ECM interactions individually, poly(ethylene glycol) (PEG) hydrogels with tunable mechanics and incorporated functionalities (tethered ECM ligands and growth factors) mimicking natural ECM properties have been designed [17,18]. Unlike polyacrylamide, the unpolymerized components of these gels are cytocompatible. Therefore, such tailored hydrogel systems can also be used to encapsulate cells and study stiffness in three dimensions (3D) [19,20]. Other common synthetic materials used are based on sugars, such as hyaluronan, dextran or alginate [21,22,23•]. Because of the abundance of available functional groups along the polymer backbone, sugars offer more flexibility than PEG in terms of chemical modifications with crosslinking moieties or bioactive molecules. These materials are just now allowing researchers to examine the relative contributions of ECM stiffness versus density of adhesive ligands.

Hydrogels have attracted a lot of attention as fully synthetic model substrates, partially due to their porous nature ensuring the supply of nutrients, growth factors, and oxygen to the cell, thus mimicking the structure of natural tissues. However, one major drawback is that porosity changes with variations in material stiffness (Figure 1b), impairing, for example, the diffusion of small molecules in stiff matrices [24]. Additionally, these

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020

synthetic matrices only recapitulate some of the properties of *in vivo* ECM. In many two dimensional (2D) studies, cells adhere to an ECM coating bound to the hydrogel surface and hence, sense an integrated stiffness dictated by the mechanics of the material itself, but also the mechanical properties of the ECM protein film (which often times is made of fibrous units with heterogeneous stiffness distributions), and how that ECM coating is tethered to the underlying substrate. The importance of ligand presentation has been demonstrated for both growth factor and integrin-mediated signaling [25,26]. For example, cells cultured on rigid surfaces functionalized with RGD via PEG linkers of different length showed a decrease of cell–substrate interactions for longer spacers [27,28], indicating that the mechanical feedback of the adhesive ligand is crucial for integrin-dependent signaling. In synthetic hydrogels, ECM tethering is greatly influenced by the pore size, which changes as hydrogel stiffness is varied. A recent study has shown that cells cultured on polyacrylamide hydrogels with covalently anchored collagen were influenced by the tethering mechanics of collagen. Stiffer hydrogels with smaller pore sizes offer more anchoring points for each collagen fibril, leading to increased mechanical feedback at each cell adhesion [29•]. The nanotopography of surfaces, as well as the arrangement of adhesive ligands have previously been shown to influence cell fate [30,31], and thus changes in gel crosslinking appear to impact cell function in part through their indirect effects on matrix tethering.

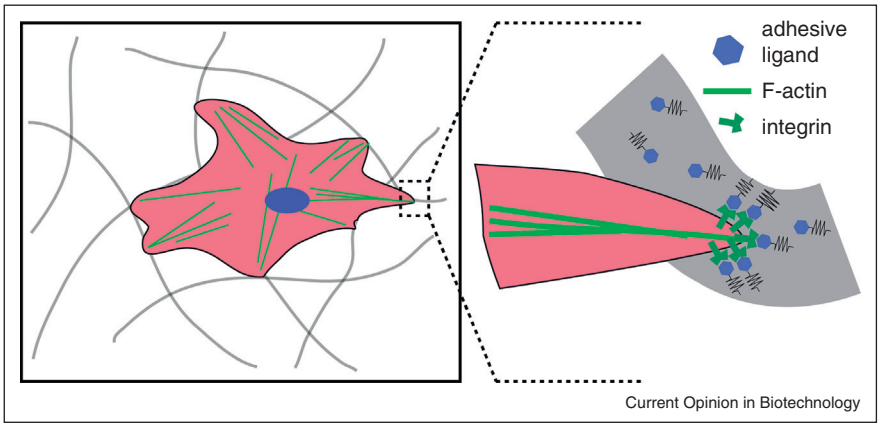
These complex mechanisms by which cells could be transducing changes in matrix structure and mechanics underscore a need to decouple mechanical properties from ligand presentation in hydrogel systems. To address this, Spatz and co-workers have developed gold nanoparticle embedded PEG hydrogel substrates with tunable stiffness and independent control over biomolecule arrangement on the nanometer scale [32], providing a platform to study mechanotransduction events and adhesion-dependent signaling independently. Additionally, our group has developed poly(dimethylsiloxane) (PDMS) elastomer substrates with vertically arrayed microposts that bend laterally when a cell attaches and exerts force to the substrate [33,34••]. The micropost stiffness is tuned solely by the height of the post, while keeping adhesive and other material bulk and surface properties constant. While these systems provide a means to show that stiffness can modulate cell function, other materials approaches will be needed to advance these studies into more complex settings, such as 3D culture.

