Controlling Stem Cell Fate with Material Design

By *Ross A. Marklein* and *Jason A. Burdick**

Advances in our understanding of stem cell interactions with their environment are leading to the development of new materials-based approaches to control stem cell behavior toward cellular culture and tissue regeneration applications. Materials can provide cues based on chemistry, mechanics, structure, and molecule delivery that control stem cell fate decisions and matrix formation. These approaches are helping to advance clinical translation of a range of stem cell types through better expansion techniques and scaffolding for use in tissue engineering approaches for the regeneration of many tissues. With this in mind, this progress report covers basic concepts and recent advances in the use of materials for manipulating stem cells.

1. Introduction

Stem cells are becoming an important component of approaches for regenerative medicine, especially within the rapidly expanding field of tissue engineering. Tissue engineering aims to develop biologically inspired 3D constructs that integrate with native tissue and/or stimulate the body's innate repair mechanisms to regenerate damaged tissue and restore function.[1] Due to an aging population and demand for a higher quality of life, the emergence of tissue engineering as a solution to repair a multitude of tissues is evident. Within the tissue-engineering paradigm, the selection of the appropriate cells, materials, and biological molecules will ultimately determine success or failure. With their ability to proliferate, self-renew, and differentiate, stem cells are becoming a promising cell source for these applications.

The successful incorporation of stem cells into tissue engineering strategies is contingent upon a thorough knowledge of factors influencing stem cell behavior. Uncommitted stem cells in the developing embryo, for example, are subjected to regional differences in their microenvironments, which result in the formation of every tissue in the human body. Through an understanding of the cues that drive stem cell fate decisions, it may be possible to incorporate these cues into the design of future 3D microenvironments to optimize and facilitate tissue repair and regeneration. These cues include soluble/immobilized factors, chemical and physical signals from the extracellular matrix (ECM), cell morphology, and external stresses. Furthermore, it is not only the simple presence of these cues that is crucial to a stem cell's response, but also their spatial and temporal context. Due to the complex nature of stem cell fate decisions and the constant "crosstalk" among different signals, it is necessary to design 3D microenvironments that consider the interplay of these diverse cues.

Biomaterials design is expanding with new material syntheses and processing techniques to enhance the complexity of 3D environments in order to direct stem cell lineage commitment.[2,3] These materials can be utilized as cell delivery vehicles, scaffolds for cell adhesion, surfaces for cell culture, and a source of soluble/immobilized factors, among others. Microenvironments can be designed to feature an intense signal to drive differentiation, or a myriad of signals that address the biologically relevant sequence of events leading to lineage commitment (Fig. 1). An understanding of materials science and chemical syntheses allows for the creation of biomaterials that can manipulate stem cells for specific tissue engineering applications. Much of this work has focused on mesenchymal stem cells (MSCs), possibly due to the ease of culture and widespread applicability in regenerative medicine, yet this technology is widely applicable to numerous stem cell types. This progress report will focus on general concepts of using materials to control stem cells, as well as provide examples of recent advances within this rapidly expanding field.

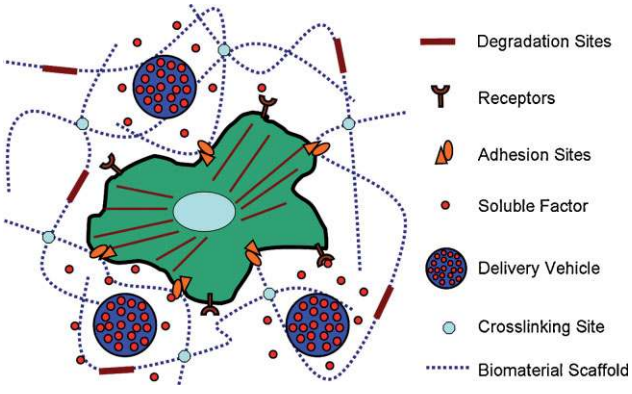

Figure 1. The stem cell microenvironment. Material control can be exerted at many levels through adhesion, cell factor binding, material degradation and mechanics, and cell morphology to manipulate stem cell interactions and fate.

[*] Prof. J. A. Burdick, R. A. Marklein
Department of Bioengineering, University of Pennsylvania
210 S 33rd Street, Philadelphia, PA 19104 (USA)
E-mail: burdick2@seas.upenn.edu

DOI: 10.1002/adma.200901055

2. Biomaterial Structure and Chemistry as Differentiation Cues

The use of biomaterials as scaffolds is a fundamental and important component of tissue engineering since these materials serve as templates for tissue formation and are engineered depending on the tissue of interest. These scaffolds not only provide mechanical and 3D structural support for cells, but can also provide cues to induce tissue repair. The structure, morphology, degradation and presentation of bioactive sites are all important parameters in material design for these applications and may signal the differentiation of stem cells.

2.1. Structures of Biomaterials for 3D Cellular Environments

Biomaterial scaffolds take on a variety of structures based on their material composition and processing to form 3D environments for cell delivery or invasion. These materials consist of natural polymers such as collagen, hyaluronic acid (HA), fibrin, or alginate, or synthetic polymers such as polyethylene glycol (PEG), dextran, or polyvinyl alcohol and can be formed into hydrogels, fibrous structures, and macroporous scaffolds.[4,5] Figure 2 illustrates examples of the formation and structure of each of these scaffold types. The biomaterial structure controls how a cell interacts with the material and is important in stem cell fate decisions since the presentation of cues and cellular morphology are dependent on this structure.

2.1.1. Hydrogels

Hydrogels are comprised of insoluble networks of crosslinked polymers with high water contents (>90%).[6] Hydrogels with the ability to encapsulate stem cells have been used for applications such as cartilage[7,8] and cardiac[9,10] tissue regeneration. In order to achieve tissue formation, stem cells must either be encapsulated within or recruited to the hydrogel. Cells can be encapsulated in hydrogels through various means including self-assembly, ionic crosslinking, and radical polymerizations.[11] For example, the water-soluble photoinitiator I2959 (Irgacure, 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-1-propanone) can be used to initiate crosslinking upon exposure to UV light with materials containing acrylate or methacrylate groups.[5,12] It is important to note that potential side effects to UV light should be thoroughly assessed, particularly with stem cells that may be susceptible to damage. Hydrogels are advantageous for cell encapsulation due to the high water content and diversity in chemistry and properties that can be obtained. It is important to consider the viability of stem cells during the encapsulation process and with culture, including the diffusion of nutrients and wastes to and from the cells. Hydrogels are dependent on factors such as the charge and chemistry of the polymer and crosslinking density. Additionally, interpenetrating networks (IPNs) can be used to further alter hydrogel properties by combining properties of each polymer.[13] One class of hydrogels that is gaining interest for stem cell encapsulation is HA based gels. HA is a natural polymer that was initially used as an implantable biomaterial to study wound healing and biocompatibility in order to monitor vascularization, inflammatory responses, and matrix secre-

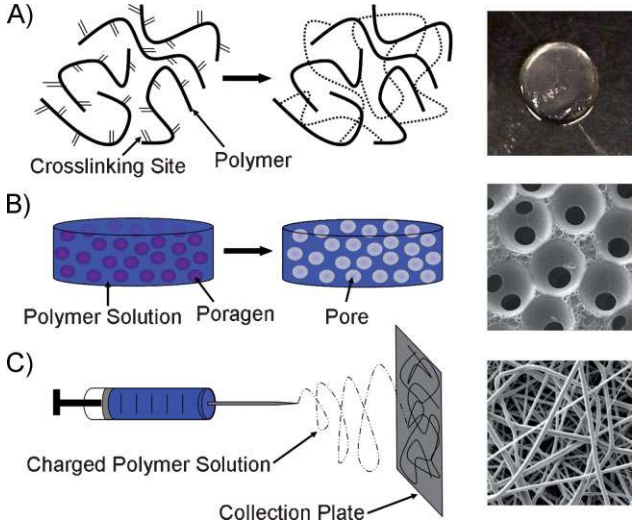

Figure 2. Scaffold fabrication and morphology. A) Polymers with reactive groups are crosslinked to form a highly swollen hydrogel network. B) Porous network formation through a poragen leaching process. C) Polymer electrospinning where an electric field causes a charged polymer solution to travel from a syringe to a grounded surface leaving distinct nano/micrometer-sized fibers.

