Cellular and Molecular Bioengineering, Vol. 1, No. 1, March 2008 *(© 2008)* pp. 15–23
DOI: 10.1007/s12195-008-0005-y

Patterning Cell and Tissue Function

COLETTE J. SHEN, JIANPING FU, and CHRISTOPHER S. CHEN

Department of Bioengineering, University of Pennsylvania, 510 Skirkanich Hall, 210 South 33rd Street, Philadelphia, PA 19104, USA

(Received 22 January 2008; accepted 30 January 2008; published online 6 March 2008)

Abstract—Highly organized structures are a defining feature of biological tissues, from vascular and neural networks to hexagonal liver lobules and striated muscle fibers. This spatial organization of cells and their surrounding extracellular matrix (ECM) is an essential aspect to the development, maintenance, and function of tissues and organs. We discuss available strategies that have been developed for spatially arranging cells within ECM environments—by patterning cell–ECM and cell–cell adhesion, soluble cues, and substrate mechanical properties—and how such strategies can subsequently affect cell and tissue function. These approaches to recreate organized structures *in vitro* ultimately will play a key role in engineering the recapitulation of tissue function and thereby further efforts in regenerative medicine.

Keywords—Micropatterning, Microfabrication, Cell adhesion, Extracellular matrix, Mechanical forces, Tissue engineering.

INTRODUCTION

A central goal of cell and tissue engineering is to understand how to recapitulate functions observed in native tissues. A common strategy has been to simply expose cells in culture to soluble, adhesive, and mechanical cues observed *in vivo*, as a means to reproduce a desired function. For example, vascular endothelial growth factor (VEGF), known to be a potent stimulant for angiogenesis, has been embedded in biomaterials or expressed in the form of gene therapies to promote vascular network ingrowth.[15,69] In addition, exposure to physical forces such as fluid flow[20,45] and mechanical stress[30] has been demonstrated as yet another stimulus in the surrounding microenvironment that can enhance cell and tissue function.

While using factors from the native microenvironment to modulate cell function has proven to be a valuable strategy for simple, single cell type cultures, it has also become clear that spatially homogeneous environments are unlikely to yield the complex structures, cell–cell interactions, and multicellular functions that are essential for many tissues. Indeed, because the *spatial organization* of cells and multicellular structures appears to play a significant role in coordinating many cellular functions, it has become clear that the spatial organization of exogenous factors also must be considered in order to support the development and maintenance of relevant multicellular structures. As evidenced *in vivo*, highly organized patterns and structures can be seen throughout biological tissues, and these structures have likely evolved to provide tissues with optimal functional capabilities: branching vascular networks efficiently perfuse even the farthest reaches of tissues and organs, striated fibers in skeletal muscle allow for both contractile and tensile strength, and hexagonal liver lobules provide conduits for perfusion, metabolic waste removal, and bile excretion. Furthermore, the specific arrangement of cells not only gives rise to physical capabilities of these larger functional units, but also complex paracrine communication amongst such cells to in turn stabilize their biochemical and phenotypic function. Thus, structure itself—or how cells are organized as individuals and arranged with respect to each other in space—is inextricably linked to the function of those cells and tissues.

Determining how such cellular organization arises is a key question for developmental biology that can also aid tissue engineers in recreating these spatial cellular arrangements necessary for cell and tissue function. Both adhesive and soluble cues coordinately guide patterning locally and at a distance: in the case of vascular and neural tissues, ephrin receptor tyrosine kinases and their surface-bound ephrin ligands serve as short-range adhesive guides, netrins function as soluble chemoattractants (or repellents), and semaphorins along with their neuropilin and plexin receptors can provide even longer range guidance cues that together can direct the spatial patterning of vessels and nerves.[7] In *Drosophila*, gradients of the morphogen Bicoid

Address correspondence to Christopher S. Chen, Department of Bioengineering, University of Pennsylvania, 510 Skirkanich Hall, 210 South 33rd Street, Philadelphia, PA 19104, USA. Electronic mail: chrischen@seas.upenn.edu

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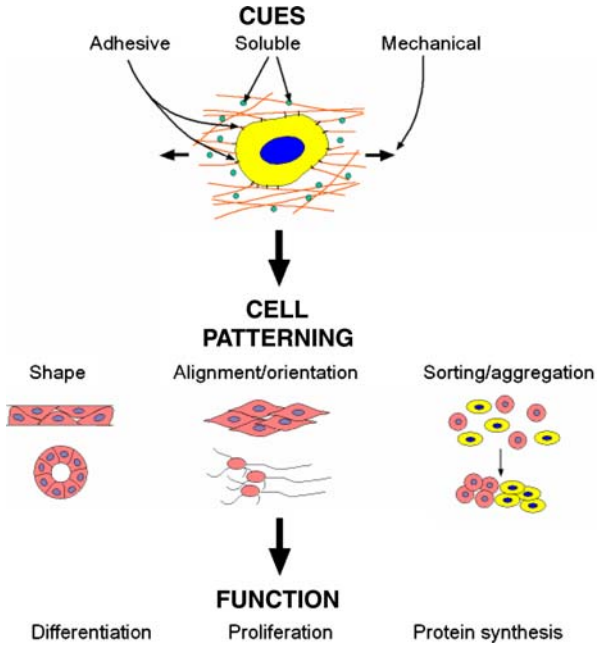

FIGURE 1. Adhesive, soluble, and mechanical cues can be used to spatially organize cells and obtain the desired tissue function.

determine cell fate along the anteroposterior axis.[14] The expression of Twist, a regulator of anterior gut formation, toward the ventral side of the embryo is controlled by both soluble morphogen gradients and mechanical forces.[17,50] Migration of the *Drosophila* oocyte to the posterior of the ovary—critical for body-axis formation of the embryo—is driven by differential adhesive affinities of cells in the ovary.[41] Similarly, differential expression of cadherin subtypes leads to segregation of neurons into different motor pools representing distinct functions in the chick spinal cord.[61]

It is clear from such examples of *in vivo* patterning that adhesive, soluble, and mechanical cues are all used to arrange cells spatially to direct their functions (Fig. 1) and thus could be applied to reconstruct functional tissues for regenerative medicine. Here, we review strategies that have been developed to employ adhesive, soluble, and mechanical cues to direct cellular patterning and function *in vitro* and discuss the many challenges that remain in their application in the context of tissue engineering toward achieving greater complexity in the interaction between cells and their environment.