The described studies with model matrices demonstrate that bulk modulus is only one of many physical parameters that can impact how cells sense the ECM. *In vivo*, cells are exposed to more complex hierarchical fibrous matrices that introduce additional considerations for stiffness sensing. In contrast to uniform and mechanically isotropic hydrogels, fibrous ECM varies in structure and mechanics at the nano-scale, cell-scale and bulk scale, suggesting that signals from different cellular sensing mechanisms may be integrated to evoke a functional output.

Designer ECMs

To gain a deeper mechanistic understanding of cellular stiffness sensing, the field has begun to explore materials which combine the complex physical features of natural matrices (Figure 2) with the tunability of synthetic matrices, which offer full and independent control over mechanical as well as various adhesive properties. Recently developed peptide hydrogels which undergo multi-hierarchical self-assembly [35,36,37•], hold great promise for such applications. Gelain *et al.* have designed 3D self-assembling peptide nanofiber scaffolds with incorporated functional motifs for adhesion, differentiation and bone marrow homing. Adult mouse neural stem cells displayed

Figure 2



Hierarchical structure of 3D fibrous matrices, leading to different levels of cellular stiffness sensing. Focal adhesion formation is impacted by the micron-scale (left), and integrin-mediated signaling affected by the nano-scale (right).

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020

enhanced survival without extra soluble factors [38], demonstrating the biocompatibility of such materials. In order to allow for stiffness control, Stupp and co-workers have developed peptide amphiphiles with systematically modified amino acid sequences self-assembling into nanofiber hydrogels of varying mechanical properties. By varying the number of valines and alanines present, matrix stiffness was changed [39]. However, with this approach changes in mechanics correlate with changes in the chemical structure of the building blocks, influencing peptide folding and possibly availability of incorporated biofunctional ligands. A recent report describes the design of a PEG hydrogel system with incorporated collagen mimetic peptide sequences which undergo triple helical assembly, thus serving as crosslinking sites [40]. Such a composite material offers the mechanical flexibility of synthetic PEG matrices, while adopting the nanofibrous structure of natural ECMs. Additionally, crosslinks are thermally reversible, potentially leading to materials with dynamically tunable stiffness.

Indeed, the availability of dynamic materials which soften or stiffen over time, via external stimuli or cell-mediated processes, enables the study of stiffness sensing in a more natural setting, as *in situ* changes in matrix mechanics are known to guide tissue development [41]. Matrix metalloproteinase-sensitive hydrogels allow for cell-mediated matrix cleavage and active cellular remodeling, rendering the material invasive and supportive of multicellular network formation [42–44]. In order to precisely control mechanical properties by external stimuli in the presence of cells, PEG-based photodegradable hydrogels undergoing local changes of network crosslink density upon irradiation have been developed [45••]. Cells embedded within the hydrogel initially adopted a rounded morphology, but started to spread after irradiation and gel degradation. In subsequent studies, the team showed that valvular interstitial cell (VIC) activation into myofibroblasts, which only occurs on stiff substrates, can be reversed upon softening of the substrate [46]. Taking a different approach, Burdick and co-workers have fabricated methacrylate-functionalized hyaluronic acid hydrogels which are crosslinked in two sequential steps [47•]. First, soft hydrogel networks are prepared using dithiothreitol as crosslinker, and variable times after cell seeding, hydrogels are further crosslinked by light. Differentiation of hMSCs into adipocytes and osteoblasts was determined by the pre-culture period on the surface of soft hydrogels. Osteogenic differentiation was favored when gels were stiffened earlier, in contrast to gels never stiffened or stiffened later which encouraged adipogenesis, demonstrating a platform to guide stem cell fate based on dynamic changes in matrix elasticity. In a followup study, HA hydrogels with dynamically tunable degradation properties have been used to interrogate the mechanism underlying hMSC fate decisions in 3D environments [48]. Cell encapsulation within gels permissive or restrictive to cell-mediated degradation exhibited high and low degrees of cell spreading, correlating with high and low traction forces, resulting in favored osteogenesis versus adipogenesis, respectively. Delayed secondary covalent crosslinking after the initial cell spreading phase switched the matrix from degradable to restrictive and locked cells in a spread state, while still promoting adipogenesis. This example shows that changes in crosslinking modulate material degradability as well as 3D matrix stiffness, and that degradability effects cell fate independent of stiffness, illustrating the utility of dynamic materials to study the interplay between such parameters and cellular responses.