Ross Marklein is currently a Bioengineering PhD student at the University of Pennsylvania under the supervision of Jason Burdick. He earned his B.S. in Biomedical Engineering at Georgia Institute of Technology in 2007. He currently is developing a hydrogel system with spatially controlled mechanical properties to control stem cell behavior. His research interests include stem cell mechanotransduction, dual crosslinked hydrogel networks, and stem cell morphological control.

Jason A. Burdick is the Wilf Family Term Assistant Professor in the Department of Bioengineering at the University of Pennsylvania. His laboratory develops biodegradable polymers to control stem cell behavior and for tissue engineering and drug delivery applications. He has received a K22 Award from the NIH, a Fellowship in Science and Engineering from the Packard Foundation, an Early Career Award from the Coulter Foundation, and a CAREER Award from the NSF.

tion.[14,15] While HA does not possess any inherent crosslinking ability, chemical modification allows for crosslinking.[16,17]

2.1.2. Fibrous Scaffolds

Although hydrogels provide a highly controlled 3D microenvironment for cells, the nature of this scaffold does not entirely mimic the structure of native ECM. In particular, the crosslinked polymer network does not possess a fibrillar architecture that is prevalent in ECM components such as collagen and fibrin.[18,19] One common method to create scaffolds with a fibrous morphology is the process of electrospinning. This method involves extruding a charged polymer solution through a blunt needle, which is attracted to a grounded material due to a large potential difference.[18] Electrospinning has been used to produce fibrous scaffolds from a wide range of polymers with diverse properties, both synthetic and natural, for a range of tissue applications.[20] Another attractive feature is that the fibers can be aligned by spinning on a rotating mandrel to produce anisotropy in both the bulk physical properties and in cellular morphology and matrix production.[21,22] However, one of the limitations of this technique is the potentially poor cell infiltration into the scaffold, either when seeded or when implanted. As demonstrated by Baker et al.,[23] it is possible to combine multiple polymer jets and a rotating mandrel to create electrospun scaffolds that have desired anisotropic mechanical properties, as well as enhanced MSC infiltration (due to removal of ''sacrificial fibers''). In general, the diversity in materials that can be obtained with fibrous morphologies and the potential advantages of the structure makes these scaffolds useful for controlling stem cells.

2.1.3. Macroporous Scaffolds

One of the most widely used biomaterial structures for tissue engineering involves macroporous scaffolds, which can form interconnected porous networks that allow for cellular infiltration and tissue formation. These scaffolds are often formed with leachable components (such as salt crystals or microspheres) around which the desired polymer forms a scaffold.[24] Upon removal of the leachable components, a 3D structure can be obtained with varying parameters such as pore size, porosity, and interconnectivity. Linnes et al.[25] created a macroporous scaffold based on fibrinogen using sintered PMMA microspheres, which allowed for a highly porous, interconnected 3D microenvironment that upon addition of thrombin or genipin significantly increased in stability and mechanics. In another study, poly-(ε-caprolactone) scaffolds (formed using a gas foaming technique) with varied pore size and interconnectivity were created to monitor osteogenesis of dura mater stem cells.[26] In the case of large pore sizes, cells may interpret the environment as 2D; however, the macrostructure of the scaffold allows for the creation of a 3D tissue as cells synthesize and interact with secreted matrix.

2.2. Chemical Signals in Biomaterials

Stem cells may interact with biomaterials through surface receptors such as integrins and cell adhesion molecules.[27] The selection of a biomaterial must take into consideration the inherent cell adhesivity of a material (e.g., in the case of natural materials) or the ability to confer additional biofunctionality in order to elicit a particular response from stem cells. Adhesion may be desirable or undesirable depending on the desired differentiation path and native cell environment. There are a wide range of techniques to control adhesion, including altering the hydrophobicity of a material to influence protein adsorption or by tethering proteins or their analogs directly to a material. Beyond adhesion, other chemical cues may be included to manipulate stem cell interactions and differentiation, either directly or indirectly by controlling protein interactions.

2.2.1. Cell Adhesion Motifs

A simple and common technique in many tissue engineering strategies is to incorporate analogs of native ECM components into scaffolds in order to control stem cell interactions. The fibronectin binding domain arginine–glycine–aspartic acid (RGD) has been widely used to promote binding sites for $\alpha v\beta 3$ integrins in applications such as osteogenesis and chondrogenesis.[28,29] The effects of RGD concentration and its spatial organization have been investigated and determined to be regulators of stem cell morphology, proliferation, and differentiation.[30] While RGD is used as a ''default'' binding site for biomaterials, efforts have been made to investigate the contextual presentation of RGD within fibronectin and its effect on stem cell behavior. Martino et al.[31] demonstrated that the presentation of certain fibronectin domains, including RGD and its synergy sequence PHSRN, can significantly affect MSC spreading and proliferation. Additionally, other sequences are being investigated for cell specific differentiation such as laminin-derived IKVAV and YIGSR.[32]

2.2.2. Chemistry of Biomaterials

More indirect approaches (e.g., controlling hydrophobicity) toward addressing cell recognition of biomaterials have produced interesting results. For example, by altering the hydrophobicity of a surface, the formation and differentiation potential of embryonic stem cells (ESCs) within embryoid bodies (EBs) could be tuned to promote desirable EB size and composition.[33] In another study, Benoit et al.[34] altered the microenvironment by introducing different small molecules such as phosphates, carboxylic acids, and aliphatic chains (very hydrophobic). The presence of these molecules led to increased MSC expression of bone, cartilage, and fat associated markers of differentiation, respectively.

It is often difficult to predict how a stem cell will respond to its environmental cues and thus methods have been developed to rapidly screen biomaterials and stem cell interactions.[35–37] The use of a combinatorial library of biomaterials formed from different acrylate and methacrylate monomers proved to be useful for identifying environments suitable for uniform ESC differentiation into epithelial cells.[38] Figure 3 shows one example of a screening of the influence of material chemistry on ESC differentiation. Further combinatorial studies were performed on MSCs, neural stem cells (NSCs), and primary articular chondrocytes using monomers with varied degradation, hydrophobicity, molecular weight, and crosslinking.[39] This method allows for determination of ideal microenvironments for stem cell differentiation and can also be coupled with other induction factors (as discussed later) to screen thousands of possible scenarios for controlling stem cell behavior.[40] Rapid screening techniques are useful in that they can identify unique environments that cannot