ADHESIVE CUES FOR CELL PATTERNING

Cell adhesion to the ECM and neighboring cells plays a critical role in regulating many cellular functions, such as proliferation, differentiation, and migration. In the absence of adhesion, many cell types do not appear to respond to the presence of even saturating levels of soluble growth factors.[18,26] Interestingly, this effect of adhesion can be graded (and therefore controlled): Ingber and colleagues showed progressively increasing densities of the ECM protein, fibronectin, immobilized on substrates can progressively increase rates of cell proliferation, and low densities resulted in cell rounding, detachment, and apoptosis.[26,27,51] Building on these findings, researchers engineered substrates with micropatterned adhesive and non-adhesive regions to prescribe the area of cell–ECM contact and cell shape and therefore provide a more exacting approach to modulate adhesion. Using this approach, numerous groups have found that changing the degree of cell adhesion can modulate many cellular processes, including hepatocyte and osteoblast differentiation,[22,55] endothelial cell proliferation and apoptosis (Fig. 2a),[9] and even the lineage fate of mesenchymal stem cells (MSCs) (Fig. 2b).[40]

While such studies of single cells cultured on patterned adhesive regions have been instrumental in demonstrating the contribution of cell–ECM adhesion to cell function, cells *in vivo* often exist in multicellular structures. Thus, one begs the question of whether adhesion to ECM leads to the same responses when cells are in these more complex multicellular contexts. Interestingly, while adhesion and spreading of single endothelial cells dictated a switch between growth versus apoptosis, culturing endothelial cells on lines of fibronectin such that multicellular interactions were permitted resulted in differentiation into capillary tube-like structures at intermediate degrees of spreading and loss of the apoptotic effect at low cell spreading (Dike *et al.*, 1999, unpublished results).[12] Proliferation within multicellular sheets of cells patterned in different geometric shapes only occurred in certain regions in the structure, such as corners of flat sheets and valleys in undulating sheets.[43] Emergent patterns of mechanical stress within these multicellular structures appear to be responsible for dictating these proliferative patterns, highlighting the complex nature of predicting how cell adhesion could impact cell function within a multicellular context. Despite the importance of adhesive interactions to cell function, it is still poorly defined whether the identity of the matrix protein and respective receptors must be optimized to engage specific cellular behaviors. In part, this is because most substrates can adsorb cell-produced matrix, such that one can only prescribe the initial conditions of the experiment. In the few instances where inert surfaces have been developed, promiscuous ligands such as the RGD peptide are most often used, leading to little additional insight in the importance of different integrins or matrices in engineering tissue function. Thus, much additional work is to be done in this area.

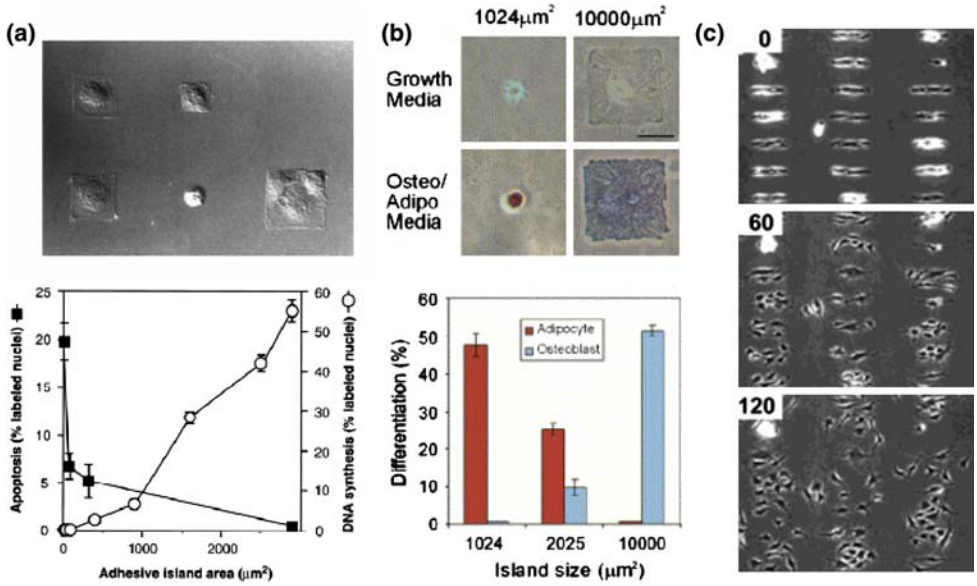

FIGURE 2. Patterning of adhesive cues regulates cell function. The degree of cell–ECM adhesion, controlled by varying sizes of micropatterned islands of fibronectin, regulates (a) endothelial cell growth versus apoptosis, with greater proliferation in more highly spread cells (largest island shown has width of 40 μm), and (b) MSC differentiation, with less spread cells undergoing adipogenesis and more spread cells undergoing osteogenesis. (c) Electroactive switching allows for dynamic control of cell–ECM adhesion, here releasing endothelial cells from patterned constraints. Time (in minutes) after voltage pulse is indicated (Part (a) from Chen, C.S. et al., Geometric control of cell life and death, Science 276:1425–1428, 1997, reprinted with permission from AAAS; Part (b) reprinted from Dev. Cell, Vol. 6, McBeath, R. et al., Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment, 483–495, © 2004, with permission from Elsevier; Part (c) reprinted with permission from Jiang, X. et al., Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements, J. Am. Chem. Soc., 125:2366–2367, 2003, © 2003 American Chemical Society.).

