REVIEW

Bridging the Gap: From 2D Cell Culture to 3D Microengineered Extracellular Matrices

Yanfen Li and Kristopher A. Kilian*

Historically the culture of mammalian cells in the laboratory has been performed on planar substrates with media cocktails that are optimized to maintain phenotype. However, it is becoming increasingly clear that much of biology discerned from 2D studies does not translate well to the 3D microenvironment. Over the last several decades, 2D and 3D microengineering approaches have been developed that better recapitulate the complex architecture and properties of in vivo tissue. Inspired by the infrastructure of the microelectronics industry, lithographic patterning approaches have taken center stage because of the ease in which cell-sized features can be engineered on surfaces and within a broad range of biocompatible materials. Patterning and templating techniques enable precise control over extracellular matrix properties including: composition, mechanics, geometry, cell–cell contact, and diffusion. In this review article we explore how the field of engineered extracellular matrices has evolved with the development of new hydrogel chemistry and the maturation of micro- and nano- fabrication. Guided by the spatiotemporal regulation of cell state in developing tissues, techniques for micropatterning in 2D, pseudo-3D systems, and patterning within 3D hydrogels will be discussed in the context of translating the information gained from 2D systems to synthetic engineered 3D tissues.

1. Introduction

Nature has developed intricate processes in which the form and function of tissues arise in multicellular organisms. Starting from a single cell, a complex array of biophysical and biochemical cues guide the segregation of our earliest progenitors into distinct germ layers that ultimately develop into the multitude of specialized cells of the adult organism. This process is regulated by many extrinsic and intrinsic factors and central to these processes is a complex orchestration between the composition of the surrounding extracellular matrix (ECM), its viscoelastic properties, spatiotemporal gradients of soluble factors, and interactions with neighboring cells. The interplay of these parameters influence cell state, function, and coordinated

assembly to precisely control tissue formation. Understanding the context in which the ECM and its cellular constituents coordinate to establish complex architectures and build functioning tissue is of great importance in developmental biology, but is also necessary in the design of materials for medicine. Here we will explore the progress and promise of engineered materials to control cellular outcomes in vitro, from new assays for cell biology to complex 3D materials that recapitulate the function of tissues (**Figure 1**).

The ECM is the noncellular component of tissues and is comprised of a combination of polysaccharides, growth factors, and proteins including collagen, fibronectin, laminin, and elastin. The ECM guides a host of cell and tissue level functions including regulation of cellular architecture,[1] directing tissue-specific stem cell specification,[2,3] guiding cell migration,[4] maintaining homeostasis,[5] and influencing tissue development,[6–9] including controlling branching morphogenesis.[10,11] One major function of the ECM is to regulate cell shape and its connectivity to surrounding cells which in turn regulates the cellular epigenetic state, gene expression, and function.[6,12] While all the cells arise from a single fertilized egg, major morphological and functional differences can be seen in different tissue types. For instance, neurons have small bodies with long axons to transfer electric signals over long distances, myocytes are long and tubular with contracting myofibrils with a shape that is optimized for generating force along the direction of the cell, and adipocytes are round with large vacuoles which is optimum for lipid storage[13–15] (**Figure 2**).

The shape of a cell is intimately connected to its function and it is acknowledged that cell shape is due in part to ECM mechanics and composition.[16–19] For example, during pregnancy the fibronectin distribution in the ECM changes from a fibrillar pattern during estrus to a patched pattern before disappearing on day six of pregnancy which regulates the shape of the stromal fibroblasts to change from elongated cells at estrus to a round morphology on day six.[1] This ECM remodeling serves to support the decidual cell's morphological differentiation and creates an environment which permits the invasion and establishment of the placenta.[20] Numerous other examples exist of how ECM influences cells and tissues ranging from microscale influence on cellular morphology[17,21,22] and proliferation[16,23,24]

Y. Li, Prof. K. A. Kilian
Department of Materials Science and Engineering
Department of Bioengineering
Institute for Genomic Biology
Micro and Nanotechnology Laboratory
University of Illinois at Urbana-Champaign
Urbana, IL 61801, USA
E-mail: kakilian@illinois.edu

www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS

www.advhealthmat.de

REVIEW

to macroscale guidance of the stem cell niche[2,25,26] and tissue formation.[18,19,27,28] This dynamic organization and reorganization of the extracellular matrix proteins during embryogenesis and morphogenesis leads to distinct and organized tissue structures composed of a variety of cell shapes performing distinct functions. From cues that shape the early embryo to dynamic morphogenesis in the adult organism, the partitioning of cells into functional structures necessarily requires differential organization that is coordinated by the properties of the matrix.

The sensitivity of the early embryo to its surrounding microenvironment during development has been appreciated for some time,[29–32] however only recently have we begun to decipher the complex interactions in the microenvironment that guide cellular processes. At the beginning of embryogenesis, the blastula reorganizes from the symmetric blastula into an asymmetrical gastrula during gastrulation.[33] The ECM is essential in guiding the movement of cells from the primitive streak of the blastula to form the germ layers ectoderm, mesoderm, and endoderm. The migrating cells of a chick embryo attaches onto the laminin in the ECM, the first glycoprotein to appear, and is then guided to the ventral surface of the epiblast.[34] In zebrafish gastrulation, fibronectin and laminin fibrils align in the direction of membrane protrusion formation to polarize mesoderm cells and guide migration which helps to shape the embryo.[8] When expression of fibronectin is knocked down, there is a disruption of cell convergence and extension proving that the ECM is essential to gastrulation.

Another aspect of embryogenesis guidance by the ECM is the compliance of the surrounding tissue. As an embryo develops, the stiffness of the embryo increases dramatically[35] and the stiffness of its environment can impact embryo development. When Rinaudo and co-workers cultured mouse embryos on 1 kPa gels representing the uterine epithelium, they observed developmental differences between those cultured on stiff petri dishes.[36] This included increased frequency of development from zygote to the two-cell stage and from two-cell to blastocyst stage, increased hatching frequency, and increased placentae size once transplanted back into recipient females. Regional stiffness differences of embryos also begin to appear early on in embryogenesis including stiffening of the blastula wall[37] and stiffening of the marginal zone[38] and the notochord[39] during gastrulation. This asymmetric stiffening can be guided by differences in stiffness of the ECM[37] and by its orientation, namely, the orientation of fibrillary fibronectin.[40] To further understand these in vivo observations, additional in vitro studies with embryonic stem cells (ESC) has shed light on the influence of the ECM. Softer substrates promote self-renewal and pluripotency of ESCs and create more homogeneous cell populations[41,42] in addition to increasing cell traction at the basal surface.[43] However, stiffer substrates promote cell growth and differentiation.[44,45]

The ECM continues to play an important role in guiding cell and tissue geometry during processes like branching morphogenesis, during which the epithelial trees in the lung, kidney, mammary, and salivary glands are created[46] (**Figure 3**A). Branching involves repetitive epithelial cleft and bud formation[47,48] and the ECM can provide both mechanical cues and also serve to stabilize newly formed branches. During initial salivary gland formation, focal adhesion kinase (FAK) acts as a mechanosensor and is required for the assembly of ECM fibrils within a growing

Yanfen Li is a Ph.D Candidate in the Department of Bioengineering at the University of Illinois at Urbana-Champaign, where she also obtained her B.A degree in Bioengineering in 2012. She is a NSF Graduate Research fellow and her research is focused on understanding the influence of biomaterials on cellular mechanics and cytoskeletal organization.

Kristopher A. Kilian is Assistant Professor of Materials Science at the University of Illinois at Urbana-Champaign and Director of the Laboratory for Bioinspired Interfacial Design, where his group develops model systems for exploring tissue form and function. He received his PhD in chemistry at the University of New South Wales in 2007, and completed postdoctoral studies at the University of Chicago in 2011.

cleft.[49] These clefts then lead to the assembly of fibronectin fibrils via Rho-associated kinase (ROCK)-induced actomyosin contraction.[50] Fibronectin is also critical for initiation of epithelial branching where fibrillary fibronectin accumulate in cleft forming regions and suppresses cadherin cell–cell adhesions.[10] Other ECM components like collagen play a stabilizing role and can be found in the stalks of the forming branches.[51]

In addition to embryo development and initial tissue formation, important changes in tissue morphology occur during normal and pathological processes. One example is the epithelial-to-mesenchymal transition (EMT) where cuboidal, polarized epithelial cells attached to the basement membrane undergo a physiological change to adopt an elongated mesenchymal cell morphology with increased migratory capacity and increased production of ECM components[52–54] (Figure 3B). EMT is an important process during gastrulation,[55] tissue repair,[56] and cancer progression.[57,58] The ECM composition plays a role here too where it has been shown that type I collagen gels can induce EMT of thyroidepithelial cells.[59,60] Other in vitro studies showed that laminin can suppress EMT of mammary epithelial cells,[61] whereas fibronectin enhances EMT of human bronchial epithelial cells[62] and can direct migration of EMT induced mesothelial cells.[63] The same was seen for primary alveolar epithelial cells, where fibronectin promoted EMT, whereas laminin and collagen promoted apoptosis instead of EMT.[64]

The mechanical properties of the cell and tissue microenvironment can also play a role in morphogenesis where a stiffer
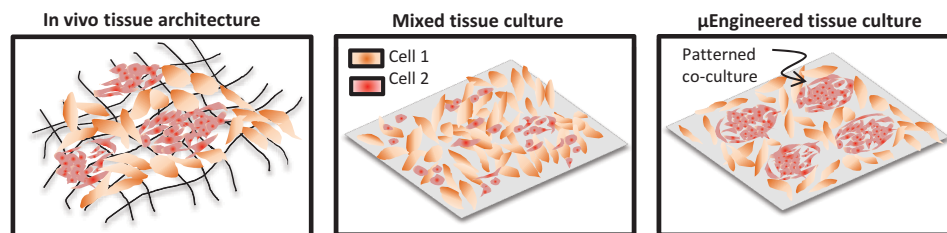
Figure 1. Approach to recapitulate structures in vitro using 2D microengineering.

environment can promote EMT of murine mammary gland cells and Madin–Darby canine kidney epithelial cells.[65] In the breast cancer microenvironment, the increase in and alignment of collagen fibrils increases the stiffness of the cancer microenvironment.[66,67] This increase in stiffness then drives EMT of breast tumor cells, increasing tumor invasion potential and metastasis.[68] A recent study by Huang and co-workers showed that soft fibrin gels will promote the growth of a subpopulation of tumor initiating melanoma cells, suggesting that soft matrices may prove important for amplifying specific cell types.[69] From these studies it is clear that the interplay between mechanical properties and matrix composition will guide a range of cellular processes in a context dependent fashion.

As the influence of individual ECM components is becoming increasing clear, the synergy of these factors can sometimes produce fascinating results. When natural organs and tissues are decellularized to form scaffolds, the complex ECM proteins are left behind to guide spatiotemporal organization of multiple cell types. Gershlak et al. decellularized rat hearts of different developmental stages to study differences in ECM composition and stiffness between fetal, neonatal, and adult hearts.[70] It was found that stiffness increases approximately twofold between fetal and neonatal but not between neonatal and adult hearts. In addition, the composition of the ECM was significantly different. When these ECM components were cross-linked into polyacrylamide gels of differing stiffness, mesenchymal stem

cells (MSC) responded differently depending on the ECM composition. From an increase in stiffness from 9 to 48 kPa, MSCs showed a decrease in stress generated by gels while on adult ECM but increase in stress while on fetal ECM with a further influence on differentiation potential. In addition, cell traction force increases with substrate stiffness on fetal ECM but not neonatal or adult ECM. This work shows that both ECM composition and substrate stiffness influences tissue form and function through pathways that are regulated through multiple biophysical and biochemical cues. Establishing these structure–function relationships that govern the formation of tissue in vivo is challenging at best due to the dynamic environment and limitations associated with analyzing excised tissue.[71] Depending on the substrate stiffness, the effect of ECM composition may be different and unraveling the complexity of the natural ECM environment is a complex endeavor that will be best assessed using combinatorial strategies.