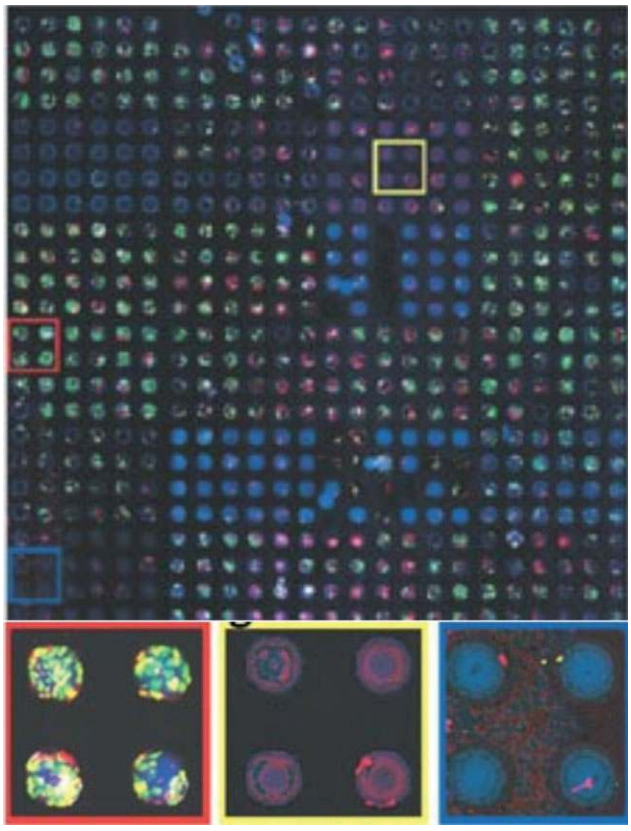

Figure 3. Investigating stem cell and material interactions with polymer arrays. Top: Human ESCs cultured on a polymer in the presence of RA for 6 days and then stained for cytokeratin 7 (green), vimentin (red), and nuclei (blue). Bottom: Three examples of polymers highlighted from above array. Reproduced with permission from [38]. Copyright 2004, Nature Publishing Group.

be predicted based on material structure and chemistry. The materials in these studies are also inexpensive and much easier to synthesize than scaffolds possessing complex chemistries and cell recognition sites.[41–43] These studies indicate that biomaterial design does not need to exactly mimic native tissue, but rather possess the fundamental characteristics that promote desired stem cell behavior.

2.2.3. Natural and Synthetic Biomaterials

A major advantage of using naturally derived materials is that they possess desired cell recognition sites to control cellular behavior such as adhesion and degradation. For example, fibrin hydrogels consist of polymerized fibrinogen, which possesses multiple direct binding sites, as well as sites that bind growth factors, fibronectin, HA, and von Willebrand factor.[19] The addition of thrombin to fibrinogen allows for the formation of fibrin hydrogels consisting of nanometer scaled fibers that can be recognized by cells. Early studies using dorsal root ganglia demonstrated the effects of varied fibrin network formation on neurite extension by adding biorecognition molecules and factor XIII, which participates in covalent crosslinking.[44] PEGylated fibrinogen has been used by several groups in order to utilize the stem cell recognition of fibrinogen while also allowing for more control and variation of network degradation and mechanics.[25,45,46] Another route for creating desirable 3D microenvironments for stem cells is to harness the potential regenerative properties of stem cell-derived biomaterials. Nair et al.[47,48] developed a biomaterial from acellularized EBs using Triton-X/DNAse treatments to remove cellular components while maintaining the ECM components such as collagen IV, laminin, and fibronectin. EBs induced toward a specific lineage and subsequently acellularized could create a stem cell-derived biomaterial with desired morphogenic cues for a given tissue engineering application.

HA is another naturally occurring material (i.e., polysaccharide) consisting of repeating disaccharide units and has been implicated in many stem cell fate decisions.[49] Extensive work by Shu et al.[14,50] has involved chemically modifying HA to confer additional cell recognition, degradability, and crosslinking ability. The presence of hydroxyl and carboxyl groups allows for chemical modification of the HA backbone with methods such as carbodiimide chemistry. Another useful modification of HA (and other hydroxyl containing polymers) is the addition of methacrylates or acrylates, which allows for radical polymerization.[16] Significant work has been performed using photocrosslinkable HA hydrogels for stem cell encapsulation, specifically involving cartilage tissue engineering.[7,51]

While natural materials provide inherent instructive cues for stem cells, limitations of these materials include a possible immune response, potential loss of biological activity during processing, and insufficient mechanical properties. In many cases, synthetic materials are used as ''blank slates'' that can be modified to confer biofunctionality and promote stem cell differentiation. One of the most common synthetic materials used as a backbone for hydrogel systems is PEG. Due to its hydrophilicity and ease of modification, highly swollen hydrogels can be formed that also contain cell recognition sites.[52] For example, PEG coupled to poly(L-Lysine) promotes greater neural progenitor survival and differentiation to mature neural phenotypes than unmodified PEG hydrogels.[53] This is potentially due to the charged amino side chains present in lysine, which allow for cell adhesion and survival and can also provide sites for further chemical modification. A recent study demonstrated the temporally controlled presentation of cell binding using PEG hydrogels coupled with a matrix metalloproteinase (MMP)-cleavable RGD peptide.[54] The motivation behind this study was that initiation of chondrogenesis is dependent on fibronectin, but persistence of this binding inhibits long-term chondrogenesis.[55,56] The incorporation of an MMP-13 cleavable linker resulted in increased glycosaminoglycan production, as well as a greater percentage of collagen II positive cells compared to undifferentiated MSCs.

2.3. Scaffold Degradation

While biomaterials may consist of either natural or synthetic materials, it is generally accepted that they serve as a temporary scaffold and, as new tissue is formed, they should degrade. Therefore, it is necessary to design materials that degrade over a timescale that corresponds with a given application (i.e., formation of mature, functional tissue). Structurally, scaffold degradation allows for cellular infiltration, as well as ECM

synthesis and distribution. The ideal degradation profile, in terms of tissue mechanical properties, may be a decrease in scaffold mechanical properties over time, with the concurrent synthesis of ECM by cells.[1] While this may be oversimplified, it is important to address biodegradability of biomaterials when designing a scaffold. Beyond structural importance, scaffold degradation also controls temporal properties, including the presentation of chemical and mechanical cues at different times in development and regeneration.

Cell-mediated degradation is best evidenced by naturally occurring MMP degradation of ECM components such as collagen. In more synthetic materials, MMP-sensitive sequences can be incorporated as crosslinkers, which degrade once the encapsulated or migrating cells begin to secrete MMPs.[57–59] These sequences are typically used to promote degradation of the biomaterial as the cells begin to secrete matrix components and remodel their surroundings. Stem cells secrete specific MMPs that correlate with their lineage commitment (e.g., MMP-3 for ESC cardiogenesis, MMP-9 for NSC commitment, and MMP-13 for chondrogenesis).[54,60,61] Scaffolds possessing MMP-1 sensitive sites promoted greater cell infiltration and matrix deposition than scaffolds without these sites when implanted in a cranial defect.[58] Therefore, biomaterials have been designed to incorporate these sequences in order to allow for cell spreading/infiltration and matrix remodeling.[54,58,62] Importantly, the ability to remodel and spread in matrices may be a signal in controlling differentiation and lineage commitment in stem cells, both through cell–cell interactions and morphological cues.