In many instances, it also remains to be determined what ligand type and density optimizes functional maintenance of different cell types within distinct regions in a putative tissue construct.

While the field has demonstrated a role for adhesive patterning to control cell function, can we employ differential adhesive cues to achieve sorting of cells into distinct, functional populations in engineered tissues? Cell sorting into distinct tissue layers occurs throughout development and is evident in the segregation of endothelial and muscle layers in arterial blood vessels. A first step may be to collect a single cell type into a functional aggregate, as juxtacrine and paracrine signaling have been shown to promote survival, differentiation, and coordinate excitation of groups of cells.[3,44,54] To aggregate cells, approaches involving differential cell attachment and haptotaxis on gradients of adhesive ligands have been proposed.[6,8,49,56] To sort multiple cell types, then, cross-gradients of different adhesive ligands preferred by different cell types potentially could be employed. It may also be possible to sort two populations simply by creating a single gradient of the same adhesive ligand, as different cell types have been shown to require different levels of substrate adhesiveness for attachment.[37] Additionally, we may also be able to exploit differential cell–cell adhesion to sort cell populations. *In vitro*, differential cadherin expression has been shown to cause sorting of mixed populations of chick embryonic cells in which high expressers separate from low expressers.[57] It may be possible for two randomly dispersed cell types expressing different cadherin subtypes to sort out spontaneously in an engineered tissue construct, or this process can be aided by artificially expressing varying levels of cadherins in different cell types. Thus, while the bulk of effort in engineering substrates has been directed toward controlling cell adhesion and function, there is also ample opportunity to redirect some of these tools specifically to engineer cellular and multicellular structure.

In addition to these static approaches to pattern cells, recent tools have been developed that allow one to alter adhesive cues dynamically during the course of culture. For example, one could introduce high adhesive surfaces to induce cells first to proliferate and then, once an appropriate mass of cells has been obtained, a new ligand or ligand density could be triggered, inducing cell differentiation. Recently, electroactive switching on culture surfaces has been shown to control the adhesiveness of independent regions and thus allow for cell expansion and temporally patterned co-cultures (Fig. 2c).[28,66,67]

In summary, adhesive cues have been used to achieve tight spatial control over cell–ECM and cell–cell interactions in anchorage-dependent cells to control

cell function. Given the importance of adhesion in regulating cells and the rapid advances in biomaterials engineering, we are likely to see continued fast paced development in realizing their potential to play a leading role in engineering complex tissues.

SOLUBLE CUES FOR CELL PATTERNING

Soluble cues in the form of growth factors and other chemokines regulate a myriad of cell functions, from proliferation and differentiation to motility and protein synthesis. Further, varying the level of soluble factor stimulation can lead to different cellular responses. For example, while physiological levels of VEGF can induce the growth of functional capillaries, higher levels present in pathological conditions such as tumors result in abnormal, leaky vessels, while even higher artificial levels obtained *in vitro* can at times suppress vessel formation by saturating receptors and preventing subsequent signaling induced by receptor dimerization.[23,63] In addition, many cell types are able to sense directional cues from gradients of soluble molecules and respond with asymmetric changes in cell morphology and motility.[52,62] Thus, restriction of set, desired levels of soluble cues to defined regions and presentation of soluble factor gradients can both be explored for purposes of spatially organizing cellular responses in engineered tissue constructs.

In vitro, spatial control of soluble cues is typically accomplished through microfluidic platforms, as any concentration differences would rapidly disappear in static systems. Whitesides and colleagues demonstrated the ability of laminar fluid flow in microfluidic channels to localize soluble molecule delivery to adhered cells due to the ability of different fluid streams to flow parallel to each other without mixing when joined into a single stream.[58,59] In recent work by Jeon and coworkers, physically connected but fluidically isolated compartments obtained by a microfluidic flow-induced hydrostatic pressure difference allowed for differential soluble factor stimulation of axons and somata from the same neurons (Fig. 3a), thus recreating *in vivo* conditions where different parts of neurons may experience distinct microenvironmental cues due to their length.[46,60] Similar microfabricated devices have also been used to generate spatially and temporally controlled gradients of soluble factors to direct cell migration[33,64] and promote stem cell proliferation and differentiation proportional to soluble growth factor concentration (Fig. 3b).[11] Dynamic flow systems, then, are powerful tools for spatially controlling soluble cellular stimuli and thus cellular responses *in vitro*.

While the above examples demonstrate the ability of externally introduced soluble factors to regulate cell