Conclusions and future directions

Despite the wealth of literature studying cell behavior on hydrogel substrates with tunable stiffness, it is only now becoming appreciated that even the most sophisticated materials approaches to modulate stiffness also influence other physicochemical factors that themselves could impact cell function. Thus, additional challenges remain to decipher the true contribution of stiffness versus other materials properties that could be used to engineer cell behaviors. Simultaneously, an understanding of cellular mechanotransduction events in the more complex hierarchical materials of natural tissues is still lacking. In particular, future efforts will have to focus on studying how stiffness sensing occurs on different scales, as differing mechanics at various size scales is a feature intrinsic to the heterogeneous and anisotropic structure of *in vivo* ECM (Figure 2). With the recent development of new materials capable of structural control at the nanometer, micrometer, and bulk level, stiffness at the single integrin, focal adhesion and inter-focal adhesion scale can be tuned, potentially leading to a full hierarchical understanding of the molecular stiffness sensing process. With increasing structural complexity, it will also be important to implement molecular sensors as a feedback tool linking changes in matrix structure or conformation and intracellular response (e.g. FRET-based sensor for matrix protein folding upon cellular ECM remodeling [49]), allowing observers to follow dynamic mechanotransduction events in real time. Finally, to gain deeper insight into the role of stiffness sensing for tissue function, studies will have to probe the impact of stiffness in a broader context by incorporating additional physical and chemical factors which will likely lead to enhanced cell responses due to synergistic effects (e.g. [50]). A full study of the mechanical parameters governing cell–ECM interactions will enable the design of improved materials for tissue engineering applications, and will provide novel model systems to decipher how matrix mechanics control normal and disease physiological processes.

Acknowledgements

The authors thank Brendon Baker, Michele Wozniak and Jason Burdick for thoughtful discussions and suggestions. This work was supported in part by grants from the National Institutes of Health (EB00262, EB08396, GM74048).

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Discher DE, Janmey P, Wang YL: **Tissue cells feel and respond to the stiffness of their substrate**. *Science* 2005, **310**:1139-1143.

2. Wozniak MA, Chen CS: **Mechanotransduction in development: a growing role for contractility**. *Nat Rev Mol Cell Biol* 2009, **10**:34-43.

3. Paszek MJ, Weaver VM: **The tension mounts: mechanics meets morphogenesis and malignancy**. *J Mammary Gland Biol Neoplasia* 2004, **9**:325-342.

4. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, Reinhart-King CA, Margulies SS, Dembo M, Boettiger D *et al.*: **Tensional homeostasis and the malignant phenotype**. *Cancer Cell* 2005, **8**:241-254.

5. Georges PC, Hui JJ, Gombos Z, McCormick ME, Wang AY, Uemura M, Mick R, Janmey PA, Furth EE, Wells RG: **Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis**. *Am J Physiol Gastrointest Liver Physiol* 2007, **293**:G1147-G1154.

6. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, Fong SF, Csiszar K, Giaccia A, Weninger W *et al.*: **Matrix crosslinking forces tumor progression by enhancing integrin signaling**. *Cell* 2009, **139**:891-906.

7. Samuel MS, Lopez JI, McGhee EJ, Croft DR, Strachan D, Timpson P, Munro J, Schroder E, Zhou J, Brunton VG *et al.*: **Actomyosin-mediated cellular tension drives increased tissue stiffness and beta-catenin activation to induce epidermal hyperplasia and tumor growth**. *Cancer Cell* 2011, **19**:776-791.

8. • Liu J, Tan Y, Zhang H, Zhang Y, Xu P, Chen J, Poh YC, Tang K, Wang N, Huang B: **Soft fibrin gels promote selection and growth of tumorigenic cells**. *Nat Mater* 2012, **11**:734-741.
Tumorigenic cells are selected by culturing single cancer cells in soft fibrin matrices.

9. Alcaraz J, Xu R, Mori H, Nelson CM, Mroue R, Spencer VA, Brownfield D, Radisky DC, Bustamante C, Bissell MJ: **Laminin and biomimetic extracellular elasticity enhance functional differentiation in mammary epithelia**. *EMBO J* 2008, **27**:2829-2838.

10. Ingber DE: **Fibronectin controls capillary endothelial cell growth by modulating cell shape**. *Proc Natl Acad Sci USA* 1990, **87**:3579-3583.