Hydrolysis represents another major route for scaffold degradation that can be utilized to facilitate tissue formation or alter scaffold properties with time. By incorporating hydrolytically degradable units into a scaffold or by altering the amount of a given degradable unit, a desired degradation profile can be achieved. For example, although cells can secrete hyaluronidases, which have the ability to degrade HA, this degradation does not occur on a time scale that promotes adequate matrix deposition in covalently crosslinked HA gels. Hydrolytically degradable lactic acid (LA) units can be incorporated into the HA backbone in order to allow for a controlled degradation rate and increased matrix production.[63] Additionally, LA groups have also been incorporated into non-degradable PEG hydrogels in order to facilitate scaffold degradation and promote neural precursor differentiation into neurons and glial cells.[64] In another PEG system, the step growth polymerization of dithiothreitol (DTT) and PEG diacrylate (PEGDA) formed acrylate terminated PEG-DTT with a range of molecular weights. Varying the extent of polymerization allowed for different molecular weights, which resulted in varied degradation and swelling properties. MSC morphology and viability were found to be dependent on network degradability as cells encapsulated in more degradable gels were more viable and spread.[65]

3. Controlled Presentation and Delivery of Differentiation Factors

In standard stem cell cultures, growth factors are simply added to culture media to induce a differentiation program. Significant advances have been made in understanding how these factors can control stem cell fates in controlled in vitro cultures.[66] While this method of simply adding a cocktail of factors to cells can be quite powerful, it is typically not possible for implantable materials and does not account for desirable spatial presentation. Thus, efforts are being made to control the spatial and temporal presentation of these factors in order to mimic the native tissue development. From a materials perspective, differentiation factors can be added directly to the medium for in vitro cultures (including with bioreactors), physically entrapped or sequestered within a scaffold, or encapsulated in micro/nanoparticles for controlled release.

3.1. Soluble Factor Delivery

The ability to easily manipulate and control the addition of soluble factors to culture medium makes this approach the most well characterized effector of stem cell differentiation. In combination with the morphology of clusters of cells (e.g., 2D surface for osteogenesis, 3D pellets for chondrogenesis), much is known about stem cell differentiation using standard tissue culture approaches. These factors not only participate in the commitment of cells, but also the decision of cells to remain quiescent or undifferentiated. For example, Leukemia inhibitory factor (LIF) is commonly employed to prevent ESCs from differentiating and is added to ESC cultures in order to allow them to proliferate without spontaneously differentiating.[67] Typically, the goal with biomaterials is to aid in stem cell differentiation; however, there is also interest in materials that prevent differentiation for use as substrates in cell culture.

The addition of growth factors to cultures (either added to culture media or via material delivery) can act in synergy with other tissue engineering strategies to optimize stem cell differentiation and tissue formation. For example, bone morphogenic protein-2 (BMP-2) has been incorporated into HA hydrogels along with MSCs to promote osteogenesis, as noted by increased osteocalcin and CD31 expression compared to controls without BMP-2.[17] The TGF-β family of proteins are well documented chondrogenic factors and are typically added to scaffolds in combination with encapsulated stem cells.[7,68] However, it is not only the addition of this growth factor that is crucial to chondrogenesis, but also the temporal presentation. Long term exposure to TGF-β2 resulted in greater GAG and collagen II production and an upregulation in Sox-9 when compared to MSCs with shorter exposure time.[69] As mentioned previously, high throughput screening can also be a useful tool for determining which factors are regulators of stem cell fate so that they can be incorporated into tissue engineering applications.[40,70]

3.2. Immobilized Factors

Similar to coupling cell adhesion motifs to scaffolds, differentiation factors can be specifically immobilized on a biomaterial surface to elicit a desired response. This is a common theme in nature as stem cell niches contain covalently and non-covalently bound factors that maintain the cell's undifferentiated state. Stem cell factor (SCF) and LIF are membrane-bound cytokines found in niches that support undifferentiated stem cells.[32] LIF can be

added to inhibit ESC differentiation, but the immobilization of LIF can also affect ESC commitment.[71] LIF immobilized to a non-woven polyester fabric (NWPF) using carbodiimide chemistry was shown to support a greater percentage of undifferentiated ESC colonies when compared to the NWPF only groups. The immobilized LIF was shown to be bound in its active form and had a similar effect (in terms of pluripotency maintenance) to adding soluble LIF to the culture medium. Another study immobilized both LIF and SCF in order to observe the threshold behavior of certain factors on stem cell maintenance.[72] Additionally, growth factors such as TGF-β1 have been immobilized on surfaces to promote chondrogenesis of MSCs rather than simply adding it to the culture.[73,74]

While the ability to covalently tether factors to biomaterials has shown great promise, another technique involves a more biomimetic approach by which growth factors are sequestered using non-covalent means. Heparin is a sulfated proteoglycan that has the ability to bind and sequester growth factors and thus slow their release while maintaining their biological activity. Specifically, heparin can bind TGF-β proteins and influence stem cell differentiation into chondrocytes, which has been demonstrated using poly(N-isopropylacrylamide-*co*-acrylic acid) thermoresponsive hydrogels.[75] In this system, MSCs in gels containing heparin-bound TGF-β3 had significantly greater upregulation of chondrogenic markers of differentiation, specifically collagen II, Sox-9, and aggrecan. However, the applicability of this system depends specifically on the binding affinity of the protein to heparin. A similar strategy was used with a porous PLGA scaffold in which both dexamethasone and heparin-bound TGF-β1 were incorporated and its chondrogenic potential evaluated using MSCs.[76] Heparin-binding has also been utilized in electrospinning applications so that cells not only experience the desired fibrous morphology and adhesive properties of the electrospun material, but also the added effect of immobilized factors. Casper et al.[77] covalently linked both heparin and perlecan (another sulfated proteoglycan associated with mesenchymal tissues) to collagen and gelatin electrospun scaffolds using EDC/NHS carbodiimide chemistry. Using fibroblast growth factor-2 (FGF-2) as their model growth factor, they demonstrated that both heparin and perlecan effectively bound FGF-2, but perlecan displayed better binding at lower concentrations. FGF-2 is secreted by osteoblastic cells and is present in the early stages of bone repair and its biological activity is significantly enhanced by heparan sulfate binding. This method could prove useful in bone regeneration applications along with the inclusion of other heparan sulfate binding proteins such as BMP-2 and platelet-derived growth factor (PDGF).

3.3. Encapsulated Delivery Vehicles

Another means to control the presentation of differentiation factors to the stem cell microenvironment is through the use of biodegradable delivery vehicles. These vehicles can take the form of polymeric microparticles as well as the scaffold itself, which can be tailored to release encapsulated factors. Release is controlled through both diffusion and degradation, and thus material design is essential for released molecule presentation to stem cells.

3.3.1. Controlled Release from Scaffolds

A direct method to release differentiation factors to the stem cell microenvironment is through encapsulation within the 3D scaffold. As biodegradability is a desired property of biomaterials, many researchers have utilized this degradation to not only allow for remodeling of the microenvironment and ECM synthesis, but to also allow for local delivery of factors to aid in stem cell commitment and tissue repair.

Due to their highly swollen state, hydrogels are able to rapidly deliver factors to surrounding tissue or to encapsulated cells within the hydrogel. Cardiogenesis can be affected by controlled release of basic FGF (bFGF) from gelatin hydrogels with or without cardiosphere derived cells (CDCs) or MSCs.[78] bFGF release significantly enhanced vascularization, as well as myocardial perfusion and contractility. While coupling the delivery of bFGF with CDCs resulted in greater myocardiocyte differentiation and engraftment than bFGF treatment alone, MSCs did not exhibit the same additive effects of combined growth factor and cell transplantation on recovery of myocardial function. In order to promote greater chondrogenesis of MSCs encapsulated in hydrogels, TGF-β3 has been encapsulated during the hydrogel formation process in order to locally deliver the factor for in vivo and in vitro tissue formation.[7,79] In one example, MSCs were encapsulated in methacrylated HA (to allow for photocrosslinking) and either polymerized in situ with TGF-β3 or pre-cultured for 2 weeks in growth medium containing TGF-β3 and subsequently implanted subcutaneously.[7] Pre-cultured constructs exhibited higher collagen II, aggrecan, and chondroitin sulfate expression compared to constructs encapsulated with TGF-β3 and negative controls without growth factor. These results emphasize the importance of sustained release of a factor to stem cells in order to elicit the desired differentiation and tissue formation response. One approach to control release from hydrogels is by modifying the degradation rate of the network structure. Using a PEG dimethacrylate system incorporating hydrolytically degradable lactide units into the PEG backbone, Benoit et al.[80] demonstrated a highly regulated delivery of fluvastatin, which stimulates BMP-2 production and osteogenic differentiation. The release rate and dose were controlled by adjusting the lactide repeat unit length and initial fluvastatin concentration, respectively. The incorporation of controlled release into this network resulted in increased ALP, collagen I, and BMP-2 production by encapsulated human MSCs.