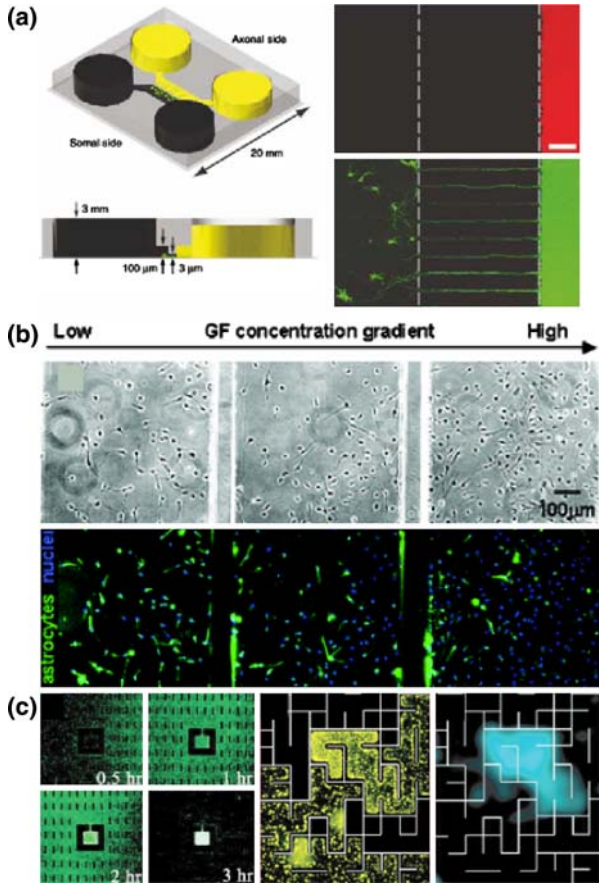

FIGURE 3. Spatial control of soluble cues can be used to direct cell behavior. (a) A microfluidic platform is used to culture neuronal axons and somata in fluidically separate compartments through a hydrostatic pressure gradient. When applied to the axonal compartment only, dextran remains in the compartment (right, top), while CellTracker Green backtracks through axons to the somata (right, bottom) (scale bar = 100 μm). (b) A gradient of growth factor regulates neural stem cell differentiation, with lower concentrations promoting greater astrocyte differentiation. (c) Chemotaxis toward paracrine signals promotes bacterial accumulation and quorum sensing. Bacteria accumulate into a central enclosure (left) and more enclosed areas of a microfluidic maze (middle), where chemotactic signals tend to be most amplified. Intrinsic luminescence indicates active quorum sensing in areas where the cells have reached a critical density (right) (Part (a) reprinted by permission from Macmillan Publishers Ltd: (Nature Methods) Taylor, A.M. et al., A microfluidic culture platform for CNS axonal injury, regeneration and transport, Nature Methods 2:599–605, 2005, © 2005; Part (b) from Chung, B.G. et al., Human neural stem cell growth and differentiation in a gradient-generating microfluidic device, Lab. Chip 5:401-406, 2005, reproduced by permission of The Royal Society of Chemistry; Part (c) from Park, S. et al., Motion to form a quorum, Science 301:188, 2003, reprinted with permission from AAAS.).

organization and function, recent experiments in bacterial population dynamics illustrate the importance of paracrine signaling—where cells respond to short-range cues originating from neighboring cells. In a behavior referred to as quorum sensing, bacteria

collectively alter their gene expression when their density reaches a critical level. To recreate this process *in vitro*, Park *et al.*[47] studied the growth of bacteria in microfluidic devices and found that bacterial cells accumulated in enclosed areas of a microfluidic maze where the chemotactic signals did not disperse and thus were most amplified (Fig. 3c). This study clearly demonstrated the possibility of using spatially defined chemotactic signals—derived from the cells in culture themselves—for aggregating cell populations to control their collective behavior.

Although *in vitro* studies of tissue formation on scaffolds can be performed simply by adding growth factors to cell culture media, translation of these studies to *in vivo* tissue engineering applications requires the use of proper delivery systems so that soluble factors can be expressed at the appropriate concentration, location, and duration to support proper cellular infiltration and differentiation. Inductive growth factor delivery can generally be achieved by two different approaches: controlled release, which allows for replacement of factors that are cleared or internalized and subsequently degraded, and immobilization to the substrate, which prevents clearance and internalization. With both these strategies, growth factor delivery can be controlled spatially—for example limited to certain regions of the scaffold or presented as a gradient. Spatial control of delivery can also be dictated by the cells themselves, as methods for producing materials that release the immobilized factor in response to enzymatic activity of a migrating cell have also been developed.[53,69] Exquisite spatial control can especially be obtained through immobilization to the substrate, as diffusion effects are minimized. However, with this strategy consideration must be given to potential differences in cellular response to free versus substrate-bound factors, as growth factor-receptor internalization can modulate signaling significantly.[1] Thus, soluble cues can serve as powerful long-range directors of cellular organization and function, with strategies being devised to deliver and maintain these signals in tissue engineered constructs.

MECHANICAL CUES FOR CELL PATTERNING

Cell function is classically thought to be directed and controlled by extracellular stimuli primarily in the form of soluble molecules that can bind to cell surface receptors or adhesive interactions from the ECM and cell–cell adhesion. Recently, there have been increasing lines of evidence suggesting that mechanical properties (e.g., rigidity) of the ECM to which a cell adheres can also mediate many aspects of cell function, including proliferation, differentiation, and migration. Increasing ECM rigidity has been associated with enhanced proliferation of fibroblasts and vascular smooth muscle cells[13] and greater tumor malignant potential.[48] Varying ECM rigidity also induces MSCs to differentiate into different tissue types corresponding to the tissues' relative mechanical rigidity *in vivo* (Fig. 4a).[16] Thus, many of the same functions that are regulated by increased cell adhesion to the ECM appear to be analogously enhanced by more rigid substrates.