11. Mason BN, Starchenko A, Williams RM, Bonassar LJ, Reinhart-King CA: **Tuning three-dimensional collagen matrix stiffness independently of collagen concentration modulates endothelial cell behavior**. *Acta Biomater* 2013, **9**:4635-4644.

12. Rault I, Frei V, Herbage D, AbdulMalak N, Huc A: **Evaluation of different chemical methods for cross-linking collagen gel, films and sponges**. *J Mater Sci Mater Med* 1996, **7**:215-221.

13. Syedain ZH, Bjork J, Sando L, Tranquillo RT: **Controlled compaction with ruthenium-catalyzed photochemical cross-linking of fibrin-based engineered connective tissue**. *Biomaterials* 2009, **30**:6695-6701.

14. Pelham RJ Jr, Wang Y: **Cell locomotion and focal adhesions are regulated by substrate flexibility**. *Proc Natl Acad Sci USA* 1997, **94**:13661-13665.

15. Lo CM, Wang HB, Dembo M, Wang YL: **Cell movement is guided by the rigidity of the substrate**. *Biophys J* 2000, **79**:144-152.

16. Engler AJ, Sen S, Sweeney HL, Discher DE: **Matrix elasticity directs stem cell lineage specification**. *Cell* 2006, **126**:677-689.

17. Mann BK, West JL: **Cell adhesion peptides alter smooth muscle cell adhesion, proliferation, migration, and matrix protein synthesis on modified surfaces and in polymer scaffolds**. *J Biomed Mater Res* 2002, **60**:86-93.

18. Hern DL, Hubbell JA: **Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing**. *J Biomed Mater Res* 1998, **39**:266-276.

19. Burdick JA, Anseth KS: **Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering**. *Biomaterials* 2002, **23**:4315-4323.

20. Mann BK, Gobin AS, Tsai AT, Schmedlen RH, West JL: **Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering**. *Biomaterials* 2001, **22**:3045-3051.

21. Burdick JA, Prestwich GD: **Hyaluronic acid hydrogels for biomedical applications**. *Adv Mater* 2011, **23**:H41-H56.

22. Levesque SG, Shoichet MS: **Synthesis of enzyme-degradable, peptide-cross-linked dextran hydrogels**. *Bioconjug Chem* 2007, **18**:874-885.

23. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, • Bencherif SA, Rivera-Feliciano J, Mooney DJ: **Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate**. *Nat Mater* 2010, **9**:518-526.
Studies cellular stiffness sensing in 3D hydrogels.

24. Lu S, Ramirez WF, Anseth KS: **Photopolymerized, multilaminated matrix devices with optimized nonuniform initial concentration profiles to control drug release**. *J Pharm Sci* 2000, **89**:45-51.

25. Alberti K, Davey RE, Onishi K, George S, Salchert K, Seib FP, Bornhauser M, Pompe T, Nagy A, Werner C *et al.*: **Functional immobilization of signaling proteins enables control of stem cell fate**. *Nat Methods* 2008, **5**:645-650.

26. Fan VH, Tamama K, Au A, Littrell R, Richardson LB, Wright JW, Wells A, Griffith LG: **Tethered epidermal growth factor provides a survival advantage to mesenchymal stem cells**. *Stem Cells* 2007, **25**:1241-1251.

27. Beer JH, Springer KT, Coller BS: **Immobilized Arg-Gly-Asp (RGD) peptides of varying lengths as structural probes of the platelet glycoprotein IIb/IIIa receptor**. *Blood* 1992, **79**:117-128.

28. Houseman BT, Mrksich M: **The microenvironment of immobilized Arg-Gly-Asp peptides is an important determinant of cell adhesion**. *Biomaterials* 2001, **22**:943-955.

29. Trappmann B, Gautrot JE, Connelly JT, Strange DG, Li Y, • Oyen ML, Cohen Stuart MA, Boehm H, Li B, Vogel V *et al.*: **Extracellular-matrix tethering regulates stem-cell fate**. *Nat Mater* 2012, **11**:642-649.
Tethering of ECM proteins to polyacrylamide hydrogels impacts on epidermal and mesenchymal stem cell differentiation.

30. Dalby MJ, Gadegaard N, Tare R, Andar A, Riehle MO, Herzyk P, Wilkinson CD, Oreffo RO: **The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder**. *Nat Mater* 2007, **6**:997-1003.

31. Selhuber-Unkel C, Lopez-Garcia M, Kessler H, Spatz JP: **Cooperativity in adhesion cluster formation during initial cell adhesion**. *Biophys J* 2008, **95**:5424-5431.