3.3.2. Controlled Delivery Using Particles

Both micro- and nanoparticles have received considerable attention in applications such as cancer therapeutics and biomedical imaging modalities, but are also useful for the delivery of molecules to stem cells.[81,82] Since stem cells undergoing lineage commitment require a specific spatio-temporal presentation of factors, efforts have been made to incorporate these particles into biomaterials for controlled release rates. It is also important to consider the activity of the encapsulated factor upon release, which is dependent upon the process for encapsulation.

Microparticles can also be utilized without a biomaterial scaffold in order to control the stem cell microenvironment. Micro- and nanoparticles have been injected with and without stem cells into injury sites to promote both neurogenesis and chondrogenesis.[83,84] Using a water-in-oil-in-water (W/O/W)

emulsion technique, ciliary neurotrophic factor and brain-derived neurotrophic factor were encapsulated in poly(lactic-co-glycolic acid) (PLGA) microspheres to allow for sustained release and aid in regeneration of central nervous tissue and retinal tissue, respectively. By coating larger O/W PLGA microspheres encapsulating one factor (dexamethasone, DEXA) with smaller W/O/W emulsion microspheres encapsulating another factor (dehydroepiandrosterone, DHEA), the release of multiple factors is possible.[85] The negative charges of the carboxyl on PLGA microspheres containing DHEA are electrostatically attracted to the positive charge of poly(ethyleneimine) incorporated into the DEXA-loaded microspheres. This minimally invasive injection of microspheres and stem cells could prove to be advantageous as the cells form cartilage tissue around the microspheres and then fill in the voids once they degrade.

Microspheres can also be utilized in ESC differentiation to allow for more control over the 3D microenvironment within EBs. EBs consist of an aggregate of pluripotent stem cells that possess the ability to differentiate into all the germ layers (endoderm, mesoderm, ectoderm). However, within this aggregate, the microenvironment varies by location due initially to cell–cell contact and diffusional constraints and later by local matrix and paracrine factor secretion.[86] Efforts have been made to influence the aggregation of ESCs into EBs in order to create more uniform EB populations, but significant improvements are needed in order to exercise more control over differentiation within these aggregates.[87,88] Encapsulation of differentiation factors into microspheres and incorporating them into differentiating EBs could allow for more control over the ESC microenvironment. Carpenedo et al.[89] demonstrated a highly controlled method of incorporating retinoic-acid (RA) loaded PLGA microspheres into EBs. Rotary suspension culture was used to allow for uniform EB formation and to facilitate the microsphere incorporation. Compared to normal EBs and EBs incorporating unloaded microspheres, EBs containing RA-loaded microspheres exhibited a very homogeneous and organized structure. Furthermore, EBs incorporating RA-loaded microspheres exhibited a completely different structure than EBs exposed to soluble RA. Figure 4 illustrates these morphological differences in EBs exposed to different microenvironments as microsphere-mediated delivery of RA led to an increase in endoderm/epiblast organization as compared to the non-cystic unorganized EBs exposed to soluble RA. The desired cellular morphology, whether it is uniform or heterogeneous, of the EBs is dependent on the application. This method of locally delivering factors within a differentiating EB bypasses the limitations associated with soluble delivery as it has been shown that a dense shell containing collagen I, tight cell–cell junctions, and basement membrane hinder diffusive transport.[90]

Angiogenesis is a critical process with the formation of many tissue types because it allows for adequate nutrient supply and integration with native tissue. In tissue engineering applications, it is necessary to not only stimulate the differentiation of stem cells into the specialized tissue cell of interest, but to also allow for formation of vasculature in the tissue.[91] Two growth factors intimately involved in the process of vascularization are vascular endothelial growth factor (VEGF) and PDGF. However, it is not only the presence of these two factors that influences angiogenesis, but also their temporal presentation. VEGF is responsible for the initiation of angiogenesis and involves endothelial cell activation and proliferation while PDGF is required after VEGF activation in order to allow for blood vessel maturation through recruitment of smooth muscle cells. Richardson et al.[92] developed a dual growth factor release system in which VEGF is encapsulated in the porous PLGA scaffold and PDGF is encapsulated in PLGA microspheres dispersed throughout the scaffold. Based on release kinetics, they demonstrated an initial rapid release of VEGF and a delayed release of PDGF, which contributed to greater maturation of vessels as evidenced by α-smooth muscle actin compared to VEGF or PDGF factor addition only. In a similar system, BMP-2 and BMP-7 loaded into PLGA microspheres at different concentrations (and thus different release rates) was investigated as a system for bone tissue regeneration.[93] The sequential delivery of BMP-2 and BMP-7 in porous PLGA scaffolds resulted in enhanced osteogenic differentiation of MSCs as evidenced by cell proliferation and alkaline phosphatase (ALP) activity.

While PLGA microparticles have received the most attention as delivery vehicles for stem cell applications, other notable microencapsulating carriers exist. Naturally derived materials such as alginate, chitosan, and gelatin have been used to encapsulate factors based on their biocompatibility and ability to crosslink by ionic and chemical means.[94–96] Based on a given application, the release kinetics can be tailored by altering the polymer composition, method of formation and encapsulation, and post-formation processing (such as coating or complexing with other materials).

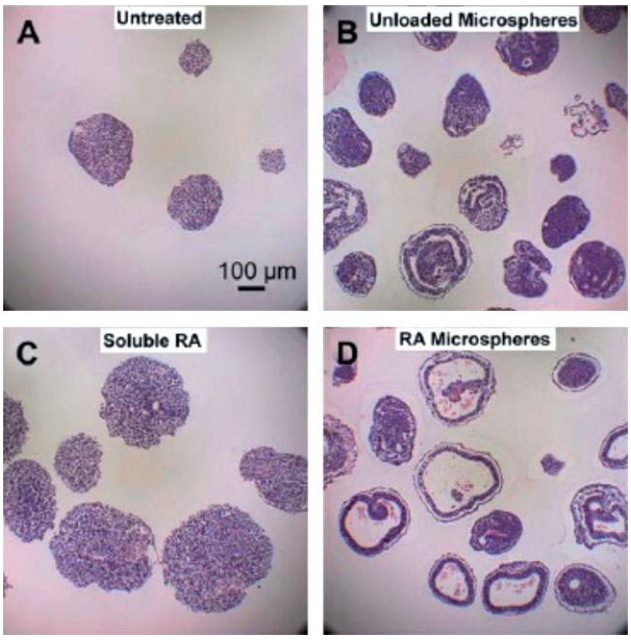

Figure 4. Microsphere molecule delivery. Hematoxylin–Eosin staining of EBs in A) untreated, B) unloaded incorporated microspheres, C) soluble retinoic acid delivery, D) retinoic acid loaded incorporated microsphere groups, indicating that controlled and local RA delivery controls the morphology of EBs. Reproduced with permission from [89]. Copyright 2009, Elsevier.

4. Material Control of Cell Morphology

Fully differentiated cells take on a variety of well-recognized shapes both in vivo and during in vitro culture ranging from striated, contractile myoblasts to spherical chondrocytes, to highly branched neurons. While there has been considerable research concerning the adoption of specific cell morphologies as a result of differentiation, the concept of cell morphology as an effector of differentiation, and not simply a consequence, has only recently received significant attention.