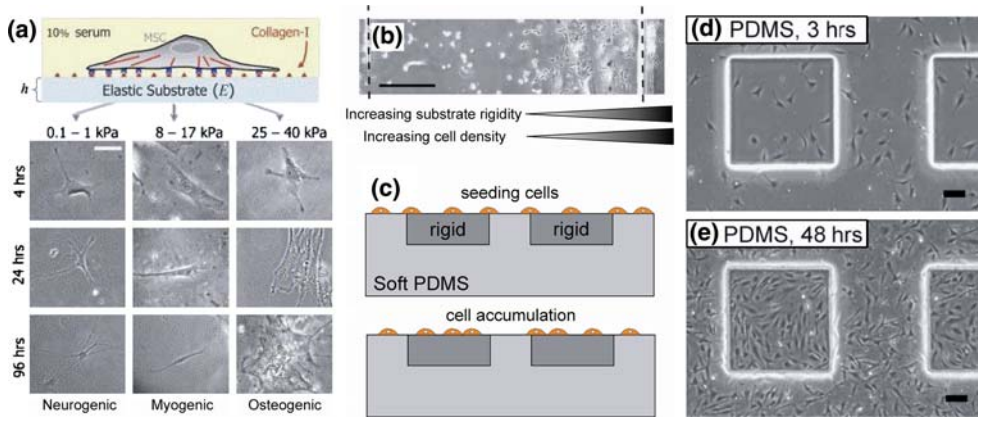

FIGURE 4. Tissue cells can sense and respond to variations in ECM rigidity. (a) Elasticity of polyacrylamide gels can direct MSC lineage specification. Gels of low, intermediate, and high stiffness are neurogenic, myogenic, and osteogenic, respectively. (b) Vascular smooth muscle cells accumulate toward the stiff end of a soft-to-stiff gradient collagen-coated polyacrylamide gel (scale bar = 500 μm). (c–e) 3T3 fibroblasts accumulate on the more rigid patterned regions of poly-(dimethylsiloxane) (PDMS) substrates over 48 h (scale bars = 100 μm) (Part (a) reprinted from Cell, Vol. 126, Engler, A.J. et al., Matrix elasticity directs stem cell lineage specification, 677–689, © 2006, with permission from Elsevier; Part (b) from Zaari, N. et al., Photopolymerization in microfluidic gradient generators: microscale control of substrate compliance to manipulate cell response, Adv. Mater. 16:2133–2137, 2004, © Wiley-VCH Verlag GmbH & Co. KGaA, reproduced with permission; Part (c) from Gray, D.S. et al., Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus, J. Biomed. Mater. Res. A 66:605–614, 2003, © 2003, reprinted with permission of John Wiley & Sons, Inc.).

We can potentially employ spatial control of mechanical rather than adhesive cues, then, as an additional parameter to create more complex arrangements of such cellular functions in tissue engineered constructs. As with adhesive and soluble gradients, it is possible that cell sorting can be obtained through mechanotaxis, the migration of cells toward stiffer regions of substrates, as demonstrated by fibroblasts and vascular smooth muscle cells (Fig. 4b).[36,65,68] As different cell types may have varying migratory responses to mechanical gradients—with some more sensitive to mechanical cues and some less migratory in general—different cell populations can potentially be induced to aggregate in distinct regions of the substrate, allowing for control of collective cell function by differential mechanical properties. Aggregation of cells into spatially defined regions of desired rigidity can also be achieved by patterning substrates whose mechanical properties can be tuned independent of their adhesive properties. Our laboratory has developed a polymeric cell culture system with spatially patterned substrate rigidity, in which endothelial cells and fibroblasts accumulate on more rigid regions of the substrates (Fig. 4c–e).[19] In both spatially constrained and gradient systems, the mechanical properties of the substrate can be tailored to promote the function desired in individual cell types, i.e., tissue-specific differentiation of MSCs or proliferation versus quiescence of smooth muscle cells.

In addition to spatial control of a substrate's intrinsic mechanical properties, the application of mechanical forces in the form of directional strain or shear stress can also be used to influence cell function. Endothelial cell and myoblast alignment, important for recreating the functional vascular lumen monolayers and muscle fibers seen *in vivo*, and directional endothelial sprouting, important for guided tissue vascularization, have been promoted by mechanical strain applied to fibrin gels.[38,39] Endothelial cell gene expression can also be modulated by shear stress gradients created by converging-width flow channels.[31] These examples clearly demonstrate the importance of spatial control of external mechanical forces—in addition to intrinsic substrate mechanical properties—on cell and tissue form and function.

CONCLUSIONS

Using principles derived from the strategies for cell patterning described here, we are gradually developing the capability to regenerate the spatial organization required for optimal cell function *in vitro*. To this end, several key challenges need to be addressed at this stage: How can we best translate techniques developed in two-dimensional (2D) experimental systems to three-dimensional (3D) tissue constructs? How can we combine various soluble, adhesive, and mechanical patterning strategies and tailor them for specific cell and tissue types? What is the best strategy to combine multiple cell types that are typically found together in tissues? Finally, once cells are organized into desired patterns and structures, are we in fact obtaining all functions expected from the tissue?

The transition from 2D to 3D cell organization is crucial for reconstructing physiological tissue environments. Several challenges accompany this transition, though, such as taking into consideration the mechanical properties, adhesive ligand type and density, and porosity of the natural or synthetic ECM used. Specifically for spatial patterning, consideration must be given to creating adhesive or mechanical patterns that vary in both the horizontal and vertical directions, as well as controlling soluble chemical factor diffusion through and binding to the ECM. Significant progress has been made recently in developing solutions for these challenges, enabling the design of 3D patterned constructs. Two-photon photolithography has been used to create spatial patterns and gradients of varying adhesive and mechanical properties within bulk non-adhesive materials to control cell localization, spreading, and mechanics.[21] Techniques for creating arrays of 3D cellular clusters of controlled size have been developed, demonstrating control over chondrocyte matrix synthesis by modulating cell–cell adhesion in 3D[2] and creating uniform embryoid bodies for stem cell differentiation.[29]

While initial efforts should focus on thoroughly developing and characterizing simple systems involving single patterning strategies and cell types, it is ultimately through combinations of these that we can engineer organotypic, functional tissue constructs. In addition to local ECM cues, chemokines can be introduced both in soluble form through exogenous addition or paracrine signaling from neighboring cells and in immobilized form attached to the ECM. Combined gradients of fibronectin and the growth factor VEGF immobilized on a solid surface have been shown to enhance directional endothelial cell migration.[35] The different time scales over which these cues exert their effect can also be used to control cell behavior on different levels: while adhesive cues can organize cells on surfaces within minutes to hours,[10] mechanical cues control cell arrangement over days,[19] potentially allowing two different axes of control over cells on the same surface. The relative influence of each patterning strategy in combination needs to be carefully controlled, though, as shear flow-induced mechanical forces have been demonstrated to overcome endothelial cell haptotaxis above a threshold rate.[24]

Tissues are comprised of multiple cell types that communicate with each other through soluble paracrine signaling, direct adhesive interactions, and transmission of mechanical force. Studies done to recreate these interactions *in vitro* demonstrate enhancement of hepatocyte function by fibroblasts in micropatterned co-cultures[4,5] and improved endothelial sprouting in 3D fibrin gels when cultured below a layer of fibroblasts.[42] Different properties between cell types should be taken into consideration when designing integrated constructs. In response to pro-migratory cues, for example, endothelial and epithelial cells have longer persistence time than fibroblasts and so will exhibit cohort migration to form continuous structures (e.g., capillary blood vessels), while fibroblasts migrate randomly to fill in connective tissue space.[25]