Over the past decade, the maturation of nano and microengineering technologies for both "soft" and "hard" materials have enabled researchers to precisely control cellular assemblies in vitro.[72,73] These approaches can be used to mimic the in vivo microenvironment toward deconstructing the cues that orchestrate cellular assembly, while providing platforms that are amenable to modern microscopy techniques. More recently, dynamic hydrogel systems that can pattern material properties such as protein composition and stiffness in real time demonstrate the promise of synthetic materials that may recapitulate aspects of in vivo systems such as morphogenesis. In this review article we explore the evolution of 2D cell culture systems to engineered 3D model tissues, with a particular focus on translating information gained in 2D to inform the design of 3D materials. We highlight the importance of integrating multiple biophysical and biochemical cues to guide cellular processes in the laboratory. This review is not intended to be comprehensive but rather demonstrates the trend in translating 2D assays to 3D biomaterials; for details on recent advances in methods and materials to direct cell fate, readers are referred recent reviews.[74–76]
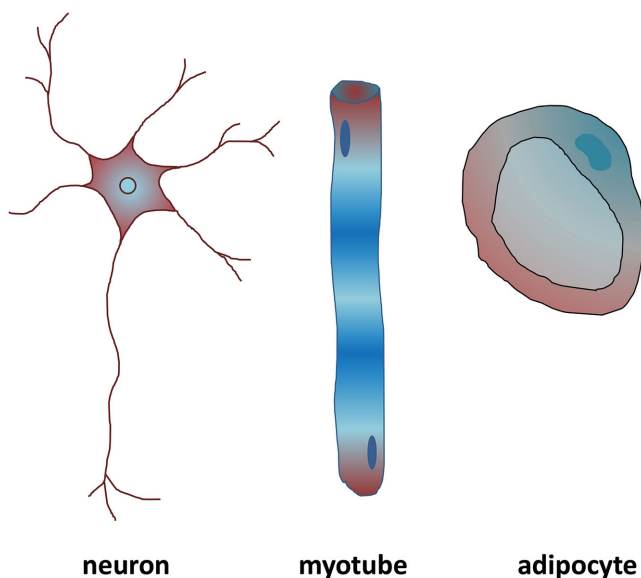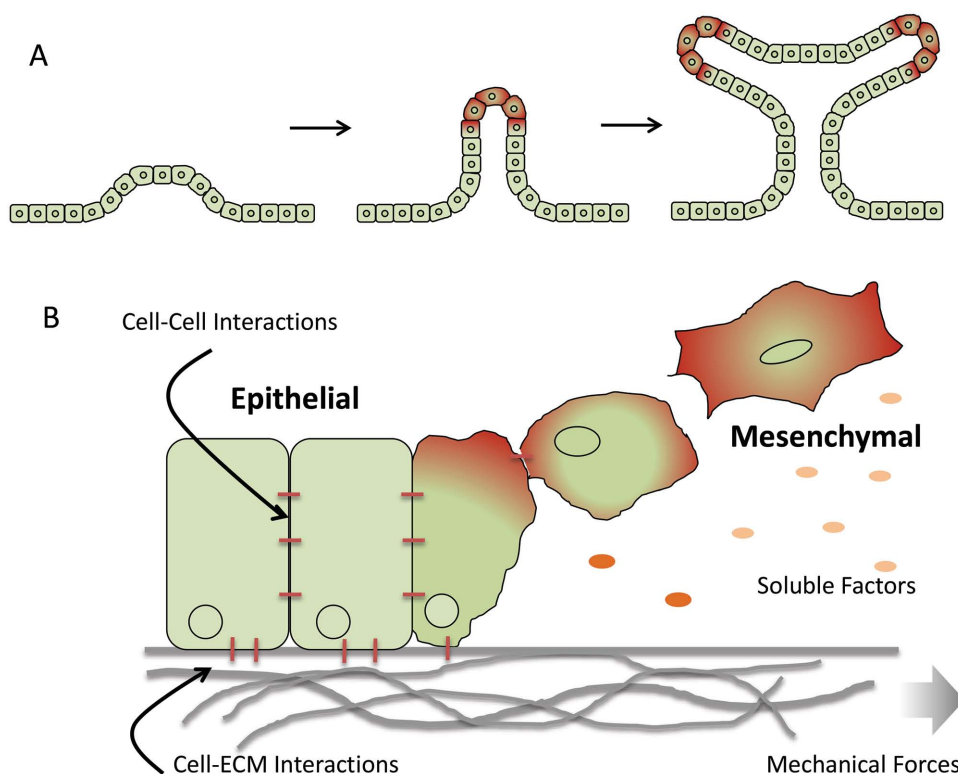
2. Recreating the Form and Function of Cells and Tissues on 2D Materials

2.1. Micropatterning Single Cells to Explore Geometry–Function Relationships

Cell shape influences a variety of cellular activities including proliferation,[16,77,78] migration,[79] and the regulation of lineage specific gene expression.[21,80–85] In order to decouple these

neuron   myotube   adipocyte

Figure 2. Representations of cells with different shapes found in tissue.

Materials
Views
www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

REVIEW

Figure 3. Depiction of A) branching morphogenesis where soluble and insoluble signals coordinate the formation of hierarchical structures in developing tissue; B) the epithelial-to-mesenchymal transition.

relationships, the microelectronics industry has provided a wealth of tools to modify the surface of materials with spatial definition in order to precisely control the shape of single cells via the presentation of ECM components including proteins and peptides.

One common approach is the technique of soft lithography (i.e., microcontact printing), developed by Kumar and Whitesides[86] to spatially pattern chemistry. This technique involves the use of a structured pattern mask formed via photolithography to cast an elastomer which can be used to transfer a specific chemistry to a surface (**Figure 4**). Microcontact printing has also been used to pattern chemistry on glass substrates via silane monomers[87] and proteins.[88] For more detail on soft lithography, we refer the reader to several reviews.[89–91]

Other patterning techniques include localized self-assembled monolayer (SAM) replacement which involves using a microfluidic device to remove regions of inert alkanethiol and replacing it with "active" alkanethiol,[92] dip pen lithography which uses an atomic-force microscope (AFM) tip to deposit molecules onto a surface,[93] and various other related strategies (**Table 1**).

To improve SAM stability, researchers have explored patterning under liquid medium or using inks with different properties such as low diffusion, reactive SAMs, or supramolecular interactions.[94]

Using these techniques, the surface positioning and composition of ECM proteins can be precisely tuned with micrometer scale resolution and single cells can be captured in patterns to study the influence of geometric cues on cellular processes.[90,91,112] After initial attachment of the cells onto the substrate, the cells then acquire their new cell shape in

two stages. First, the most distal contacts of the cell define the apices of the cell shape. Second, the cell borders link and minimize the distance between the two apices. Nonadhesive regions are overcome by the formation of stress fibers and the accumulation of focal adhesions. These stress fibers work against the membrane tension in the cell border.[113] It was shown that irrespective of the shape of the adhesive region, compressive stress is maximum at the cell center and vanishes at the cell boundary, vanishing more rapidly at regions of high curvature or at sharp corners. Sheer stress is essentially zero for isotropic shapes. For anisotropic shapes, sheer stress also builds up at the center of the shape.[114] The actomyosin contractility generated by the patterning of fibroblasts was shown to influence rotation of the nucleus which can be controlled via apical actin fibers absent in circular geometries but present in elongated geometries.[115] In a striking example of how patterning can influence subcellular architecture, Bornens and co-workers demonstrated how the positioning of protein patterns under a cell can guide adhesion and cytoskeletal tension.[113] When the location of adhesive and nonadhesive regions are manipulated within the same given convex envelope, cells on different ECM patterns might have similar shape but different cytoskeletal networks (**Figure 5**A).

Controlling cell shape will guide the organization of the cytoskeleton and adhesion architecture, and thereby influence cell behavior.[116–118] In addition to microfilaments, cell shape was recently shown to guide the formation and localization of intermediate filaments.[119] When cells are patterned in defined geometries, vimentin intermediate filaments (VIF) are primarily perinuclear in contrast to the microfilaments which

REVIEW
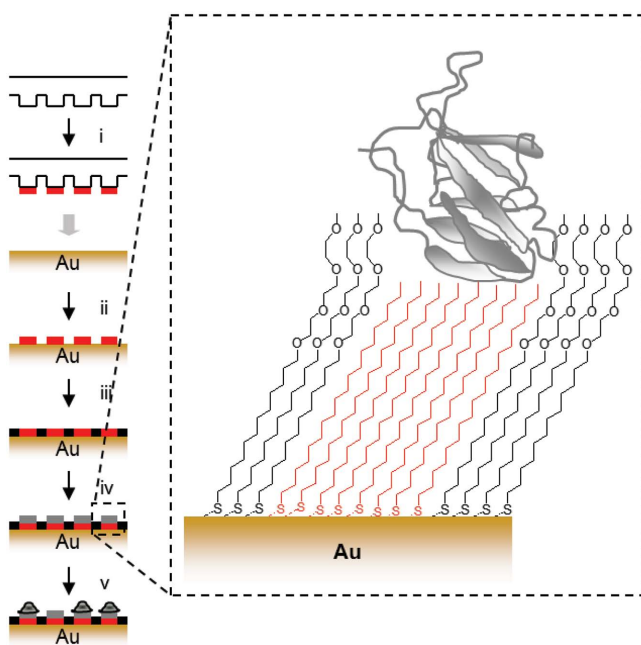
Figure 4. Soft lithography strategy for patterning cells. (i) A polydimethylsiloxane (PDMS) stamp is inked with octadecanethiol, (ii) printed onto the gold surface, (iii) the intervening regions are passivated with a tri(ethylene glycol) diluent, (iv) matrix protein is physisorbed to hydrophobic regions, and (v) cells are captured specifically to protein coated islands.

localize to the circumference of the cell. Microtubules (MT) tend to be evenly distributed throughout the cell with the exception of shapes approximating a teardrop, where filaments localize at the blunt edge (Figure 5B). In these cases with asymmetric shapes, VIF and MT tend to avoid concentrated areas of MF which biases the cell motility pattern. Similarly, patterning cells in asymmetric shapes will influence cell polarity and exert control over protrusion[120] and directional cell movement.[121,122] When cultured in the presence of platelet-derived growth factor (PDGF), cells preferentially extend lamellipodia, filopodia, and microspikes from shape corners, where tractional forces are also concentrated.[123] Other cellular functions, such as the contractile strength of vascular smooth muscle cells (VSMC), are also geometry dependent.[124,125] Patterned VSMCs showed a greater contractile range and decreased contractile strength when elongated due to morphological changes in its nucleus.

In addition to subtle geometric cues, the degree to which a cell can spread will influence adhesion, cytoskeletal tension, and intracellular signaling. Cells cultured on smaller areas will have a higher rate of apoptosis, while spread cells show an increased rate of DNA synthesis, resulting in decreased apoptosis.[126] With an increase in cell spreading, there is also an increase in nuclear volume and chromatin decondensation.[77] This is coupled with epigenetic changes including an increase in histone H3 acetylation at lysine 9. An increase in actomyosin contractility, e.g., in triangles versus circles, is also associated with an increase in polymerized actin and a decrease in nuclear levels of histone deacetylase 3 resulting in decondensed chromatin and global histone

Table 1. Summary of strategies for micropatterning hard and soft materials.

Substrate	Surface chemistry	Adhesive ligand	Cell type	Reference
Gold	Mixed alkane thiolates	Peptides (RGD)	Capillary endothelial cells	[95]
	Adsorption/agarose[a]	Protein (fibronectin)	Pulmonary artery endothelial cells, smooth muscle cells	[96]
	Gold-thiol	Chemistry (1-octadecanethiol)		[97]
	Gold-thiol	Protein (collagen)		[93]
Glass	Silane	Protein (collagen IV, fibronectin, laminin)	Neuroblastoma	[98]
	Adsorption/BSA[a]	Protein (fibronectin)	Adrenal capillary endothelial	[99]
	Adsorption/PEG[a]	Protein (collagen)	Neuron, glia cells	[100]
Glass/PDMS	Adsorption/pluronic[a]	Protein (collagen)	Hepatocytes, fibroblast	[88]
Graphene	Reduced graphene oxide	Reduced graphene oxide	Mesenchymal stem cell	[101]
Hydrogel (PA)	NHS acrylate	Protein (fibronectin)	Fibroblast	[102]
	Sulfo-SANPAH	Protein (collagen)	Airway smooth muscle cell	[103]
	Adsorption	Protein (fibronectin)	Mammary gland epithelial cell	[104]
	Hydrazine hydrate	Protein (collagen)	Fibroblast	[105]
	Physical crosslinking	Protein (collagen I, fibronectin, laminin)	Fibroblast	[106]
Hydrogel (PEG)	Polymer brush	Protein (BSA)	Hippocampal neurons	[107]
	Gold-thiol/cysteamine	Peptide (cRGD)	Fibroblast	[108]
	Thiol-ene	Peptide (RGD)	Fibroblast	[109]
	Biotin-avidin	Peptide (G11GRGDS, G5CSRARKQAASIKVAVSADR)	Bovine aortic endothelial cells, nerve cells	[110]
Polystyrene	Adsorption/poly(NIPAAm)[a]	Protein (fibronectin)	Rat hepatocytes	[111]