4.1. Cell Adhesion Regulates Morphology

The importance of cell adhesion to materials not only involves the general support of cells and signal transduction (as mentioned in previous sections), but can also dictate cellular morphology. For instance, the effects of integrin binding and cytoskeletal organization on cell morphology and chondrogenesis were investigated using RGD-coupled agarose and alginate gels.[29,56] Increased RGD concentrations in alginate gels resulted in a diminished expression of chondrogenic genes and deposition of collagen II and proteoglycans by encapsulated MSCs. Furthermore, soluble RGD peptide addition helped recover the chondrogenic potential since it competed with bound ligands in the gel.[56] In a follow up study, RGD coupled agarose gels were used to investigate the effect of morphology and cytoskeletal organization on MSC chondrogenesis.[29] Increased cell spreading and differences in cytoskeleton arrangement were observed in gels with higher RGD concentrations. The addition of a potent inhibitor of actin polymerization (cytochalasin D) prevented the inhibitory effects of RGD on chondrogenesis, which reinforces the concept that integrin binding and coupling with the cytoskeleton can play a pivotal role in MSC differentiation.

The distribution of cell binding molecules also influences stem cell morphology and lineage commitment. Specifically, the formation of focal adhesion complexes has been well documented to involve integrin clustering and inside–out coupling with the actin cytoskeleton.[97,98] Comisar et al.[30] studied the effects of different ligand presentations on pre-osteoblast morphology and osteogenic differentiation. RGD was covalently coupled to alginate gels using carbodiimide chemistry and the degree of substitution was varied to create alginate chains with a range of peptide modifications. By changing the ratio of modified to unmodified alginate for different degrees of substitution, they were able to control the total bulk RGD density, as well as the spacing of adhesive ''islands.'' Cell morphology and osteogenic differentiation were found to be dependent on ligand spacing, while proliferation was found to be dependent on bulk RGD density. Lower ligand spacing favored focal adhesion formation and cell spreading, while higher spacing resulted in greater osteocalcin expression. The effects of bulk RGD on proliferation were shown to be biphasic, as an increase in RGD led to a maximal proliferation rate beyond which any increase in RGD density resulted in diminished proliferation.

The organization of a stem cell's cytoskeleton as a result of its microenvironment can also have a pronounced effect on lineage specification. Non-muscle myosins (NMMs) have been implicated in the regulation of cell morphology and NMMIIs are particularly implicated in stem cell morphological processes.[99–101] Myoblast alignment and striation, which are crucial to its contractility, are a result of the cell's adhesion and surrounding microenvironment. Specifically, the roles of NMMIIA and NMMIIB include involvement in the formation of myoblast bipolar morphology and prevention of over-elongating differentiating myotubes, respectively.[102] The importance of polarization is also evident as neurons exhibit a preferential directionality that is required for their functionality. Aligned fibrous scaffolds and micropatterned surfaces have been used to direct neural progenitor cells to adopt the appropriate cell morphologies due to either fiber morphology or the presence of desired adhesion molecules.[103] The addition of gelatin to PCL electrospun fibers resulted in enhanced neurite outgrowth and alignment of NSCs (C17.2 cells) in the direction of the electrospun fibers. The presence of alignment in collagen and collagen/carbon nanotube structures also resulted in preferential ectoderm differentiation of ESCs compared to non-aligned gelatin scaffolds, which showed differentiation into all three germ layers.[104] The synergy between adhesion and neural progenitor alignment was also demonstrated using a co-culture of hippocampal progenitor cells (HPCs) and astrocytes in the presence of patterned laminin substrates.[105] The NSC niche involves specific cell–cell and cell–matrix contact and this study demonstrated that the presence of both factors (i.e., alignment and cellular interactions) influenced progenitor morphology and resulted in greater expression of a neural marker of differentiation (β3-tubulin). Similar results were found in a study involving MSCs differentiating into cardiac muscle cells.[106] Co-culture of these predifferentiated cells on aligned substrates with cardiomyocytes resulted in greater electrical conduction and upregulation of cardiogenic markers of differentiation compared to co-cultures on isotropic substrates. While adhesion to specific molecules can initiate a differentiation program, the presentation of these adhesion sites allows for proper coupling of cell morphological and signal transduction pathways.

4.2. Control of Cell Shape Directs Differentiation

The physical control of stem cell binding and morphology results in profound effects on stem cell behavior, including differentiation. Controlling materials through crosslinking, feature sizes, and topography represent various means to influence cell morphology, and thus differentiation.

4.2.1. Extent and Type of Crosslinking Controls Cell Morphology

Within a 3D scaffold such as a hydrogel, the ability of a cell to spread and adopt a specific morphology can be influenced by the crosslinking density, which is either static or dynamic using non-degradable or degradable components, respectively. PEG hydrogels have been modified by several groups with varied crosslinking (e.g., length of crosslinker or incorporation of IPNs) and to incorporate hydrolytic and cell-sensitive degradation to modulate stem cell spreading.[107–109] As stated above, the mesh size can be used to control features such as ECM distribution by encapsulated stem cells. As an additional example, MSCs encapsulated in degradable PEG-*co*-cyclic acetal gels exhibited different morphologies based on the crosslinking density. For

example, gels that were more swollen promoted a more spindled morphology than highly crosslinked gels.[110] Cell viability was high in all formulations and cell morphology was directly correlated to crosslinking density as cells were more spread in less crosslinked networks.

Network degradation plays a temporal role in both the restriction of cell morphology and ECM synthesis of stem cells. Biomaterials provide initial adhesion and mechanical cues that influence cell morphology and signal transduction and the subsequent commitment and formation of tissue is contingent upon proper material degradation. HA hydrogels have been developed that not only influence stem cell fate decisions, but have controlled degradation to enhance ECM distribution.[63] For instance, a comparison of two non-degradable hydrogel networks (methacrylated HA and PEG diacrylate) indicated that where MSCs are maintained in a spherical shape, other factors such as cell recognition sites (such as CD44 receptor binding to HA) and growth factors (such as TGF-β3) can control differentiation.[7] Further modifications to HA to control cell morphology have included incorporating MMP-sensitive cleavage sites, which allow for MSC spreading compared to the rounded morphology found in non-degradable crosslinked gels.[111] In a system involving vinyl-terminated 4-arm PEG, the inclusion of MMP-degradable sites allowed for spreading and the adoption of a smooth muscle cell phenotype for MSCs.[112] In this gel, both MSCs and SMCs acquired a spindled, elongated shape that influenced cytoskeletal organization and adoption of the desired smooth muscle cell phenotype. It is expected that these same trends of crosslinking and degradation are important for all types of stem cells, yet this area has focused primarily on MSCs.

4.2.2. Differentiation Effects of Feature Sizes

Cell morphology can also be controlled by the size of features on a 2D substrate or the size of individual components of a scaffold. The growth and differentiation of EBs in microwells of defined size has provided definitive evidence of how feature size influences stem cell fate decisions.[113] Compared to traditional suspension culture, EBs cultured in PEG-coated wells of different diameters showed remarkably lower levels of variability in terms of SSEA-1 and α-fetoprotein expression. EB size homogeneity is crucial for applications in which other material effects on stem cell differentiation are being studied, in order to eliminate unaccountable variability.[33,86,87,89,114] The restriction of cell spreading on functionalized surfaces has also produced interesting results concerning stem cell differentiation. MSCs cultured on fibronectin-coated islands of various sizes resulted in commitment of cells to adipocytes on smaller islands and osteoblasts on larger islands.[115] Surfaces with varying degrees of hydrophobicity and terminal end groups were also studied to determine how EB size and morphology affect ESC differentiation (similar to the microwell study).[33] After separating EB populations based on size, it was discovered that intermediate-sized EBs (100–300 μm) showed the highest viability, lowest apoptotic rate, and highest differentiation potential.