The final and perhaps most important challenge in efforts to engineer patterned tissue constructs is *ensuring functional output*. Our goal should be to create the simplest construct possible that can provide the maximum function attainable, and one place to start is to identify the few key cell–cell or cell–ECM interactions *in vivo* that are most responsible for tissue function. Bhatia and colleagues have designed a 3D photopatterned hydrogel construct containing hepatocytes such that its hexagonal architecture can mimic *in vivo* liver lobule structure and maximize perfusion, which is essential for highly metabolic hepatocytes.[34] Langer and coworkers have demonstrated enhanced vascular network formation in engineered skeletal muscle constructs with the addition of fibroblasts, and this prevascularization improved perfusion of implanted constructs.[32] As these engineered structures continue to evolve to higher levels of complexity, tissue function will also be enhanced proportionally to strengthen our efforts in engineering tissues for regenerative medicine.

ACKNOWLEDGMENTS

This work was supported in part by the NIH (EB00262, HL73305, GM74048). Shen acknowledges support from the Paul and Daisy Soros Foundation.

REFERENCES

[1]Aguilar, R. C., and B. Wendland. Endocytosis of membrane receptors: two pathways are better than one. *Proc. Natl. Acad. Sci. USA* 102:2679–2680, 2005.

[2]Albrecht, D. R., G. H. Underhill, T. B. Wassermann, R. L. Sah, and S. N. Bhatia. Probing the role of multicellular organization in three-dimensional microenvironments. *Nat. Methods* 3:369–375, 2006.

[3]Bernstein, S. A., and G. E. Morley. Gap junctions and propagation of the cardiac action potential. *Adv. Cardiol.* 42:71–85, 2006.

[4]Bhatia, S. N., U. J. Balis, M. L. Yarmush, and M. Toner. Probing heterotypic cell interactions: hepatocyte function in microfabricated co-cultures. *J. Biomater. Sci. Polym. Ed.* 9:1137–1160, 1998.

[5]Bhatia, S. N., M. L. Yarmush, and M. Toner. Controlling cell interactions by micropatterning in co-cultures: hepatocytes and 3T3 fibroblasts. *J. Biomed. Mater. Res.* 34:189–199, 1997.

[6]Burdick, J. A., A. Khademhosseini, and R. Langer. Fabrication of gradient hydrogels using a microfluidics/photopolymerization process. *Langmuir* 20:5153–5156, 2004.

[7]Carmeliet, P., and M. Tessier-Lavigne. Common mechanisms of nerve and blood vessel wiring. *Nature* 436:193–200, 2005.

[8]Carter, S. B. Principles of cell motility: the direction of cell movement and cancer invasion. *Nature* 208:1183–1187, 1965.

[9]Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber. Geometric control of cell life and death. *Science* 276:1425–1428, 1997.

[10]Chen, C. S., M. Mrksich, S. Huang, G. M. Whitesides, and D. E. Ingber. Micropatterned surfaces for control of cell shape, position, and function. *Biotechnol. Prog.* 14:356–363, 1998.

[11]Chung, B. G., L. A. Flanagan, S. W. Rhee, P. H. Schwartz, A. P. Lee, E. S. Monuki, and N. L. Jeon. Human neural stem cell growth and differentiation in a gradient-generating microfluidic device. *Lab. Chip* 5:401–406, 2005.

[12]Dike, L. E., C. S. Chen, M. Mrksich, J. Tien, G. M. Whitesides, and D. E. Ingber. Geometric control of switching between growth, apoptosis, and differentiation during angiogenesis using micropatterned substrates. *In Vitro Cell. Dev. Biol. Anim.* 35:441–448, 1999.

[13]Discher, D. E., P. Janmey, and Y. L. Wang. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310:1139–1143, 2005.

[14]Driever, W., and C. Nusslein-Volhard. The bicoid protein determines position in the *Drosophila* embryo in a concentration-dependent manner. *Cell* 54:95–104, 1988.

[15]Elbjeirami, W. M., and J. L. West. Angiogenesis-like activity of endothelial cells co-cultured with VEGF-producing smooth muscle cells. *Tissue Eng.* 12:381–390, 2006.

[16]Engler, A. J., S. Sen, H. L. Sweeney, and D. E. Discher. Matrix elasticity directs stem cell lineage specification. *Cell* 126:677–689, 2006.

[17]Farge, E. Mechanical induction of Twist in the *Drosophila* foregut/stomodeal primordium. *Curr. Biol.* 13:1365–1377, 2003.

[18]Gospodarowicz, D., G. Greenburg, and C. R. Birdwell. Determination of cellular shape by the extracellular matrix and its correlation with the control of cellular growth. *Cancer Res.* 38:4155–4171, 1978.

[19]Gray, D. S., J. Tien, and C. S. Chen. Repositioning of cells by mechanotaxis on surfaces with micropatterned Young's modulus. *J. Biomed. Mater. Res. A* 66:605–614, 2003.

[20]Hahn, M. S., M. K. McHale, E. Wang, R. H. Schmedlen, and J. L. West. Physiologic pulsatile flow bioreactor conditioning of poly(ethylene glycol)-based tissue engineered vascular grafts. *Ann. Biomed. Eng.* 35:190–200, 2007.