[a]Additive to prevent nonspecific protein adhesion.
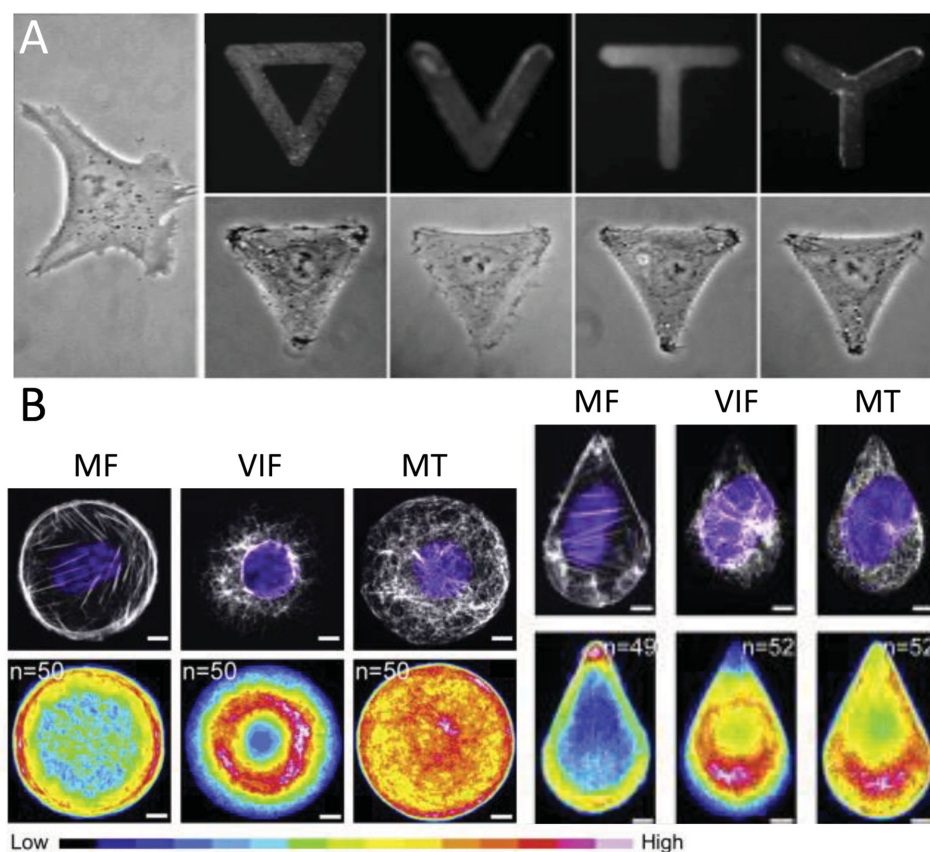
Figure 5. A) Adherent fibroblasts take on the adhesive geometry printed on the substrate. Reproduced with permission.[113] 2006, Wiley Periodicals, Inc. B) Microfilaments (MF), vimentin intermediate filaments (VIF), and microtubules (MT), differentially organized within a cell in response to geometry. Reproduced with permission.[119] 2014, Elsevier.

acetylation.[82] Micropatterning has also been used to direct EMT signaling in single cells. Epithelial cells confined to small islands showed reduced metalloproteinase induced EMT but not TGFβ transforming growth factor beta (TGFβ) induced EMT.[81] When allowed to spread, epithelial cells also increased expression of myofibroblast markers in contrast to when cell spreading is restricted, epithelial-myofibroblast transition is prevented via myocardin-related transcription factor (MRTF)-A signaling.[127]

To understand how cell shape influences developmental processes, in vitro stem cell systems have provided a powerful tool in deciphering the role of geometric cues in guiding lineage outcomes.[15,128,129] Recently we demonstrated that restricting the spreading of single MSCs will enhance and preserve the multipotent phenotype in culture through control of cytoskeletal tension and actomyosin contractility.[130] Chen and co-workers showed that spread cells, which experience high actomyosin contractility, tend to adopt an osteogenesis outcome while rounded cells, which experience lower actomyosin contractility, prefer to undergo adipogenesis when exposed to soluble media supplements.[15] In addition to spreading, MSCs cultured in shapes of the same area but increasing aspect ratio, or pentagonal shapes with variable subcellular curvature at the perimeter, differentially undergo adipogenesis or osteogenesis depending on the geometry that fosters the lowest or highest cytoskeletal tension, respectively.[128] Human mesenchymal stem cells (hMSCs) cultured in micropatterns and exposed to TGFβ3 upregulate myogenic genes when spread, and chondrogenic genes when shape is restricted[129] (**Figure 6**).

2.2. Deconstructing Morphogenesis Using 2D Micropatterning

Single cell patterning gives us great insight into the relationship between cell shape, adhesive structures, and cytoskeletal tension during cellular processes. Micropatterning can also be used for multicellular systems to explore cell–cell contact, force transmission, and for signaling both to adjacent cells and across large populations. Cells can be patterned in individual two-cell patterns or up to thousands of cells can be patterned into a tissue sheet. Patterning of cells in these larger geometries will help us define how cells interact with each other and how they behave in large tissue structures. Nelson and Chen patterned endothelial cell doublets in a bowtie configuration, and showed how vascular endothelial-cadherin inhibits growth by decreasing cell spreading through changes in cell adhesion to the ECM. This cadherin induced proliferation signal can be inhibited by blocking actin–myosin generated tension.[96,131] The two-cell bowtie configuration can be adjusted for higher cell–cell contact by the addition of triangles to the bowtie shape; as the number of cell–cell contacts increase, proliferation decreases.[132]

REVIEW
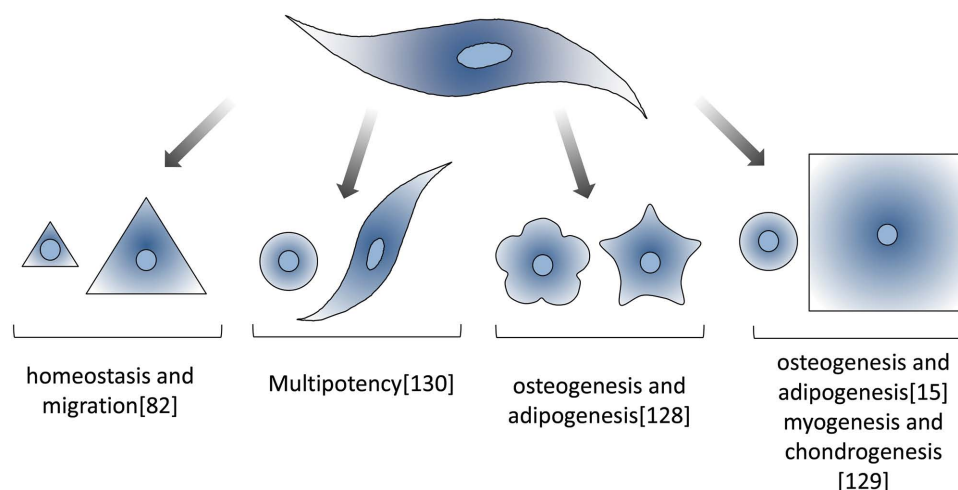
Figure 6. Cells respond to geometric cues to coordinate and regulate a variety of biological activities.

Spatial patterns of cellular growth generate mechanical stresses that help to deform tissues into their specific forms. The forces experienced by the cells can be predicted by finite-element models of multicellular mechanics which are then confirmed and measured directly by using a micromechanical force sensor array. Chen and co-workers used a combination of micropatterning large populations of cells and finite element analysis to show how a gradient of force is spatially organized in large patterns from the perimeter to the center[134] (**Figure 7**A). Changing the shape of perimeter features in patterned epithelial sheets was shown to promote EMT of epithelial cells in regions of high tension.[135] This is due to several factors. Cells at the edge of patterened shapes experience reduced cell–cell adhesion in addition to increased mechanical stress from the contractile tension generated by the cellular sheet.[134] These stress gradients are then propagated by intercellular transmission of the actomyosin cytoskeleton and can influence cell function and development. At regions of higher stress at pattern perimenters, MRTF-A localized to the nucleus, combined with decreased cell–cell contact and increased tension, promotes EMT of epithelial cells in that region. Human adipose derived stem cells grown in a ring showed increased proliferation in the outer edges, where cells were large and spread, and increased differentiation in the inner edges where cells were small and elongated.[136] hMSC from bone marrow were shown to respond to perimeter geometric cues during lineage specification and commitment when exposed to mixed differentiation promoting media.[137,138] In regions of higher stress, hMSCs were shown to differentiate toward the osteoblast lineage, while in regions that fostered a lower degree of stress, hMSCs preferred to undergo adipogenesis (Figure 7B). Ding and co-workers further explored the role of cell–cell contact and density within multicellular sheets.[139] They seeded varying densities of MSCs on identically sized adhesive islands to create populations of cells, which differed in their cell–cell contact frequency and cell sizes. When exposed to sole osteogenic or sole adipogenic media, adipogenesis increased with cell density but osteogenesis was unaffected. When exposed to both media, as density was increased, adipogenesis increased and osteogenesis decreased.

Large-scale patterning also allows us to study complex migration patterns which play a pivotal role in biological systems for regulating various processes such as gastrulation, morphogenesis, and tissue organization. The random motion of cells can be controlled and directed with asymmetric "ratchet" microgeometries, which induces a controlled cell polarity. These ratchet shapes can be controlled to guide cells of different types into different directions, which could be useful in cell sorting.[79,122] The geometrical confinement of cells into circles induces a persistent, coordinated, and synchronized rotation of the cells that depends on their density and the size of the circles. The speed of such rotating large-scale movements slows down as the density increases. The rotating cells move as a solid body, with a uniform angular velocity[133] (Figure 7C). Warmflash et al. showed that confinement of human embryonic stem cell (hESCs) to a disk shaped geometry was sufficient to recapitulate germ layer patterning with the addition of bone morphogenetic protein 4 (BMP4).[140] The disk shape is representative of the disk-shaped epithelium at gastrulation and, when seeded with a density comparable to the cell number at the initial gastrula, the colony of hESCs self-organized and differentiated into the three germ layers. Studies such as these allow us to separate different biophysical cues and gain insight into their influences on tissue development.

Micropatterning through soft lithography has enabled exploration of a wide array of complex cellular processes. It allows for the precise control of individual cell shapes to study the interplay between geometry, intracellular signaling, and function. The techniques are reproducible and allow for the creation of complex geometries, where different factors such as angle and curvature can be tuned and varied. However, limitations in the variation of viscoelasticity and matrix dynamics have led researchers to investigate other materials systems.

2.3. Micropatterned Hydrogels: Integrating Biophysical and Biochemical Cues in 2D

The majority of 2D patterning approaches employ rigid substrates that do not faithfully represent the deformable matrices

Materials
Views
www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

REVIEW
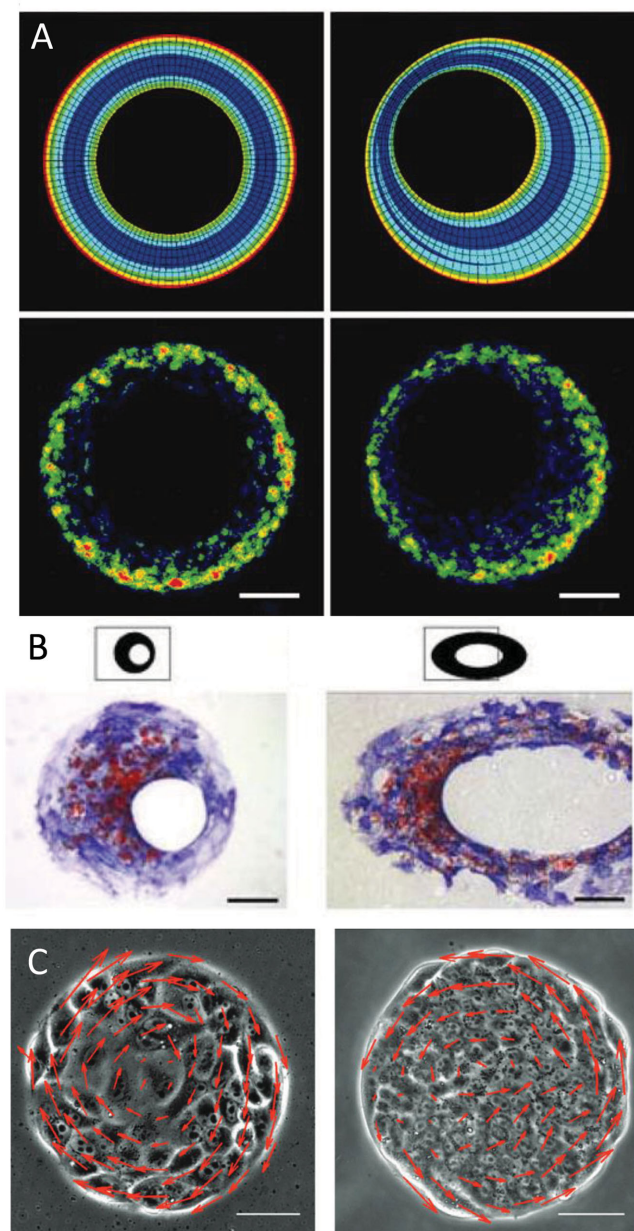
Figure 7. A) Finite element modeling of a contractile monolayer of cells (top) demonstrates variable regions of stress which correlate with proliferation (bottom; bromodeoxyuridine staining). Adapted with permission.[134] 2005, National Academy of Sciences. B) Different regions of stress in tissue islands are shown to coordinate osteogenesis (blue; alkaline phosphatase) and adipogenesis (red; Oil Red O). Adapted with permission.[137] 2008, Wiley Periodicals, Inc. C) Patterned cells show directed migration within micropatterned islands. Reproduced with permission.[133] 2013, Royal Society of Chemistry.

observed in vivo. Hydrogels are an appealing scaffold material for cell and tissue studies due to their high water content and tailored chemical and physical properties.[141–143] Using hydrogels to mimic in vivo microenvironments has proved useful in deconstructing the biochemical and biophysical cues that influence cellular morphology,[144,145] proliferation,[146–149]

migration,[114] and differentiation.[44,150–153] Recently, micropatterning techniques have been combined with hydrogel systems in order to study the interplay between matrix protein presentation, mechanics, and geometry.[83,105,154] Cells in vivo do not spread in the same way as cells cultured on 2D matrices, but rather adopt distinct geometries that relate to the presentation of matrix proteins and the deformability of the surrounding matrix. Cells sense the stiffness of their environment and modify their shape, proliferation, and stiffness in response. In addition, as cells spread more they can increase their inherent cortical stiffness by upregulating cytoskeletal contractility. To explore the relationship between cell geometry and substrate stiffness, Tee et al. cultured hMSCs on micropatterned polyacrylamide gels of varying stiffness and observed distinct behavior for cells on soft versus stiff substrates in the regulation of cell stiffness.[155] On soft substrates, cellular stiffness depends more strongly on matrix mechanics than on spread area. In contrast, cells that were patterned on stiff substrates show that geometry has a more pronounced role in directing cell stiffness.