Stem cells grown on fibrous scaffolds have also shown differentiation dependent behavior in terms of the fiber chemistry, size, and alignment. For example, MSCs grown on electrospun aligned PCL scaffolds showed preferential differentiation to a chondrogenic lineage on nanoscale versus microscale fibers.[116] While cells aligned in the direction of the fibers for both nano- and microscale scaffolds, the nanofibers (~500 nm diameter) promoted higher GAG and mRNA expression of collagen II and aggrecan. Similar results were observed in the case of NSCs grown on poly(L-LA) (PLLA) electrospun fibers.[117] Again, while fiber diameter did not influence the extent of alignment, NSCs were found to have a higher level of differentiation on nanofibers compared to microfibers based on neurofilament expression and neurite outgrowth. Since native ECM components possess features on the order of nanometers, these findings emphasize the importance of biomimicry when developing scaffolds for stem cell differentiation. Electrospinning allows for a great degree of control over fiber chemistry through choice of polymer, fiber size through changes in polymer concentration, and fiber alignment through design of the electrospinning apparatus.[20]

4.2.3. Topography Influences Differentiation

Electrospun fibers represent one means by which scaffold features can be designed in order to influence stem cell spreading and adhesion. In the same study that found that nanoscale fibers promoted chondrogenic differentiation of MSCs, it was also found that chondrogenic differentiation was enhanced on nanofibers over porous PCL scaffolds.[116] Similar to fibers of controlled diameter, several studies have been performed to investigate the influence of micropatterned ridges or grooves on stem cells. For example, patterned grooves 300 nm deep were formed with varying widths (10, 25, and 100 μm) and the osteogenic effects on MSCs were compared.[118] Based on gene microarray analysis, MSCs grown on 100 μm grooves showed significant upregulation in genes associated with skeletal development compared to other groove sizes. In another study, the effects of topography were found to have a greater influence on MSC neurogenesis than a potent neurogenic soluble factor RA.[119] Nanoscale grooves showed greater enhancement in MSC neural differentiation compared to smooth or microscale groove substrates. This also provides interesting insight into the concept of transdifferentiation of stem cells, particularly MSCs transdifferentiating into neuronal cells as evidenced by increased β3-tubulin, MAP2, and glial fibrillary acidic protein expression. It should be noted that the concept of transdifferentiation of MSCs into neurons is controversial and work still needs to be performed in this area.

Beyond fibers and grooves, surface roughness has also been shown to regulate stem cell behavior. MSCs on PLGA scaffolds treated with an alkalizing agent to incorporate surface roughness resulted in upregulation of ALP, bone sialoprotein, osteocalcin, and VEGF during the initial stages of MC3T3 pre-osteoblast culture compared to non-treated PLGA scaffolds.[120] In another study, MSCs grown on He-irradiated PCL showed an increase in ALP activity and collagen production compared to non-irradiated and tissue culture polystyrene (TCPS) controls.[121] The irradiation resulted in a "smoothening" of the PCL material and, most importantly, no change in surface energy that could affect protein adsorption, thus confirming the effect surface topography has on stem cell differentiation. While these studies show conflicting results, they demonstrate a clear dependence of stem cell differentiation on surface topography.

4.3. Patterned Stem Cell Morphology

The ability to spatially control stem cell spreading and subsequent fate decisions is of great importance in tissue engineering applications due to the heterogeneous nature of tissues. Specialized zonal architecture in cartilage, cardiac muscle fiber arrangement, and the varied degrees of vascularity represent critical hierarchical organizations within tissues that provide their unique functions.[122–124] Patterning of biomaterials can be achieved by spatially controlling the physical restraints surrounding a cell or by patterning adhesion molecules in order to control stem cell spreading.

One of the most prevalent methods of patterning 3D microenvironments is through the use of photopolymerization. The ability to spatially control the location, intensity, and duration of light allows for high pattern fidelity and extensive processing capabilities.[5] Complex hydrogel features can be produced by an additive-polymerization process in which a crosslinked PEG network is immersed in a solution of non-crosslinked PEGDA and subsequently exposed to UV light.[125] The use of a photomask restricts light (and consequently, crosslinking) to certain regions. In another additive polymerization process, PEGDA combined with amino-functionalized PEG allowed for multi-layered assemblies of gels that resembled microvascular networks through multiple photopolymerization steps.[126] Another means to spatially control cell morphology is through the combination of sequential crosslinking steps that occur by distinct methods. This has been demonstrated by groups using HA as the base network, which is first crosslinked with chemical crosslinks (e.g., Michael addition) and then exposed to UV light in order to crosslink remaining methacrylate or acrylate functional groups.[127] Khetan et al.[62] demonstrated with acrylated HA hydrogels that MSC spreading can be patterned based on the type of crosslinking in specific regions. Using an MMP-degradable and thiol-terminated crosslinker, a fraction of available acrylates were consumed during the initial Michael addition crosslinking step. Exposing one half of the gel to UV light effectively restricted cell spreading in these dual crosslinked regions and allowed MSCs in non-exposed regions to thoroughly spread (Fig. 5). In vasculature and nervous tissue, the maintenance of organized cell spreading is of critical importance and the ability to photopattern and control cell morphology in distinct regions could prove useful for these applications.

Patterning of cell adhesion sites can also serve to control cell morphology and stem cell differentiation within a 3D scaffold. The ability of multi-photon confocal microscopy to focus light in a specific plane (and certain regions within this plane) provides the technology to photopattern adhesive molecules within a hydrogel network.[128] Similar to the additive polymerization methods, a solution of acrylated PEG-coupled RGD peptide was allowed to equilibrate within a PEGDA network. By programming the region of interest and depth of the feature, channels of RGD adhesion sites were conferred within the PEG hydrogel. Cell spreading was restricted in regions not exposed to UV light and the coupling of RGD to exposed regions (in the form of channels) allowed for spreading and migration of cells from a fibrin cluster encapsulated within the gel. This method could be used to spatially control cell spreading and promote infiltration of recruited stem cells and vasculature.

5. Matrix Mechanics Direct Stem Cell Differentiation

Considerable evidence exists for cell mechanosensitivity, primarily in systems where cells experience external stresses, such as shear and tension, which results in changes in protein expression and, in some cases, differentiation.[129,130] Recently, the inherent mechanical properties of a material have received considerable attention with regards to controlling stem cell behavior. The stiffness of a material is governed by the structure and composition of the network components, extent of crosslinking (both physical and covalent), and the organization of the network (whether it is anisotropic or part of an IPN).