[21]Hahn, M. S., J. S. Miller, and J. L. West. Three-dimensional biochemical and biomechanical patterning of

hydrogels for guiding cell behavior. *Adv. Mater.* 18:2679–2684, 2006.
[22]Harbers, G. M., and K. E. Healy. The effect of ligand type and density on osteoblast adhesion, proliferation, and matrix mineralization. *J. Biomed. Mater. Res. A* 75:855–869, 2005.
[23]Heldin, C. H. Dimerization of cell surface receptors in signal transduction. *Cell* 80:213–223, 1995.
[24]Hsu, S., R. Thakar, D. Liepmann, and S. Li. Effects of shear stress on endothelial cell haptotaxis on micropatterned surfaces. *Biochem. Biophys. Res. Commun.* 337:401–409, 2005.
[25]Huang, S., C. P. Brangwynne, K. K. Parker, and D. E. Ingber. Symmetry-breaking in mammalian cell cohort migration during tissue pattern formation: role of random-walk persistence. *Cell Motil. Cytoskeleton* 61:201–213, 2005.
[26]Ingber, D. E. Fibronectin controls capillary endothelial cell growth by modulating cell shape. *Proc. Natl. Acad. Sci. USA* 87:3579–3583, 1990.
[27]Ingber, D. E., and J. Folkman. Mechanochemical switching between growth and differentiation during fibroblast growth factor-stimulated angiogenesis in vitro: role of extracellular matrix. *J. Cell Biol.* 109:317–330, 1989.
[28]Jiang, X., R. Ferrigno, M. Mrksich, and G. M. Whitesides. Electrochemical desorption of self-assembled monolayers noninvasively releases patterned cells from geometrical confinements. *J. Am. Chem. Soc.* 125:2366–2367, 2003.
[29]Karp, J. M., J. Yeh, G. Eng, J. Fukuda, J. Blumling, K. Y. Suh, J. Cheng, A. Mahdavi, J. Borenstein, R. Langer, and A. Khademhosseini. Controlling size, shape and homogeneity of embryoid bodies using poly(ethylene glycol) microwells. *Lab. Chip* 7:786–794, 2007.
[30]Kim, B. S., J. Nikolovski, J. Bonadio, and D. J. Mooney. Cyclic mechanical strain regulates the development of engineered smooth muscle tissue. *Nat. Biotechnol.* 17:979–983, 1999.
[31]LaMack, J. A., and M. H. Friedman. Individual and combined effects of shear stress magnitude and spatial gradient on endothelial cell gene expression. *Am. J. Physiol. Heart Circ. Physiol.* 293:H2853–H2859, 2007.
[32]Levenberg, S., J. Rouwkema, M. Macdonald, E. S. Garfein, D. S. Kohane, D. C. Darland, R. Marini, C. A. van Blitterswijk, R. C. Mulligan, P. A. D'Amore, and R. Langer. Engineering vascularized skeletal muscle tissue. *Nat. Biotechnol.* 23:879–884, 2005.
[33]Li Jeon, N., H. Baskaran, S. K. Dertinger, G. M. Whitesides, L. Van de Water, and M. Toner. Neutrophil chemotaxis in linear and complex gradients of interleukin-8 formed in a microfabricated device. *Nat. Biotechnol.* 20:826–830, 2002.
[34]Liu Tsang, V., A. A. Chen, L. M. Cho, K. D. Jadin, R. L. Sah, S. DeLong, J. L. West, and S. N. Bhatia. Fabrication of 3D hepatic tissues by additive photopatterning of cellular hydrogels. *FASEB J.* 21:790–801, 2007.
[35]Liu, L., B. D. Ratner, E. H. Sage, and S. Jiang. Endothelial cell migration on surface-density gradients of fibronectin, VEGF, or both proteins. *Langmuir* 23:11168–11173, 2007.
[36]Lo, C., H. Wang, M. Dembo, and Y. Wang. Cell movement is guided by the rigidity of the substrate. *Biophys. J.* 79:144–152, 2000.
[37]Matsuda, T., Y. Saito, and K. Shoda. Cell sorting technique based on thermoresponsive differential cell adhesiveness. *Biomacromolecules* 8:2345–2349, 2007.
[38]Matsumoto, T., J. Sasaki, E. Alsberg, H. Egusa, H. Yatani, and T. Sohmura. Three-dimensional cell and tissue patterning in a strained fibrin gel system. *PLoS ONE* 2:e1211, 2007.
[39]Matsumoto, T., Y. C. Yung, C. Fischbach, H. J. Kong, R. Nakaoka, and D. J. Mooney. Mechanical strain regulates endothelial cell patterning in vitro. *Tissue Eng.* 13:207–217, 2007.
[40]McBeath, R., D. M. Pirone, C. M. Nelson, K. Bhadriraju, and C. S. Chen. Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Dev. Cell* 6:483–495, 2004.
[41]McNeill, H. Sticking together and sorting things out: adhesion as a force in development. *Nat. Rev. Genet.* 1:100–108, 2000.
[42]Nakatsu, M. N., R. C. Sainson, J. N. Aoto, K. L. Taylor, M. Aitkenhead, S. Perez-del-Pulgar, P. M. Carpenter, and C. C. Hughes. Angiogenic sprouting and capillary lumen formation modeled by human umbilical vein endothelial cells (HUVEC) in fibrin gels: the role of fibroblasts and Angiopoietin-1. *Microvasc. Res.* 66:102–112, 2003.
[43]Nelson, C. M., R. P. Jean, J. L. Tan, W. F. Liu, N. J. Sniadecki, A. A. Spector, and C. S. Chen. Emergent patterns of growth controlled by multicellular form and mechanics. *Proc. Natl. Acad. Sci. USA* 102:11594–11599, 2005.
[44]Pallante, B. A., I. Duignan, D. Okin, A. Chin, M. C. Bressan, T. Mikawa, and J. M. Edelberg. Bone marrow Oct3/4+ cells differentiate into cardiac myocytes via age-dependent paracrine mechanisms. *Circ. Res.* 100:e1–e11, 2007.
[45]Park, J., F. Berthiaume, M. Toner, M. L. Yarmush, and A. W. Tilles. Microfabricated grooved substrates as platforms for bioartificial liver reactors. *Biotechnol. Bioeng.* 90:632–644, 2005.
[46]Park, J. W., B. Vahidi, A. M. Taylor, S. W. Rhee, and N. L. Jeon. Microfluidic culture platform for neuroscience research. *Nat. Protoc.* 1:2128–2136, 2006.
[47]Park, S., P. M. Wolanin, E. A. Yuzbashyan, P. Silberzan, J. B. Stock, and R. H. Austin. Motion to form a quorum. *Science* 301:188, 2003.
[48]Paszek, M. J., N. Zahir, K. R. Johnson, J. N. Lakins, G. I. Rozenberg, A. Gefen, C. A. Reinhart-King, S. S. Margulies, M. Dembo, and D. Boettiger. Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8:241–254, 2005.
[49]Plummer, S. T., Q. Wang, P. W. Bohn, R. Stockton, and M. A. Schwartz. Electrochemically derived gradients of the extracellular matrix protein fibronectin on gold. *Langmuir* 19:7528–7536, 2003.
[50]Ray, R. P., K. Arora, C. Nusslein-Volhard, and W. M. Gelbart. The control of cell fate along the dorsal-ventral axis of the *Drosophila* embryo. *Development* 113:35–54, 1991.
[51]Re, F., A. Zanetti, M. Sironi, N. Polentarutti, L. Lanfrancone, E. Dejana, and F. Colotta. Inhibition of anchorage-dependent cell spreading triggers apoptosis in cultured human endothelial cells. *J. Cell Biol.* 127:537–546, 1994.
[52]Ridley, A. J., M. A. Schwartz, K. Burridge, R. A. Firtel, M. H. Ginsberg, G. Borisy, J. T. Parsons, and A. R. Horwitz. Cell migration: integrating signals from front to back. *Science* 302:1704–1709, 2003.
[53]Sakiyama-Elbert, S. E., A. Panitch, and J. A. Hubbell. Development of growth factor fusion proteins for cell-triggered drug delivery. *FASEB J.* 15:1300–1302, 2001.