MSCs have been shown to undergo differentiation in response to substrate stiffness.[156] Since cells cultured on planar substrates show a high degree of spatial and geometric heterogeneity, which complicates studies aimed at correlating ECM properties to outcome, we used micropatterning to normalize cell shape across substrates of variable stiffness. By patterning MSCs in shapes with subcellular geometric cues that modulate actomyosin contractility across hydrogels of varying stiffness, we were able to discern the relationship between cell shape, matrix stiffness and the osteogenesis program.[83] Subcellular features that increase focal adhesion and nonmuscle myosin activity were shown to promote osteogenesis. In a separate study, MSCs were micropatterned on soft hydrogels to explore neurogenesis and adipogenesis.[157] MSCs that were allowed to spread developed features consistent with the neurogenesis lineage, while cells confined to small isotropic geometries tended to adhere to the adipogenesis program (**Figure 8**). To further explore how MSCs respond to shape and stiffness, we "switched" the matrix underlying the patterned cells, either from a stiff substrate with features that promote actomyosin contractility or a soft substrate with features that promote neurogenesis, and measured the response of lineage specific markers.[158] Interestingly, MSCs showed a considerable amount of plasticity in the expression of early markers. Consistent with differentiation studies, we recently demonstrated how geometric features on single cells and multicellular populations will also influence the expression of multipotency markers.[159]

By combining micropatterning approaches with a viscoelastic polymeric system, the effect of both cell shape and matrix stiffness can be explored. These techniques combine the precise spatial control found in micropatterning along with the physical tunability of polymeric systems, in order to precisely control microenvironment parameters. Nevertheless, these systems do not reflect the true dimensionality of in vivo systems, and there is a need for 3D models that can be engineered to high precision to understand how cell signaling differs from 2D to 3D.
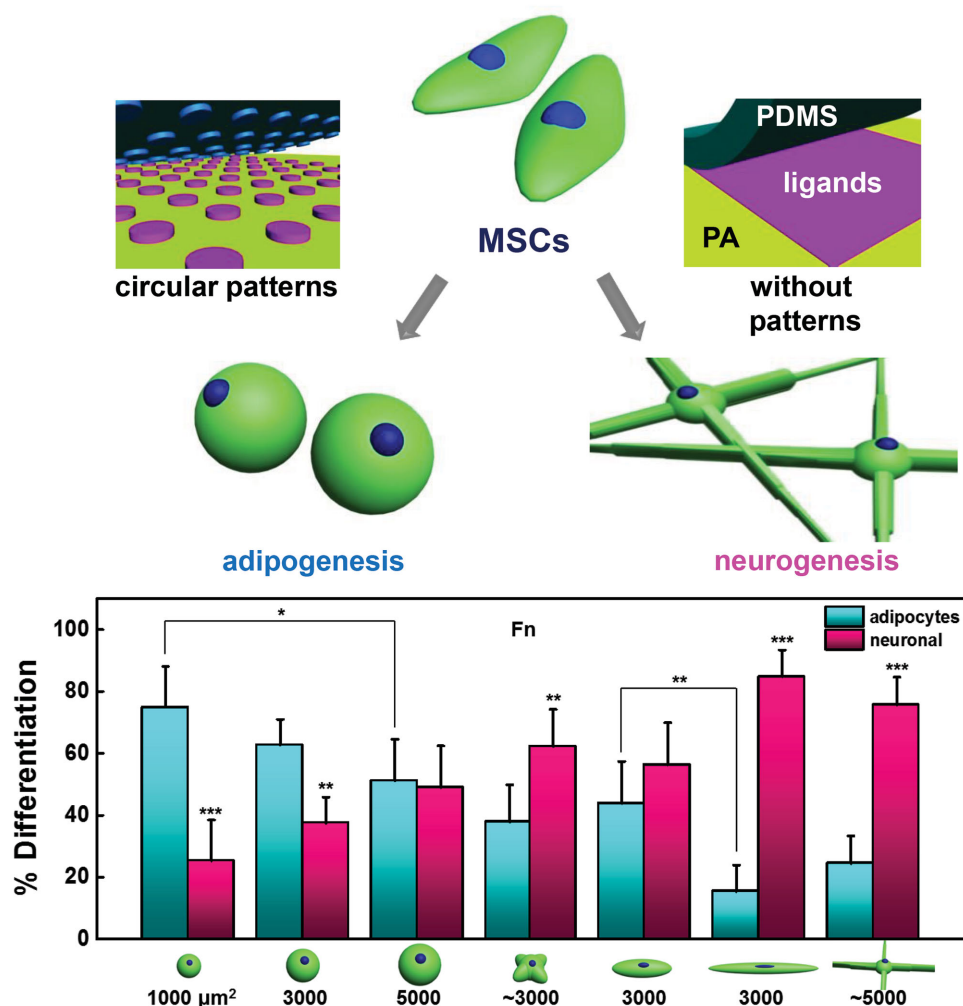
Figure 8. Geometric cues patterned on soft hydrogels guide adipogenesis and neurogenesis in the absence of soluble differentiation promoting compounds. Reproduced with permission.[157] 2013, Elsevier.
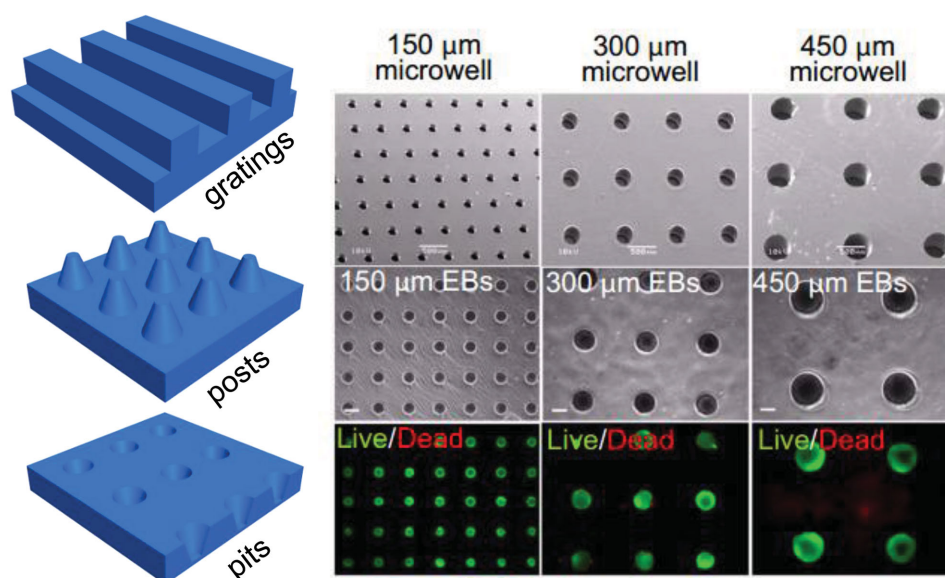
3. Microengineering 3D Biomaterials to Study and Direct Cellular Signaling and Tissue Organization

3.1. Beyond 2D: Nano and Microtemplating Pseudo-3D Environments

While 2D biomaterial systems can give us insight into many cellular functions, the addition of a third dimension enables a closer mimic to the in vivo environment. When cells are cultured in 2D, the planar substrate induces an artificial polarity between the lower and upper surfaces of the cells. Strategies aimed at a closer approximation of tissue dimensionality involve pseudo-3D or 2.5D environments, which aims to reduce the artificial polarity of 2D culture while maintaining its ease.

One example of a pseudo-3D environment is the creation of microwells, which are topographically structured surfaces that comprise a high density of micron sized cavities of a desirable geometry. They can be created via curing a gel solution, most commonly polydimethylsiloxane (PDMS) or polyethylene glycol (PEG), onto a silicon master.[160] Microwell cultured

hESC demonstrated the formation of embryoid-bodies (EBs) with a defined size that maintained undifferentiated proliferation[161,162] (**Figure 9**). hESCs cultured in microwells of different sizes show that cardiogenesis can be enhanced and endothelial cell differentiation decreased in larger EBs, as mediated by noncanonical WNT signaling.[163] Larger concave microwells were shown to increase neuronal and cardiomyocyte differentiation[164] and were more likely to form contracting EBs[163] EB-mediated differentiation of hESCs in microwell culture also show differences in gene expression related to development when compared to traditional 2D culture[165] Increased induction into mesoderm and endoderm lineages was shown for hESCs cultured in microwells due to the influence of 3D culture on many signaling pathways, including canonical Wingless type (WNT) and TGFβ signaling. The size of the embryoid body can also be controlled via controlling the size of the microwell, which leads to differences in gene expression. It was seen that EBs cultured in 100 µm wells expressed more ectoderm-associated genes, whereas EBs cultured in 500 µm microwells expressed more mesoderm related genes.

© 2015 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim *Adv. Healthcare Mater.* **2015**, *4*, 2780–2796

Makrials Views
www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

REVIEW

Figure 9. Depiction of templating approaches to fabricate microwells, grooves, posts, and pits. Right: Embryonic stem cells captured in microwells. Reproduced with permission.[163] 2009, National Academy of Sciences.

Differences in cell morphology and cytoskeletal structure were observed when human adipose-derived mesenchymal stem cells (hADSCs) were cultured in microwells of varying shapes and sizes.[166] hADSCs grown on flat surfaces were flat and fibroblastic with focal adhesions located at the outer edge of the cells and connected by strong stress fibers. Comparatively, when grown in microwells, hADSCs had a more 3D shape and cytoskeletal orientation. These cytoskeletal networks are further mediated by the shape of the microwell. Cells cultured in square microwells formed focal adhesions at the corners, connected by strong stress fibers. Conversely, cells cultured in round microwells had more homogenously distributed focal adhesions and were connected by weaker stress fibers.

Another form of pseudo-3D culture is topographic patterning to create cellular and subcellular textured surfaces consisting of micron and nanosized features including grooves, pits, and pillars.[167] This approach has been harnessed to explore and direct diverse cellular functions including neural guidance,[168,169] promotion of myotubes,[170] stem cell differentiation,[171–173] and epigenetic changes.[174–176] In addition, topographic patterning enables approximation on a materials surface the patterned features observed within the in vivo environment. For instance, the basement membrane of tissues consists of a combination of different topography including pits, pores, protrusions, striations, particulates, and fibers.[177] To mimic these features, topographic patterning is generally performed through photopatterning, printing, and micromachining. Topographical patterning of a few micrometer wide grooves was able to induce cell alignments along the groove direction.[178] hMSCs cultured on 350 nm gratings have an elongated morphology with an aligned actin cytoskeleton, while on unpatterned controls, spreading cells showed a random but denser actin cytoskeleton network with altered cytoskeletal and focal adhesion protein expression.[179] Neurite growth can also be influenced by topographic cues and will follow along surface topographies,[168,169,180] while a closely spaced array of nonadhesive PEG nanohydrogels will

promote directional axon growth while limiting the attachment of astrocytes.[169] Micropatterned PDMS channels can promote neurite alignment in adult human neural stem cells; however, smaller channels force cells to deform their cytoskeleton which is unfavorable for neurogenesis.[181]

In addition to grooves, pits and pillars can be fabricated in materials to reflect properties of native ECMs. 3T3 fibroblasts grown on micron sized pillars showed differences in morphology and migration.[182] Cells migrating on a surface with pillars are forced to encounter topographic stimuli which facilitate changes in behavior. Compared to flat surfaces, cells on pillar substrates have a more branched shape and have increased linear speed and decreased directional stability, which is likely caused by localized stability of focal adhesions. The cells anchor to pillars via focal adhesions, followed by contraction and acceleration toward the pillar. A study by Kim et al. looked at the differences between two topographic surfaces. Human epithelial cells were cultured in pillars versus pits[183] and it was observed that on pillar substrates cells migrated toward the sidewall, whereas on pit substrates cells tended to move toward the sidewalls and the bottom. These differences can be a result from the actin reorganization of the cell and the differences in focal adhesion formation at the convex and concave corners of pillar and pit substrates. For more information on topographical techniques to modulate cell signaling, we refer readers to recent reviews.[172,184–186]

The creation of pseudo-3D systems is a novel way of combining the ease of 2D systems while overcoming the main limitation of lacking a third dimension. However, it is more restrictive in the complexity of shapes and does not completely eliminate the artificial polarity introduced by 2D systems. In addition, the generation of grooves and pits could serve to limit and organize the cell's attachment sites which would not normally be restricted in vivo. While 2D and pseudo-3D biomaterials offer simple and reproducible techniques for isolating and studying fundamental components of the extracellular

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

Materials
Views
www.MaterialsViews.com

REVIEW

environment, these techniques contain the inevitable downside of over simplifying and not fully representing in vivo 3D architectures.