32. Aydin D, Louban I, Perschmann N, Blummel J, Lohmuller T, Cavalcanti-Adam EA, Haas TL, Walczak H, Kessler H, Fiammengo R *et al.*: **Polymeric substrates with tunable elasticity and nanoscopically controlled biomolecule presentation**. *Langmuir* 2010, **26**:15472-15480.

33. Tan JL, Tien J, Pirone DM, Gray DS, Bhadriraju K, Chen CS: **Cells lying on a bed of microneedles: an approach to isolate mechanical force**. *Proc Natl Acad Sci USA* 2003, **100**:1484-1489.

34. Fu J, Wang YK, Yang MT, Desai RA, Yu X, Liu Z, Chen CS: •• **Mechanical regulation of cell function with geometrically modulated elastomeric substrates**. *Nat Methods* 2010, **7**:733-736.
Elastomeric micropost arrays as an alternative tool to measure cell mechanical forces.

35. Nowak AP, Breedveld V, Pakstis L, Ozbas B, Pine DJ, Pochan D, Deming TJ: **Rapidly recovering hydrogel scaffolds from**

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020

self-assembling diblock copolypeptide amphiphiles. *Nature* 2002, **417**:424-428.

36. Shen W, Kornfield JA, Tirrell DA: **Structure and mechanical properties of artificial protein hydrogels assembled through aggregation of leucine zipper peptide domains**. *Soft Matter* 2007, **3**:99-107.

37. • O'Leary LE, Fallas JA, Bakota EL, Kang MK, Hartgerink JD: **Multi-hierarchical self-assembly of a collagen mimetic peptide from triple helix to nanofibre and hydrogel**. *Nat Chem* 2011, **3**: 821-828.
Synthetic peptide mimicking self-assembly and structural hierarchy of collagen.

38. Gelain F, Bottai D, Vescovi A, Zhang SG: **Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures**. *PLoS ONE* 2006, **1**:e119.

39. Pashuck ET, Cui HG, Stupp SI: **Tuning supramolecular rigidity of peptide fibers through molecular structure**. *J Am Chem Soc* 2010, **132**:6041-6046.

40. Stahl PJ, Romano NH, Wirtz D, Yu SM: **PEG-based hydrogels with collagen mimetic peptide-mediated and tunable physical cross-links**. *Biomacromolecules* 2010, **11**:2336-2344.

41. Mammoto T, Ingber DE: **Mechanical control of tissue and organ development**. *Development* 2010, **137**:1407-1420.

42. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA: **Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics**. *Proc Natl Acad Sci USA* 2003, **100**:5413-5418.

43. Lutolf MP, Raeber GP, Zisch AH, Tirelli N, Hubbell JA: **Cell-responsive synthetic hydrogels**. *Adv Mater* 2003, **15**:888-892.

44. Miller JS, Shen CJ, Legant WR, Baranski JD, Blakely BL, Chen CS: **Bioactive hydrogels made from step-growth derived PEG-peptide macromers**. *Biomaterials* 2010, **31**:3736-3743.

45. •• Kloxin AM, Kasko AM, Salinas CN, Anseth KS: **Photodegradable hydrogels for dynamic tuning of physical and chemical properties**. *Science* 2009, **324**:59-63.
PEG-based photodegradable hydrogels to control matrix mechanics in the presence of cells.

46. Kloxin AM, Benton JA, Anseth KS: **In situ elasticity modulation with dynamic substrates to direct cell phenotype**. *Biomaterials* 2010, **31**:1-8.

47. • Guvendiren M, Burdick JA: **Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics**. *Nat Commun* 2012, **3**:792.
Photochemically induced matrix stiffening in the presence of cells.

48. Khetan S, Guvendiren M, Legant WR, Cohen DM, Chen CS, Burdick JA: **Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels**. *Nat Mater* 2013 http://dx.doi.org/10.1038/nmat3586.

49. Little WC, Smith ML, Ebneter U, Vogel V: **Assay to mechanically tune and optically probe fibrillar fibronectin conformations from fully relaxed to breakage**. *Matrix Biol* 2008, **27**:451-461.

50. Schwartz MA, Ginsberg MH: **Networks and crosstalk: integrin signalling spreads**. *Nat Cell Biol* 2002, **4**:E65-E68.

Please cite this article in press as: Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective, Curr Opin Biotechnol (2013), http://dx.doi.org/10.1016/j.copbio.2013.03.020