[54]Singh, A. B., K. Sugimoto, and R. C. Harris. Juxtacrine activation of epidermal growth factor (EGF) receptor by membrane-anchored heparin-binding EGF-like growth factor protects epithelial cells from anoikis while maintaining an epithelial phenotype. *J. Biol. Chem.* 282:32890–32901, 2007.

[55]Singhvi, R., A. Kumar, G. P. Lopez, G. N. Stephanopoulos, D. I. Wang, G. M. Whitesides, and D. E. Ingber. Engineering cell shape and function. *Science* 264:696–698, 1994.

[56]Smith, J. T., J. K. Tomfohr, M. C. Wells, T. P. Beebe, Jr., T. B. Kepler, and W. M. Reichert. Measurement of cell migration on surface-bound fibronectin gradients. *Langmuir* 20:8279–8286, 2004.

[57]Steinberg, M. S. On the mechanism of tissue reconstruction by dissociated cells. I. Population kinetics, differential adhesiveness, and the absence of directed migration. *Proc. Natl. Acad. Sci. USA* 48:1577–1582, 1962.

[58]Takayama, S., J. C. McDonald, E. Ostuni, M. N. Liang, P. J. Kenis, R. F. Ismagilov, and G. M. Whitesides. Patterning cells and their environments using multiple laminar fluid flows in capillary networks. *Proc. Natl. Acad. Sci. USA* 96:5545–5548, 1999.

[59]Takayama, S., E. Ostuni, P. LeDuc, K. Naruse, D. E. Ingber, and G. M. Whitesides. Subcellular positioning of small molecules. *Nature* 411:1016, 2001.

[60]Taylor, A. M., M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman, and N. L. Jeon. A microfluidic culture platform for CNS axonal injury, regeneration and transport. *Nat. Methods* 2:599–605, 2005.

[61]Tepass, U., D. Godt, and R. Winklbauer. Cell sorting in animal development: signalling and adhesive mechanisms in the formation of tissue boundaries. *Curr. Opin. Genet. Dev.* 12:572–582, 2002.

[62]Van Haastert, P. J. M., and P. N. Devreotes. Chemotaxis: signalling the way forward. *Nat. Rev. Mol. Cell Biol.* 5:626–634, 2004.

[63]von Degenfeld, G., A. Banfi, M. L. Springer, R. A. Wagner, J. Jacobi, C. R. Ozawa, M. J. Merchant, J. P. Cooke, and H. M. Blau. Microenvironmental VEGF distribution is critical for stable and functional vessel growth in ischemia. *FASEB J.* 20:2657–2659, 2006.

[64]Wang, S. J., W. Saadi, F. Lin, C. Minh-Canh Nguyen, and N. Li Jeon. Differential effects of EGF gradient profiles on MDA-MB-231 breast cancer cell chemotaxis. *Exp. Cell Res.* 300:180–189, 2004.

[65]Wong, J. Y., A. Velasco, P. Rajagopalan, and Q. Pham. Directed movement of vascular smooth muscle cells on gradient-compliant hydrogels. *Langmuir* 19:1908–1913, 2003.

[66]Yeo, W. S., and M. Mrksich. Electroactive self-assembled monolayers that permit orthogonal control over the adhesion of cells to patterned substrates. *Langmuir* 22:10816–10820, 2006.

[67]Yousaf, M. N., B. T. Houseman, and M. Mrksich. Using electroactive substrates to pattern the attachment of two different cell populations. *Proc. Natl. Acad. Sci. USA* 98:5992–5996, 2001.

[68]Zaari, N., P. Rajagopalan, S. K. Kim, A. J. Engler, and J. Y. Wong. Photopolymerization in microfluidic gradient generators: microscale control of substrate compliance to manipulate cell response. *Adv. Mater.* 16:2133–2137, 2004.

[69]Zisch, A. H., M. P. Lutolf, M. Ehrbar, G. P. Raeber, S. C. Rizzi, N. Davies, H. Schmokel, D. Bezuidenhout, V. Djonov, P. Zilla, and J. A. Hubbell. Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. *FASEB J.* 17:2260–2262, 2003.