### 3.2. Patterning 3D Biomaterials for Cell and Tissue Engineering

One of the most common forms of 3D culture is to encapsulate cells within hydrogel scaffolds consisting of different ECM proteins or peptides.[187–191] This technique involves the mixing of cells and a liquid gel, allowing the solution to set into desired shapes. Hydrogel beads have been shown to keep chondrocytes in a rounded morphology while maintaining functionality.[188–190] Hydrogel beads incorporating small molecules can also influence encapsulated hMSCs to undergo osteogenesis and adipogenesis.[192] A peptide based hydrogelation strategy has also been developed where the application of sheer stress will result in a low viscosity gel but will maintain its rigidity, allowing the gel to be delivered via a syringe.[193] The geometry of the formed gel can also play a role in directing cell function. Fibroblasts, human umbilical endothelial cells (HUVEC), and myoblasts when encapsulated in long, rectangular gelatin methacrylate gels, will elongate and self-align to the gel environment.[194] Endothelial cells cultured in channels filled with collagen gel will organize into tubes with lumen which extend up to 1 cm and exhibit cell–cell junction formation characteristic of early stage capillary vessels (**Figure 10**A). The tube diameter can be controlled by varying collagen concentration or channel width, and branching can be guided by channel geometry.[195] An exciting recent study demonstrated the potential of guiding morphogenesis in synthetic 3D matrices, where single mouse embryonic stem cells were encapsulated within soft 90Pa fibrin gels and showed that the single ESC proliferated to formed embryoid bodies which organized into the three germ layers.[196]

Another technique that has shown utility for fabricating biomimetic 3D architectures is electrospinning, which involves the production of very thin continuous fibers that are capable of supporting cell attachment.[198,199] These fibers are typically generated via the application of a high voltage to a polymer liquid solution sprayed from a very thin nozzle. These fibers can be created from a variety of different synthetic and natural polymers including poly(D,L-lactide-co-glycolide) (PLGA),[199] poly(epsilon-caprolactone) (PCL),[200] and collagen.[201] The fibers can have diameters as small as a few nanometers,[200] can be very porous,[202] and can be loaded with drugs for drug delivery.[203] Peptide-amphiphile nanofibers consisting of a peptide and an alkyl tail modification have been designed to self-assemble into nanofibers in different pH conditions without the need for additional machinery.[204] These nanofiber systems mimic the natural ECM environment consisting of interwoven protein fibers, which range in size.[205] These nanofibers have been shown to influence many cell functions[206] including guiding neuron differentiation[207] and the differentiation of MSCs.[208] While nanofibers are better at mimicking the ECM environment, hydrogels are better at simulating the soft nature of tissues. Yang and co-workers developed an interesting strategy for a hydrogel based scaffold made of gelatin microribbons.[197] The scaffolds are fabricated by wet-spinning a gelatin solution into microfibers and then collapsing them into ribbon like structures via drying in acetone (Figure 10B). When human adipose derived stromal cells were encapsulated in these microribbon structures, they proliferated up to 30-fold within three weeks.

One limitation of 3D materials as compared to 2D patterning approaches is the lack of spatial control over chemistry. One possible solution to this limitation is 3D printing which offers the promise of designing and fabricating custom scaffolds and tissues for tissue engineering. Most 3D printing techniques involve the initial design of a 3D computer model, which is then converted into 2D slices to be printed by a computer slice by slice. For a more in-depth review of 3D printing techniques we would like to refer the reader to the following reviews.[209,210] A newly printed device can be used for biomedical applications as scaffolds,[211–213] be used as a mold for creation of microfluidic devices,[214] or cells can be directly printed for tissue engineering applications. Scaffolds have been fabricated by printing hard extracellular components of a tissue or organ which mimic the original composition and structure, and have been demonstrated for applications in bone tissue regeneration to increase osteogenesis and vaculogenesis.[211–213,215] These scaffolds can be made to be biodegradable, allowing them to be replaced over time by growing bone[200] or cartilage.[216]

3D printing of cells most often uses hydrogel encapsulation of cells to form a 3D structure.[217–220] Yoo and co-workers 3D printed multilayered collagen hydrogels which contained layers of human skin fibroblasts and keratinocytes.[218] Each layer was first coated with a crosslinking agent. Then, the uncrosslinked collagen hydrogel was printed followed by the cells onto the coated surface creating a hydrogel layer. 3D printed materials have the potential to combine the fine
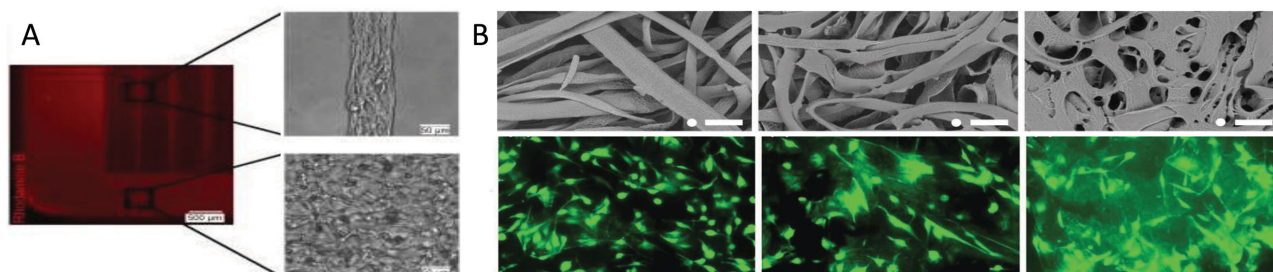
Figure 10. A) Microgrooves containing collagen enable the formation of tubes with lumen. Adapted with permission.[194] 2010, Elsevier. B) Gelatin microribbons can be used to supplement the structure in hydrogel matrices (left to right: 2.5%, 5%, 10% gelatin). Immunofluroescence images of cells adherent to the ribbons after 6 d. Reproduced with permission.[197] 2013, Wiley-VCH.

www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

REVIEW

control of 2D patterning systems with the in vivo mimicry of 3D materials to generate complex tissues with intricate microarchitecture. However, 3D printing is currently limited to a small range of materials and still cannot accomplish the micron scale tunability of 2D micropattern techniques. In addition, as the printed materials increase in size, concern regarding the vasculaturization of such materials must be addressed.

Culturing cells in 3D radically alter the interfacial interactions with the ECM compared to 2D, where cells are flattened and may lose their differentiated phenotype.[221] Cells in 3D environments also have inherently more complex cell–cell and cell–matrix interactions along with more complex dynamics for transport. Studies comparing 2D and 3D culture have shown differences in other cellular functions including cell adhesion,[222,223] migration,[224] gene expression,[225,226] and differentiation.[227,228] Cells cultured in 3D environments also show differences in cytoskeletal structure and cell attachment including: focal adhesions, cytoskeletal components, and associated signaling.[229] When fibroblast cells are cultured in 3D matrices, they form three times more adhesion sites when compared to 2D culture, with enhanced migration and proliferation rates.

Recent evidence has demonstrated how the composition of the matrix and the presentation of ligand can influence the way in which cells in 3D process signals. The seminal work by Discher and co-workers demonstrated that matrix elasticity directs stem cell lineage.[156] Naive MSCs cultured on collagen coated gels of varying stiffness show different morphology with increased neuron-specific markers on softer gels, increased myogenic markers on stiffer gels, and increased osteogenic markers on gels approximating the stiffness of pre-calcified bone. However, Huck and co-workers cultured hMSCs on PDMS and polyacrylamide (PAAm) hydrogels of different stiffness and found that cell spreading and differentiation was not affected by stiffness, but rather by a decrease in collagen crosslink concentration on gels of lower stiffness with lower anchoring density.[230] The importance of ligand on MSC differentiation was also shown in 2D culture via the use of SAMs of alkane thiolates, where changing the density and affinity of ligand was shown to influence lineage specification without changes in mechanical properties.[231] Recently, Engler and co-workers cultured human adipose derived stromal cells on poly-acrylamide gels of similar stiffness but with different porosities, and found that varying the substrate porosity did not significantly affect protein tethering or differentiation potential and that matrix stiffness remains a potent lineage directing cue.[232] From these and other studies, it is clear that there is an intimate relationship between matrix stiffness and ligand presentation, and controlling these cues in engineered extracellular matrices will be crucial for guiding cell behavior to recapitulate in vivo tissue form and function.

3.3. Toward 4D Control: Spatiotemporal Dynamics in 3D Engineered Extracellular Matrices

Culturing cells in 3D materials more closely recapitulates the in vivo environment, when compared to conventional 2D systems; however, most of these materials only offer a static image of what is otherwise a dynamic and complex environment. Recent work on stem cells has shown the cell's potential to self-organize in 3D culture.[233,234] ESCs grown in suspension with a retinal differentiation medium will self-organize and form patterns of optic-cup morphogenesis without external cues or forces.[235] Other examples of self-organized morphogenesis of ESCs or induced pluripotent stem cells (iPSC) includes the formation of glucose-responsive pancreatic islets,[236] generation of functional thyroid,[237] and functional adenohypophysis.[238] This ability for ESCs to self-pattern would be an intriguing next step for matrix engineering which could enhance the natural patterning tendencies and guide more complex cell behavior by mimicking natural ECM changes during morphogenesis in vivo. Recent advances in synthetic biomaterials has involved the design of dynamic and evolving systems which change their inherent material properties over time; thus adopting a "fourth dimension."

One example is a photodegradable hydrogel which consists of a PEG gel containing a nitrobenzyl ether-derived moiety[239] or a o-nitrobenzylether-based photodegradable monomer.[240] This gel system has been used to encapsulate fibrosarcoma cells in channels with precise release of the cells through illumination to study migratory effects. Guvendiren and Burdick developed a dynamic hydrogel which stiffens rapidly (from ≈3 to 30 kPa) in the presence of cells with the addition of light.[241] They then studied the effect of dynamic stiffening on differentiating MSCs. After the addition of media containing both adipogenic and osteogenic induction cues, the gel was stiffened for 1, 3, and 7 d. They found that adipogenic differentiation was favored the longer the stiffening event was postponed (**Figure 11**). Another hydrogel system involves a liposome loaded gel which contains liposomes loaded with gold nanorods and diethylenetriaminepentaacetic acid (DTPA) for softening the gel or CaCl2 for stiffening it.[242] The two differentially loaded liposomes release their cargo at different irradiation times, thus allowing the gel to be both stiffened and softened. When fibroblasts were cultured on their system, it was seen that stiffening inhibits fibroblast spreading (Figure 11).

Dynamic 4D systems can also include changes in topography over time. Yang and co-workers developed a stimuli-responsive hydrogel which formed macropores in response to different stimuli including temperature, small chelating molecules, and enzymes.[243] The gel was fabricated with a combination of stimuli-responsive porogens of gelatin, alginate, and hyaluronic acid. In the presence of stimuli such as temperature, macropores formed in the scaffold as the porogens were removed. When bovine articular chondrocytes (BAC) were seeded onto the scaffold, there was increased proliferation and ECM deposition upon application of stimuli along with cell release from the scaffold. This technique could be useful both for understanding the influence of a changing topography on cells and as a tool for cell delivery. The future of 4D systems will rely on advances in materials systems with controllable architecture, dynamic signaling and reversibility that emulate the complex signaling present in living tissue.

**ADVANCED
HEALTHCARE
MATERIALS**

www.advhealthmat.de

REVIEW

Materials Views
www.MaterialsViews.com
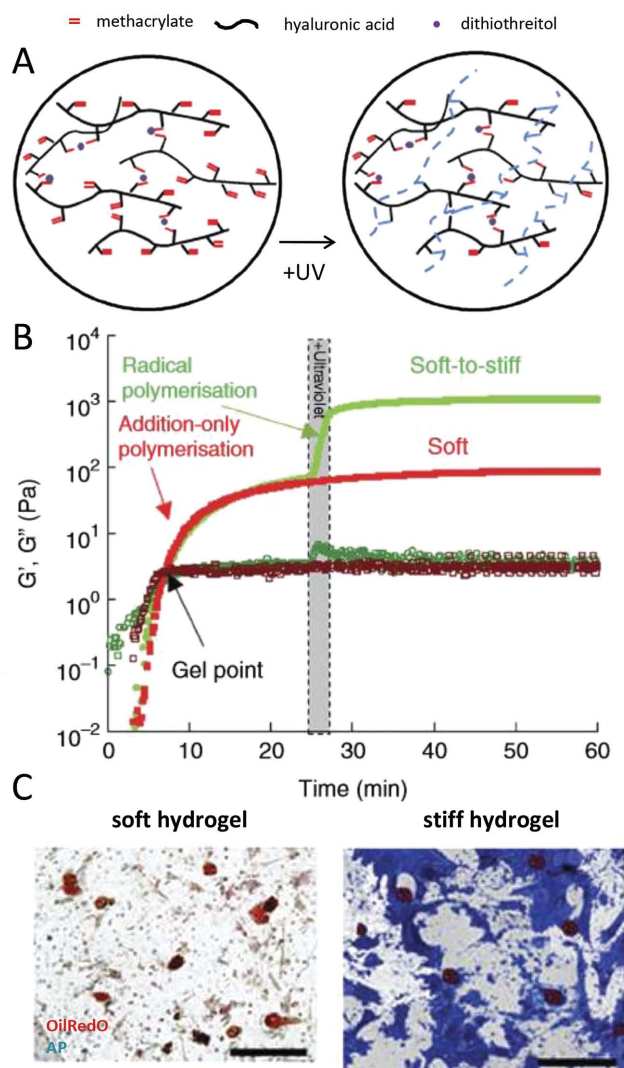
Figure 11. A) Depiction of multistage crosslinking to fabricated stiffening hydrogels. B) Mechanical characterization during stiffening. C) Histological staining of mesenchymal stem cells cultured on the soft gel (left) and after stiffening (right). Reproduced with permission.[241] 2012, Macmillan Publishers Limited.

4. Conclusions and Future Directions

Synthetic engineered extracellular matrices have proved useful in decoupling the environmental parameters that guide cellular processes and ultimately guide the form and function of tissue. From 2D plasticware to 3D dynamic hydrogels, model systems have come a long way in controlling the presentation of biochemical and biophysical cues to cells in culture toward elucidating the complex cellular orchestration underlying tissue morphogenesis. The choice of model system should reflect the questions being asked or assays being performed, as each has its own limitations.

2D patterning approaches allow for precise variation in the composition of the extracellular matrix, cell and tissue geometry, and substrate mechanical properties to discern the interplay between distinct factors. This level of control is difficult

to achieve with the majority of 3D materials. While 2D cannot fully represent the in vivo environment, it still serves as a powerful tool for understanding fundamental relationships between cell function and microenvironment parameters. Pseudo-3D platforms are useful for replicating higher order dimensionality to study the transition from 2D to 3D, where true 3D materials are difficult to yield coherent data on cell–cell and cell–ECM interactions. Pseudo-3D systems have proved a viable alternative, and show promise in mapping the differences between 2D and 3D toward translating the large number of 2D studies to 3D materials and in vivo systems. True synthetic 3D systems have matured considerably where the chemistry, mechanics, and architecture are well controlled, and thereby allow remote interrogation of cellular interactions within material encapsulates.

Recently we have seen a new trend in engineered extracellular matrices where strategies to control both spatial and temporal aspects of the material are taking center stage. Integration of new chemistries, with temporal 3D topographic patterning, has the potential to make synthetic tissue architectures a reality. Nevertheless, we believe there will always be a role for 2D studies, where context is key. Asking the right questions with appropriate materials is critical for defining the materials parameters that will orchestrate the complex interactions that regulate tissue in vivo and ex vivo. The future of engineered extracellular matrices involves creating more complex systems, which could combine multiple factors that have been studied individually with current systems, in order to understand how they interact and further enhance the influence of engineered materials on cell function. This includes combining patterning with cytokine gradients and rigidity and establishing complex multicellular cultures.

[1] F. Grinnell, *J. Cell Biol.* **1982**, *94*, 597.
[2] M. A. Hadley, *J. Cell Biol.* **1985**, *101*, 1511.
[3] F. M. Watt, W. T. S. Huck, *Nat. Rev. Mol. Cell Biol.* **2013**, *14*, 467.
[4] S. Schmidt, P. Friedl, *Cell Tissue Res.* **2010**, *339*, 83.
[5] C. Frantz, K. M. Stewart, V. M. Weaver, *J. Cell Sci.* **2010**, *123*, 4195.
[6] N. Zagris, *Micron* **2001**, *32*, 427.
[7] N. H. Brown, *Cold Spring Harb. Perspect. Biol.* **2011**, *3*, 1.
[8] A. Latimer, J. R. Jessen, *Matrix Biol.* **2010**, *29*, 89.
[9] T. Rozario, D. W. DeSimone, *Dev. Biol.* **2010**, *341*, 126.
[10] T. Sakai, M. Larsen, K. M. Yamada, *Nature* **2003**, *423*, 876.
[11] S. Stahl, S. Weitzman, J. C. Jones, *J. Cell Sci.* **1997**, *110*, 55.
[12] M.-H. Barcellas-Hoff, M. J. Bissell, *J. Cell Sci.* **1987**, *8*, 327.
[13] I. A. Meinertzhagen, S. Takemura, Z. Lu, S. Huang, S. Gao, C.-Y. Ting, C.-H. Lee, *J. Neurogenet.* **2009**, *23*, 68.
[14] P. L. Kuo, H. Lee, M. A. Bray, N. A. Geisse, Y. T. Huang, W. J. Adams, S. P. Sheehy, K. K. Parker, *Am. J. Pathol.* **2012**, *181*, 2030.
[15] R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, C. S. Chen, *Dev. Cell* **2004**, *6*, 483.
[16] D. Gospodarowicz, G. Greenburg, C. R. Birdwell, *Cancer Res.* **1978**, *38*, 4155.
[17] F. M. Watt, *Trends Biochem. Sci.* **1986**, *11*, 482.
[18] C. M. West, R. Lanza, J. Rosenbloom, M. Lowe, H. Holtzer, N. Avdalovic, *Cell* **1979**, *17*, 491.

www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

REVIEW

[19] C. M. West, H. Weerd, K. Dowdy, A. Paz, *Differentiation* **1984**, *27*, 67.

[20] B. Babiarz, L. Romagnano, S. Afonso, G. Kurilla, *Dev. Dyn.* **1996**, *206*, 330.

[21] B. M. Spiegelman, C. A. Ginty, *Cell.* **1983**, *35*, 657.

[22] J. J. Tomasek, E. D. Hay, K. Fujiwara, *Dev. Biol.* **1982**, *92*, 107.

[23] M. Öcalan, S. L. Goodman, U. Kühl, S. D. Hauschka, K. von der Mark, *Dev. Biol.* **1988**, *125*, 158.

[24] H. Senoo, R. Hata, *Kaibogaku Zasshi.* **1994**, *69*, 719.

[25] G. C. Hunt, P. Singh, J. E. Schwarzbauer, *Exp. Cell Res.* **2012**, *318*, 1820.

[26] G. Tanentzapf, D. Devenport, D. Godt, N. H. Brown, *Nat. Cell Biol.* **2007**, *9*, 1413.

[27] W. P. Daley, K. M. Yamada, *Curr. Opin. Genet. Dev.* **2013**, *23*, 408.

[28] D. J. Behonick, Z. Werb, *Mech. Dev.* **2003**, *120*, 1327.

[29] R. S. Prather, N. L. First, *J. Anim. Sci.* **1988**, *66*, 2626.

[30] V. N. Bolton, P. J. Oades, M. H. Johnson, *J. Embryol. Exp. Morphol.* **1984**, *79*, 139.

[31] I. Wilmut, D. I. Sales, *J. Reprod. Fertil.* **1981**, *61*, 179.

[32] M. C. Chang, *J. Exp. Zool.* **1950**, *114*, 197.

[33] M. H. L. Snow, *J. Embroyl. Exp. Morph.* **1977**, *42*, 293.

[34] N. Zagris, A. E. Chung, *Differentiation* **1990**, *43*, 81.

[35] M. Von Dassow, L. A. Davidson, *Dev. Dyn.* **2009**, *238*, 2.

[36] K. S. Kolahi, A. Donjacour, X. Liu, W. Lin, R. K. Simbulan, E. Bloise, E. Maltepe, P. Rinaudo, *PLoS One.* **2012**, *7*, e41717.

[37] L. A. Davidson, G. F. Oster, R. E. Keller, M. A. Koehl, *Dev. Biol.* **1999**, *209*, 221.

[38] S. W. Moore, R. E. Keller, M. A. Koehl, *Development* **1995**, *121*, 3131.

[39] D. S. Adams, R. Keller, M. A. Koehl, *Development* **1990**, *110*, 115.

[40] L. A. Davidson, R. Keller, D. W. DeSimone, *Dev. Dyn.* **2004**, *231*, 888.

[41] F. Chowdhury, Y. Li, Y.-C. Poh, T. Yokohama-Tamaki, N. Wang, T. S. Tanaka, *PLoS One* **2010**, *5*, e15655.

[42] D. Lü, C. Luo, C. Zhang, Z. Li, M. Long, *Biomaterials* **2014**, *35*, 3945.

[43] Y.-C. Poh, F. Chowdhury, T. S. Tanaka, N. Wang, *Biophys. J.* **2010**, *99*, L19.

[44] N. D. Evans, C. Minelli, E. Gentleman, V. LaPointe, S. N. Patankar, M. Kallivretaki, X. Chen, C. J. Roberts, M. M. Stevens, *Eur. Cells Mater.* **2009**, *18*, 1.

[45] N. Eroshenko, R. Ramachandran, V. K. Yadavalli, R. R. Rao, *J. Biol. Eng.* **2013**, *7*, 7.

[46] V. D. Varner, C. M. Nelson, *Development* **2014**, *141*, 2750.

[47] J. A. Davies, *Bio Essays* **2002**, *24*. 937.

[48] R. J. Metzger, M. A. Krasnow, *Science* **1999**, *284*, 1635.

[49] W. P. Daley, J. M. Kohn, M. Larsen, *Dev. Dyn.* **2011**, *240*, 2069.

[50] W. P. Daley, K. M. Gulfo, S. J. Sequeira, M. Larsen, *Dev. Biol.* **2009**, *336*, 169.

[51] C. Grobstein, J. Cohen, *Science* **1965**, *150*, 626.

[52] R. Kalluri, R. A. Weinberg, *J. Clin. Invest.* **2009**, *119*, 1420.

[53] R. Kalluri, E. G. Neilson, *J. Clin. Invest.* **2003**, *112*, 1776.

[54] E. D. Hay, *Acta Anat. (Basel)* **1995**, *154*, 8.

[55] D. Shook, R. Keller, *Mech. Develop.* **2003**, *120*, 1351.

[56] M. Parola, M. Pinzani, *Fibrogen. Tissue Repair* **2009**, *2*, 4.

[57] J. P. Thiery, H. Acloque, R. Y. J. Huang, M. A. Nieto, *Cell* **2009**, *139*, 871.

[58] B. Baum, J. Settleman, M. P. Quinlan, *Semin. Cell Dev. Biol.* **2008**, *19*, 294.

[59] E. D. Hay, *Curr. Opin. Cell Biol.* **1993**, *5*, 1029.

[60] G. Greenburg, E. D. Hay, *Development* **1988**, *102*, 605.

[61] Q. K. Chen, K. Lee, D. C. Radisky, C. M. Nelson, *Differentiation* **2013**, *86*, 126.

[62] J. Câmara, G. Jarai, *Fibrogen. Tissue Repair* **2010**, *3*, 2.

[63] Y. Yamaguchi, T. Ishigaki, K. Sano, K. Miyamoto, S. Nomura, T. Horiuchi, *Perit. Dial. Int.* **2011**, *31*, 477.

[64] K. K. Kim, M. C. Kugler, P. J. Wolters, L. Robillard, M. G. Galvez, A. N. Brumwell, D. Sheppard, H. A. Chapman, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 13180.

[65] J. L. Leight, M. A. Wozniak, S. Chen, M. L. Lynch, C. S. Chen, *Mol. Biol. Cell* **2012**, *23*, 781.

[66] S. Alowami, S. Troup, S. Al-Haddad, I. Kirkpatrick, P. H. Watson, *Breast Cancer Res.* **2003**, *5*, R129.

[67] M. W. Conklin, J. C. Eickhoff, K. M. Riching, C. A. Pehlke, K. W. Eliceiri, P. P. Provenzano, A. Friedl, P. J. Keely, *Am. J. Pathol.* **2011**, *178*, 1221.

[68] S. C. Wei, L. Fattet, J. H. Tsai, Y. Guo, V. H. Pai, H. E. Majeski, A. C. Chen, R. L. Sah, S. S. Taylor, A. J. Engler, J. Yang, *Nat. Cell Biol.* **2015**, *17*, 678.

[69] J. Liu, Y. Tan, H. Zhang, Y. Zhang, P. Xu, J. Chen, Y.-C. Poh, K. Tang, N. Wang, B. Huang, *Nat. Mater.* **2012**, *11*, 734.

[70] J. R. Gershlak, J. I. N. Resnikoff, K. E. Sullivan, C. Williams, R. M. Wang, L. D. Black, *Biochem. Biophys. Res. Commun.* **2013**, *439*, 161.

[71] E. Maes, V. Broeckx, I. Mertens, X. Sagaert, H. Prenen, B. Landuyt, L. Schoofs, *Amino Acids* **2013**, *45*, 205.

[72] Y. Ito, *Biomaterials* **1999**, *20*. 2333.

[73] M. Théry, *J. Cell Sci.* **2010**, *123*, 4201.

[74] G. H. Underhill, P. Galie, C. S. Chen, S. N. Bhatia, *Annu. Rev. Cell Dev. Biol* **2012**, *28*, 385.

[75] A. D. Celiz, J. G. W. Smith, R. Langer, D. G. Anderson, D. A. Winkler, D. A. Barrett, M. C. Davies, L. E. Young, C. Denning, M. R. Alexander, *Nat. Mater.* **2014**, *13*, 570.

[76] C. A. Custódio, R. L. Reis, J. F. Mano, *Adv. Healthcare Mater.* **2014**, *3*, 797.

[77] P. Roca-Cusachs, J. Alcaraz, R. Sunyer, J. Samitier, R. Farré, D. Navajas, *Biophys. J.* **2008**, *94*, 4984.

[78] R. G. Thakar, Q. Cheng, S. Patel, J. Chu, M. Nasir, D. Liepmann, K. Komvopoulos, S. Li, *Biophys. J.* **2009**, *96*, 3423.

[79] K. Kandere-Grzybowska, C. J. Campbell, G. Mahmud, Y. Komarova, S. Soh, B. A. Grzybowski, *Soft Matter.* **2007**, *3*, 672.

[80] J. Le Beyec, R. Xu, S.-Y. Lee, C. M. Nelson, A. Rizki, J. Alcaraz, M. J. Bissell, *Exp. Cell Res.* **2007**, *313*, 3066.

[81] C. M. Nelson, D. Khauv, M. J. Bissell, D. C. Radisky, *J. Cell. Biochem.* **2008**, *105*, 25.

[82] N. Jain, K. V. Iyer, A. Kumar, G. V Shivashankar, *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11349.

[83] J. Lee, A. A. Abdeen, T. H. Huang, K. A. Kilian, *J. Mech. Behavior Biomed. Mater.* **2014**, *38*, 209.

[84] X. Yao, R. Peng, J. Ding, *Biomaterials* **2013**, *34*, 930.

[85] M. J. Bissell, D. Farson, A. S. Tung, *J. Supramol. Struct.* **1977**, *6*, 1.

[86] A. Kumar, G. M. Whitesides, *Appl. Phys. Lett.* **1993**, *63*, 2002.

[87] C. Hoffmann, G. E. M. Tovar, *J. Coll. Interface Sci.* **2006**, *295*, 427.

[88] V. A. Liu, W. E. Jastromb, S. N. Bhatia, *J. Biomed. Mater. Res.* **2002**, *60*, 126.

[89] Y. Xia, G. M. Whitesides, *Annu. Rev. Mater. Sci.* **1998**, *28*, 153.

[90] R. S. Kane, S. Takayama, E. Ostuni, D. E. Ingber, G. M. Whitesides, *Biomaterials* **1999**, *20*, 2363.

[91] S. A. Ruiz, C. S. Chen, *Soft Matter* **2007**, *3*, 168.

[92] J. T. Koepsel, W. L. Murphy, *Langmuir* **2009**, *25*, 12825.

[93] D. L. Wilson, R. Martin, S. Hong, M. Cronin-Golomb, C. A. Mirkin, D. L. Kaplan, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13660.

[94] A. Perl, D. N. Reinhoudt, J. Huskens, *Adv. Mater.* **2009**, *21*, 2257.

[95] C. Roberts, C. S. Chen, M. Mrksich, V. Martichonok, D. E. Ingber, G. M. Whitesides, *J. Am. Chem. Soc.* **1998**, *120*, 6548.

[96] C. M. Nelson, C. S. Chen, *FEBS Lett.* **2002**, *514*, 238.

[97] R. D. Piner, *Science* **1999**, *283*, 661.

[98] B. Lom, K. E. Healy, P. E. Hockberger, *J. Neurosci. Methods* **1993**, *50*, 385.

[99] E. Ostuni, R. Kane, C. S. Chen, D. E. Ingber, G. M. Whitesides, *Langmuir* **2000**, *16*, 7811.

[100] N. E. Sanjana, S. B. Fuller, *J. Neurosci. Methods* **2004**, *136*, 151.

[101] Y.-S. Hsiao, C.-W. Kuo, P. Chen, *Adv. Funct. Mater.* **2013**, *23*, 4649.

[102] S. R. Polio, K. E. Rothenberg, D. Stamenovi, M. L. Smith, *Acta Biomater.* **2012**, *8*, 82.

[103] N. Wang, E. Ostuni, G. M. Whitesides, D. E. Ingber, *Cell Motil. Cytoskeleton* **2002**, *52*, 97.

[104] Q. Tseng, I. Wang, E. Duchemin-Pelletier, A. Azioune, N. Carpi, J. Gao, O. Filhol, M. Piel, M. Théry, M. Balland, *Lab Chip* **2011**, *11*, 2231.

[105] V. Damljanovi, B. C. Lagerholm, K. Jacobson, *Biotechniques* **2005**, *39*, 847.

[106] X. Tang, M. Yakut Ali, M. T. A. Saif, *Soft Matter* **2012**, *8*, 7197.

[107] Z. Zhou, P. Yu, H. M. Geller, C. K. Ober, *Biomacromolecules* **2013**, *14*, 529.

[108] D. Aydin, I. Louban, N. Perschmann, J. Blümmel, T. Lohmüller, E. A. Cavalcanti-Adam, T. L. Haas, H. Walczak, H. Kessler, R. Fiammengo, J. P. Spatz, *Langmuir* **2010**, *26*, 15472.

[109] C. A. DeForest, K. S. Anseth, *Nat. Chem.* **2011**, *3*, 925.

[110] N. Patel, R. Padera, G. H. Sanders, S. M. Cannizzaro, M. C. Davies, R. Langer, C. J. Roberts, S. J. Tendler, P. M. Williams, K. M. Shakesheff, *FASEB J.* **1998**, *12*, 1447.

[111] M. Yamato, C. Konno, M. Koike, Y. Isoi, T. Shimizu, A. Kikuchi, K. Makino, T. Okano, *J. Biomed. Mater. Res. A* **2003**, *67*, 1065.

[112] R. Singhvi, A. Kumar, G. Lopez, G. Stephanopoulos, D. Wang, G. Whitesides, D. Ingber, *Science* **1994**, *264*, 696.

[113] M. Théry, A. Pépin, E. Dressaire, Y. Chen, M. Bornens, *Cell Motil. Cytoskeleton* **2006**, *63*, 341.

[114] S. Banerjee, M. C. Marchetti, *New J. Phys.* **2013**, *15*, 35015.

[115] A. Kumar, A. Maitra, M. Sumit, S. Ramaswamy, G. V. Shivashankar, *Sci. Rep.* **2014**, *4*, 3781.

[116] D. Ingber, *Annu. Med.* **2003**, *35*, 564.

[117] N. Wang, J. Butler, D. Ingber, *Science* **1993**, *260*, 1124.

[118] A. J. Maniotis, C. S. Chen, D. E. Ingber, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 849.

[119] S. H. Shabbir, M. M. Cleland, R. D. Goldman, M. Mrksich, *Biomaterials* **2014**, *35*, 1359.

[120] J. James, E. D. Goluch, H. Hu, C. Liu, M. Mrksich, *Cell Motil. Cytoskeleton.* **2008**, *65*, 841.

[121] X. Jiang, D. A. Bruzewicz, A. P. Wong, M. Piel, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 975.

[122] G. Mahmud, C. J. Campbell, K. J. M. Bishop, Y. A. Komarova, O. Chaga, S. Soh, S. Huda, K. Kandere-Grzybowska, B. A. Grzybowski, *Nat. Phys.* **2009**, *5*, 606.

[123] K. K. Parker, A. L. Brock, C. Brangwynne, R. J. Mannix, N. Wang, E. Ostuni, N. A. Geisse, J. C. Adams, G. M. Whitesides, D. E. Ingber, *FASEB J.* **2002**, *16*, 1195.

[124] G. J. C. Ye, Y. Aratyn-Schaus, A. P. Nesmith, F. S. Pasqualini, P. W. Alford, K. K. Parker, *Integr. Biol. (Camb).* **2014**, *6*, 152.

[125] P. W. Alford, A. P. Nesmith, J. N. Seywerd, A. Grosberg, K. K. Parker, *Integr. Biol. (Camb).* **2011**, *3*, 1063.

[126] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425.

[127] J. W. O'Connor, E. W. Gomez, *PLoS One* **2013**, *8*, e83188.

[128] K. A. Kilian, B. Bugarija, B. T. Lahn, M. Mrksich, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4872.

[129] L. Gao, R. McBeath, C. S. Chen, *Stem Cells* **2010**, *28*, 564.

[130] D. Zhang, K. A. Kilian, *Biomaterials* **2013**, *34*, 3962.

[131] C. M. Nelson, C. S. Chen, *J. Cell Sci.* **2003**, *116*, 3571.

[132] D. S. Gray, W. F. Liu, C. J. Shen, K. Bhadriraju, C. M. Nelson, C. S. Chen, *Exp. Cell Res.* **2008**, *314*, 2846.

[133] K. Doxzen, S. R. K. Vedula, M. C. Leong, H. Hirata, N. S. Gov, A. J. Kabla, B. Ladoux, C. T. Lim, *Integr. Biol.* **2013**, *5*, 1026.

[134] C. M. Nelson, R. P. Jean, J. L. Tan, W. F. Liu, N. J. Sniadecki, A. A. Spector, C. S. Chen, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 11594.

[135] E. W. Gomez, Q. K. Chen, N. Gjorevski, C. M. Nelson, *J. Cell. Biochem.* **2010**, *110*, 44.

[136] L. Q. Wan, S. M. Kang, G. Eng, W. L. Grayson, X. L. Lu, B. Huo, J. Gimble, X. Edward Guo, V. C. Mow, G. Vunjak-Novakovic, *Integr. Biol.* **2010**, *2*, 346.

[137] S. A. Ruiz, C. S. Chen, *Stem Cells* **2008**, *26*, 2921.

[138] M. D. Treiser, E. H. Yang, S. Gordonov, D. M. Cohen, I. P. Androulakis, J. Kohn, C. S. Chen, P. V Moghe, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 610.

[139] R. Peng, X. Yao, B. Cao, J. Tang, J. Ding, *Biomaterials* **2012**, *33*, 6008.

[140] A. Warmflash, B. Sorre, F. Etoc, E. D. Siggia, A. H. Brivanlou, *Nat. Methods* **2014**, *11*, 847.

[141] J. L. Drury, D. J. Mooney, *Biomaterials* **2003**, *35*, 4337.

[142] K. Y. Lee, D. J. Mooney, *Chem. Rev.* **2001**, *101*, 1869.

[143] A. Khademhosseini, R. Langer, *Biomaterials* **2007**, *28*, 5087.

[144] T. Yeung, P. C. Georges, L. A. Flanagan, B. Marg, M. Ortiz, M. Funaki, N. Zahir, W. Ming, V. Weaver, P. A. Janmey, *Cell Motil. Cytoskelet.* **2005**, *60*, 24.

[145] L. A. S. Callahan, A. M. Ganios, E. P. Childers, S. D. Weiner, M. L. Becker, *Acta Biomater.* **2013**, *9*, 6095.

[146] C. Lee, A. Grodzinsky, M. Spector, *Biomaterials* **2001**, *22*, 3145.

[147] J. D. Mih, A. Marinkovic, F. Liu, A. S. Sharif, D. J. Tschumperlin, *J. Cell Sci.* **2012**, *125*, 5974.

[148] P. P. Provenzano, P. J. Keely, *J. Cell Sci.* **2011**, *124*, 1195.

[149] P. S. Tan, S. H. Teoh, *Mater. Sci. Eng. C* **2007**, *27*, 304.

[150] J. S. Park, J. S. Chu, A. D. Tsou, R. Diop, Z. Tang, A. Wang, S. Li, *Biomaterials* **2011**, *32*, 3921.

[151] K. Ren, T. Crouzier, C. Roy, C. Picart, *Adv. Funct. Mater.* **2008**, *18*, 1378.

[152] Y.-R. V. Shih, K.-F. Tseng, H.-Y. Lai, C.-H. Lin, O. K. Lee, *J. Bone Miner. Res.* **2011**, *26*, 730.

[153] L.-S. Wang, J. Boulaire, P. P. Y. Chan, J. E. Chung, M. Kurisawa, *Biomaterials* **2010**, *31*, 8608.

[154] K. A. K. J. Lee, A. A. Abdeen, X. Tang, T. A. Saif, *Biomaterials* **2015**, *69*, 174.

[155] S.-Y. Y. Tee, J. Fu, C. S. Chen, P. A. Janmey, *Biophys. J.* **2011**, *100*, L25.

[156] A. J. Engler, S. Sen, H. L. Sweeney, D. E. Discher, *Cell* **2006**, *126*, 677.

[157] J. Lee, A. A. Abdeen, D. Zhang, K. A. Kilian, *Biomaterials* **2013**, *34*, 8140.

[158] J. Lee, A. A. Abdeen, K. A. Kilian, *Sci. Rep.* **2014**, *4*, 5188.

[159] J. Lee, A. A. Abdeen, A. S. Kim, K. A. Kilian, *ACS Biomater. Sci. Eng.* **2015**, *1*, 218.

[160] M. Charnley, M. Textor, A. Khademhosseini, M. P. Lutolf, *Integr. Biol.* **2009**, *1*, 625.

[161] J. C. Mohr, J. J. de Pablo, S. P. Palecek, *Biomaterials* **2006**, *27*, 6032.

[162] H.-C. Moeller, M. K. Mian, S. Shrivastava, B. G. Chung, A. Khademhosseini, *Biomaterials* **2008**, *29*, 752.

[163] Y.-S. Hwang, B. G. Chung, D. Ortmann, N. Hattori, H.-C. Moeller, A. Khademhosseini, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 16978.

[164] Y. Y. Choi, B. G. Chung, D. H. Lee, A. Khademhosseini, J.-H. Kim, S.-H. Lee, *Biomaterials* **2010**, *31*, 4296.

[165] C. Hsiao, M. Tomai, J. Glynn, S. P. Palecek, *AIChE J.* **2014**, *60*, 1225.

[166] X. Xu, W. Wang, K. Kratz, L. Fang, Z. Li, A. Kurtz, N. Ma, A. Lendlein, *Adv. Healthc. Mater.* **2014**, *3*, , 1991.

[167] J. Y. Lim, H. J. Donahue, *Tissue Eng.* **2007**, *13*, 1879.

www.MaterialsViews.com

ADVANCED
HEALTHCARE
MATERIALS

www.advhealthmat.de

REVIEW

[168] S. Turunen, A.-M. Haaparanta, R. Äänismaa, M. Kellomäki, J. Tissue Eng. Regen. Med. **2013**, *7*, 253.

[169] P. Krsko, T. E. McCann, T.-T. Thach, T. L. Laabs, H. M. Geller, M. R. Libera, Biomaterials **2009**, *30*, 721.

[170] J. Gingras, R. M. Rioux, D. Cuvelier, N. A. Geisse, J. W. Lichtman, G. M. Whitesides, L. Mahadevan, J. R. Sanes, Biophys. J. **2009**, *97*, 2771.

[171] M. J. P. Biggs, R. G. Richards, N. Gadegaard, R. J. McMurray, S. Affrossman, C. D. W. Wilkinson, R. O. C. Oreffo, M. J. Dalby, J. Biomed. Mater. Res. Part A **2009**, *91*, 195.

[172] M. J. Dalby, N. Gadegaard, R. O. C. Oreffo, Nat. Mater. **2014**, *13*, 558.

[173] J. Y. Lim, A. D. Dreiss, Z. Zhou, J. C. Hansen, C. A. Siedlecki, R. W. Hengstebeck, J. Cheng, N. Winograd, H. J. Donahue, Biomaterials **2007**, *28*, 1787.

[174] P. M. Davidson, H. Özçelik, V. Hasirci, G. Reiter, K. Anselme, Adv. Mater. **2009**, *21*, 3586.

[175] F. Badique, D. R. Stamov, P. M. Davidson, M. Veuillet, G. Reiter, J. N. Freund, C. M. Franz, K. Anselme, Biomaterials **2013**, *34*, 2991.

[176] Z. Pan, C. Yan, R. Peng, Y. Zhao, Y. He, J. Ding, Biomaterials **2012**, *33*, 1730.

[177] R. G. Flemming, C. J. Murphy, G. A. Abrams, S. L. Goodman, P. F. Nealey, Biomaterials **1999**, *20*, 573.

[178] A. Curtis, C. Wilkinson, Biomaterials **1997**, *18*, 1573.

[179] E. K. F. Yim, E. M. Darling, K. Kulangara, F. Guilak, K. W. Leong, Biomaterials **2010**, *31*, 1299.

[180] N. Li, A. Folch, Exp. Cell Res. **2005**, *311*, 307.

[181] A. Béduer, C. Vieu, F. Arnauduc, J.-C. Sol, I. Loubinoux, L. Vaysse, Biomaterials **2012**, *33*, 504.

[182] M. T. Frey, I. Y. Tsai, T. P. Russell, S. K. Hanks, Y.-L. Wang, Biophys. J. **2006**, *90*, 3774.

[183] M.-H. Kim, Y. Sawada, M. Taya, M. Kino-Oka, J. Biol. Eng. **2014**, *8*, 13.

[184] M. S. Lord, M. Foss, F. Besenbacher, Nano Today **2010**, *5*, 66.

[185] L. E. McNamara, R. J. McMurray, M. J. P. Biggs, F. Kantawong, R. O. C. Oreffo, M. J. Dalby, J. Tissue Eng. **2010**, *120623*, 2010.

[186] A. Dolatshahi-Pirouz, M. Nikkhah, K. Kolind, M. R. Dokmeci, A. Khademhosseini, J. Funct. Biomater. **2011**, *2*, 88.

[187] Y. Tsuda, Y. Morimoto, S. Takeuchi, Langmuir **2010**, *26*, 2645.

[188] H. J. Häuselmann, M. B. Aydelotte, B. L. Schumacher, K. E. Kuettner, S. H. Gitelis, E. J. Thonar, Matrix **1992**, *12*, 116.

[189] J. Kisiday, M. Jin, B. Kurz, H. Hung, C. Semino, S. Zhang, A. J. Grodzinsky, Proc. Natl. Acad. Sci. USA **2002**, *99*, 9996.

[190] S. S. Mok, K. Masuda, H. J. Häuselmann, M. B. Aydelotte, E. J. Thonar, J. Biol. Chem. **1994**, *269*, 33021.

[191] S. Levenberg, N. F. Huang, E. Lavik, A. B. Rogers, J. Itskovitz-Eldor, R. Langer, Proc. Natl. Acad. Sci. USA **2003**, *100*, 12741.

[192] D. S. W. Benoit, M. P. Schwartz, A. R. Durney, K. S. Anseth, Nat. Mater. **2008**, *7*, 816.

[193] L. Haines-Butterick, K. Rajagopal, M. Branco, D. Salick, R. Rughani, M. Pilarz, M. S. Lamm, D. J. Pochan, J. P. Schneider, Proc. Natl. Acad. Sci. USA **2007**, *104*, 7791.

[194] H. Aubin, J. W. Nichol, C. B. Hutson, H. Bae, A. L. Sieminski, D. M. Cropek, P. Akhyari, A. Khademhosseini, Biomaterials **2010**, *31*, 6941.

[195] S. Raghavan, C. M. Nelson, J. D. Baranski, E. Lim, C. S. Chen, Tissue Eng. Part A **2010**, *16*, 2255.

[196] Y.-C. Poh, J. Chen, Y. Hong, H. Yi, S. Zhang, J. Chen, D. C. Wu, L. Wang, Q. Jia, R. Singh, W. Yao, Y. Tan, A. Tajik, T. S. Tanaka, N. Wang, Nat. Commun. **2014**, *5*, 4000.

[197] L. H. Han, S. Yu, T. Wang, A. W. Behn, F. Yang, Adv. Funct. Mater. **2013**, *23*, 346.

[198] A. Greiner, J. H. Wendorff, Angew. Chem.– Int. Ed. **2007**, *46*, 5670.

[199] W.-J. Li, C. T. Laurencin, E. J. Caterson, R. S. Tuan, F. K. Ko, J. Biomed. Mater. Res. **2002**, *60*, 613.

[200] H. Yoshimoto, Y. M. Shin, H. Terai, J. P. Vacanti, Biomaterials **2003**, *24*, 2077.

[201] J. A. Matthews, G. E. Wnek, D. G. Simpson, G. L. Bowlin, Biomacromolecules **2002**, *3*, 232.

[202] J. T. McCann, M. Marquez, Y. Xia, J. Am. Chem. Soc. **2006**, *128*, 1436.

[203] H. S. Yoo, T. G. Kim, T. G. Park, Adv. Drug Deliv. Rev. **2009**, *61*, 1033.

[204] J. D. Hartgerink, E. Beniash, S. I. Stupp, Proc. Natl. Acad. Sci. USA **2002**, *99*, 5133.

[205] C. Xu, R. Inai, M. Kotaki, S. Ramakrishna, Tissue Eng, **2004**,*10*, 1160.

[206] D. R. Nisbet, J. S. Forsythe, W. Shen, D. I. Finkelstein, M. K. Horne, J. Biomater. Appl. **2009**, *24*, 7.

[207] F. Yang, R. Murugan, S. Wang, S. Ramakrishna, Biomaterials **2005**, *26*, 2603.

[208] X. Xin, M. Hussain, J. J. Mao, Biomaterials **2007**, *28*, 316.

[209] H. N. Chia, B. M. Wu, J. Biol. Eng. **2015**, *9*, 4.

[210] S. V. Murphy, A. Atala, Nat. Biotechnol. **2014**, *32*, 773.

[211] S. Tarafder, N. M. Davies, A. Bandyopadhyay, S. Bose, Biomater. Sci. **2013**, *1*, 1250.

[212] F. Pati, T.-H. Song, G. Rijal, J. Jang, S. W. Kim, D.-W. Cho, Biomaterials **2015**, *37*, 230.

[213] S. Tarafder, W. S. Dernell, A. Bandyopadhyay, S. Bose, J. Biomed. Mater. Res. Part B-Appl. Biomater. **2015**, *103*, 679.

[214] K. Kamei, Y. Mashimo, Y. Koyama, C. Fockenberg, M. Nakashima, M. Nakajima, J. Li, Y. Chen, Biomed. Microdevices **2015**, *17*, 36.

[215] H. Seyednejad, D. Gawlitta, R. V. Kuiper, A. De Bruin, C. F. Van Nostrum, T. Vermonden, W. J. A. Dhert, W. E. Hennink, Biomaterials **2012**, *33*, 4309.

[216] K. C. Hung, C. S. Tseng, S. H. Hsu, Adv. Healthcare Mater. **2014**, *3*, 1578.

[217] T. Billiet, E. Gevaert, T. De Schryver, M. Cornelissen, P. Dubruel, Biomaterials **2014**, *35*, 49.

[218] W. Lee, J. C. Debasitis, V. K. Lee, J. H. Lee, K. Fischer, K. Edminster, J. K. Park, S. S. Yoo, Biomaterials **2009**, *30*, 1587.

[219] S. Khalil, J. Nam, W. Sun, Rapid Prototyp. J. **2005**, *11*, 9.

[220] J. M. Grolman, D. Zhang, A. M. Smith, J. S. Moore, K. A. Kilian, Adv. Mater. **2015**, *27*, 5512.

[221] K. von der Mark, V. Gauss, H. von der Mark, P. Muller, Nature **1977**, *267*, 531.

[222] S. X. Hsiong, N. Huebsch, C. Fischbach, H. J. Kong, D. J. Mooney, Biomacromolecules **2008**, *9*, 1843.

[223] S. H. Lee, J. J. Moon, J. L. West, Biomaterials **2008**, *29*, 2962.

[224] J. L. West, J. A. Hubbell, Macromolecules **1999**, *32*, 241.

[225] C. Fischbach, H. J. Kong, S. X. Hsiong, M. B. Evangelista, W. Yuen, D. J. Mooney, Proc. Natl. Acad. Sci. USA **2009**, *106*, 399.

[226] M. Pickl, C. H. Ries, Oncogene **2009**, *28*, 461.

[227] H. Baharvand, S. M. Hashemi, S. K. Ashtiani, A. Farrokhi, Int. J. Dev. Biol. **2006**, *50*, 645.

[228] C. N. Salinas, K. S. Anseth, Biomaterials **2008**, *29*, 2370.

[229] E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, Science **2001**, *294*, 1708.

[230] B. Trappmann, J. E. Gautrot, J. T. Connelly, D. G. T. Strange, Y. Li, M. L. Oyen, M. A. C. Stuart, H. Boehm, B. Li, V. Vogel, J. P. Spatz, F. M. Watt, W. T. S. Huck, Nat. Mater. **2012**, *11*, 642.

[231] K. A. Kilian, M. Mrksich, Angew. Chem. Int. Ed. **2012**, *51*, 4891.

[232] J. H. Wen, L. G. Vincent, A. Fuhrmann, Y. S. Choi, K. C. Hribar, H. Taylor-Weiner, S. Chen, A. J. Engler, Nat. Mater. **2014**, *13*, 979.

[233] Y. Sasai, Cell Stem Cell **2013**, *12*, 520.

[234] Y. Sasai, Nature **2013**, *493*, 318.

ADVANCED
HEALTHCARE
MATERIALS
www.advhealthmat.de

Materials
Views
www.MaterialsViews.com

REVIEW

[235] M. Eiraku, N. Takata, H. Ishibashi, M. Kawada, E. Sakakura, S. Okuda, K. Sekiguchi, T. Adachi, Y. Sasai, *Nature* **2011**, *472*, 51.

[236] H. Saito, M. Takeuchi, K. Chida, A. Miyajima, *PLoS One* **2011**, *6*, e28209.

[237] F. Antonica, D. F. Kasprzyk, R. Opitz, M. Iacovino, X.-H. Liao, A. M. Dumitrescu, S. Refetoff, K. Peremans, M. Manto, M. Kyba, S. Costagliola, *Nature* **2012**, *491*, 66.

[238] H. Suga, T. Kadoshima, M. Minaguchi, M. Ohgushi, M. Soen, T. Nakano, N. Takata, *Nature* **2011**, *480*, 57.

[239] A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59.

[240] A. M. Kloxin, M. W. Tibbitt, K. S. Anseth, *Nat. Protoc.* **2010**, *5*, 1867.

[241] M. Guvendiren, J. A. Burdick, *Nat. Commun.* **2012**, *3*, 792.

[242] R. S. Stowers, S. C. Allen, L. J. Suggs, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 1953.

[243] L. H. Han, J. H. Lai, S. Yu, F. Yang, *Biomaterials* **2013**, *34*, 4251.