5.1. Cell Mechanosensitivity

Native tissues range widely in composition (ECM components) and mechanics (0.1–1 kPa in neural tissue to on the order of GPa for fully mineralized bone tissue).[100,131] When adhesion-dependent cells are grown on materials of varying mechanics, depending on the cell type, there are noticeable differences in

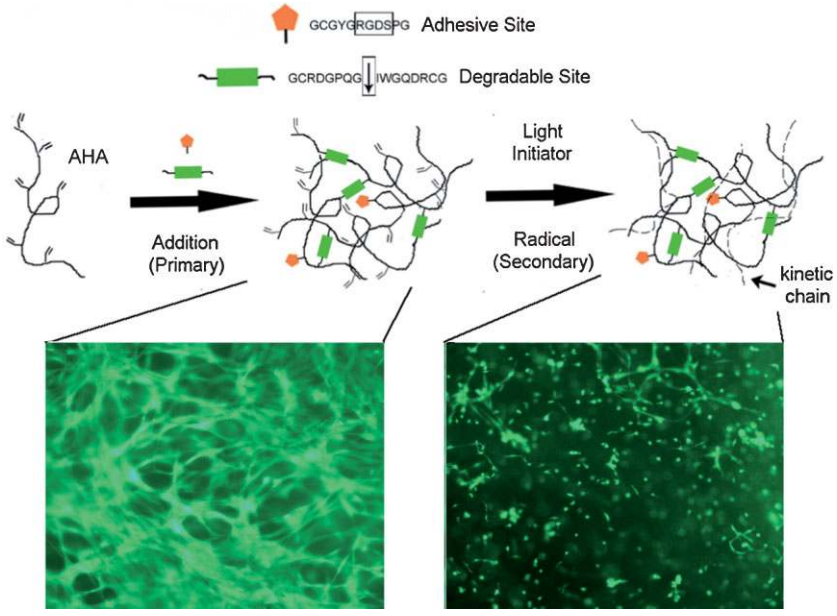

Figure 5. Controlling stem cell spreading. Sequential crosslinking of HA hydrogels containing adhesive (orange symbols) and MMP-degradable (green rectangles) sites. Encapsulated MSCs are able to remodel the matrix after the addition crosslinking (left), but not after the radical polymerization (right). This technique allows for spatial patterning of cellular spreading when light is used for the secondary radical polymerization step.

terms of cell morphology and gene expression. Initial insight into the possibility of mechanics influencing stem-cell fate decisions can be gained by observing the native tissue mechanics. Muscle tissue exhibits a stiffness of ~10 kPa and myoblasts cultured on polyacrylamide gels of varying mechanics showed optimal alignment and striation on substrates that mimicked this mechanical environment.[132] Furthermore, when myoblasts were cultured in multiple layers, cells exposed to the soft environment (on top of other myoblasts) differentiated into multi-nucleated, aligned myotubes more readily than those in contact with the rigid glass substrate in the bottom layer. Hepatocytes and neural cells exhibit similar stiffness-dependent behavior as the hepatocytes aggregate and neurons extend neurites (both indicative of their associated phenotypes) on more compliant (less stiff) matrices.[133] The consequences of aberrant tissue mechanics are apparent in situations such as myocardial infarction and liver disease, in which the stiffening of tissues results in changes in cell morphology and loss of tissue function.[100] Therefore, the mechanics of the tissue of interest should be accounted for when designing a material for tissue regeneration.

5.2. Controlling Stem Cells with Material Mechanics

The ability of stem cells to sense their 3D microenvironmental mechanics is not fully understood, although there are several well-documented factors involved in mechanosensing and mechanotransduction. Specifically, the coupling of cell adhesion molecules (such as integrins) to the cytoskeleton and the formation of focal adhesion complexes is highly dependent on matrix stiffness in both differentiated and undifferentiated cells.[134,135] The interplay of adhesion ligands and stiffness was investigated in one study to determine possible synergistic effects of the two factors on MSC differentiation.[134] MSCs grown on substrates containing collagen I, collagen IV, fibronectin, or laminin with varying stiffness were investigated for their myogenic and osteogenic potential. Osteogenesis was regulated by both stiffness and ligand type, as MSCs showed the highest upregulation in Runx2 (a transcription factor in osteoblasts) in the stiffest polyacrylamide gels containing collagen I (a major component of native bone tissue). Myogenesis, while not as stiffness dependent as osteogenesis, required a threshold stiffness (>9 kPa) before sufficient cell spreading and upregulation in MyoD1 occurred. NMM has also been implicated as part of the mechanosensing machinery. Adhesion to the matrix is governed by integrins, and coupling with the actin cytoskeleton allows the cell to form a direct link with its microenvironment, which can then be sensed through intracellular tension governed by myosin II motors. The addition of blebbistatin, a potent inhibitor of NMMII, resulted in a significant reduction in elasticity of developing zebrafish embryos and disruption of stem cell differentiation.[136] Different isoforms of NMMII also showed varied expression at different stiffnesses, but one isoform (NMMIIA) showed little variation among different stiffnesses, possibly suggesting its ubiquitous role in mechanosensing.

The effect of stiffness on stem cell differentiation is best exemplified by Engler et al.[137] in which polyacrylamide gels of varying stiffness and constant collagen I concentration were used to examine MSC behavior. Figure 6 illustrates the striking expression profiles for cells grown on gels with elasticity matching the native tissue elasticity. Cells grown on soft (0.1–1 kPa), intermediate (11 kPa), and stiff (34 kPa, similar to non-mineralized bone) gels differentiated to neurogenic, myogenic, and osteogenic lineages, respectively. Addition of blebbistatin to cultures effectively inhibited this mechanosensing by disrupting the actin cytoskeleton and intracellular tension. This lineage commitment was found to depend solely on the elasticity of the substrate since the cells were exposed to a constant collagen density and cultured in growth medium without differentiation factors.

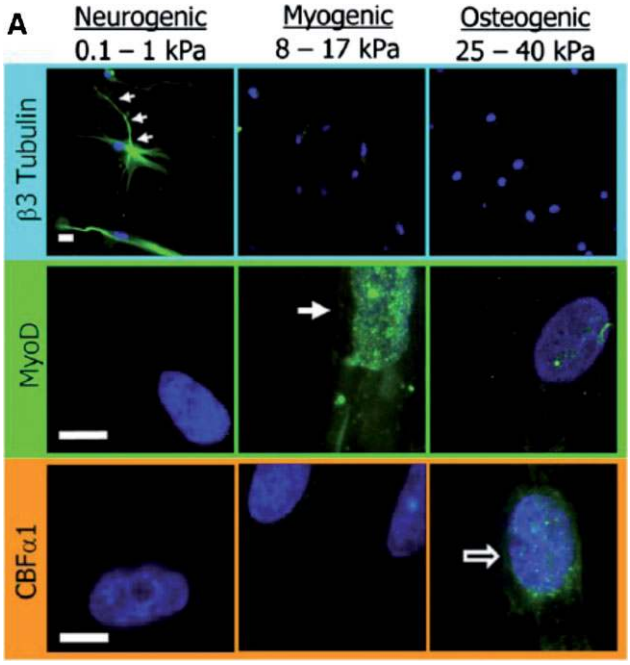

Figure 6. Matrix mechanics dictates MSC differentiation. MSCs grown on polyacrylamide gels of three stiffnesses (0.1–1 kPa, 8–17 kPa, and 25–40 kPa) expressed differentiation markers characteristic of cells found in tissues exhibiting similar stiffnesses. β3-tubulin indicates presence of neurogenic cytoskeletal filaments, myogenic differentiation factor 1 (MyoD1) a myogenic transcription factor, and core binding factor alpha 1 (CBFA1) an osteogenic transcription factor. Reproduced with permission from [137]. Copyright 2006.

The effects of mechanics on NSCs were examined using a semi-IPN network of polyacrylamide and PEG.[138] The addition of PEG to the network allows for modulation of mechanics (due to PEG hydrophilicity) without contributing to the biofunctionality of the material, as the RGD concentration was kept constant. NSCs cultured on these semi-IPNs showed differentiation profiles that correlated well with native tissue (i.e., neurons formed on softer substrates and astrocytes formed on stiffer substrates). This observed differentiation behavior is consistent with other studies in which primary neurons and astrocytes were cultured on gels of various moduli.[139,140]

Local mechanical control of stem cell microenvironments can also be accomplished by patterning colonies of cells. In MSC aggregates grown on patterned cell adhesive surfaces, patterns of differentiation were observed that corresponded with local strains

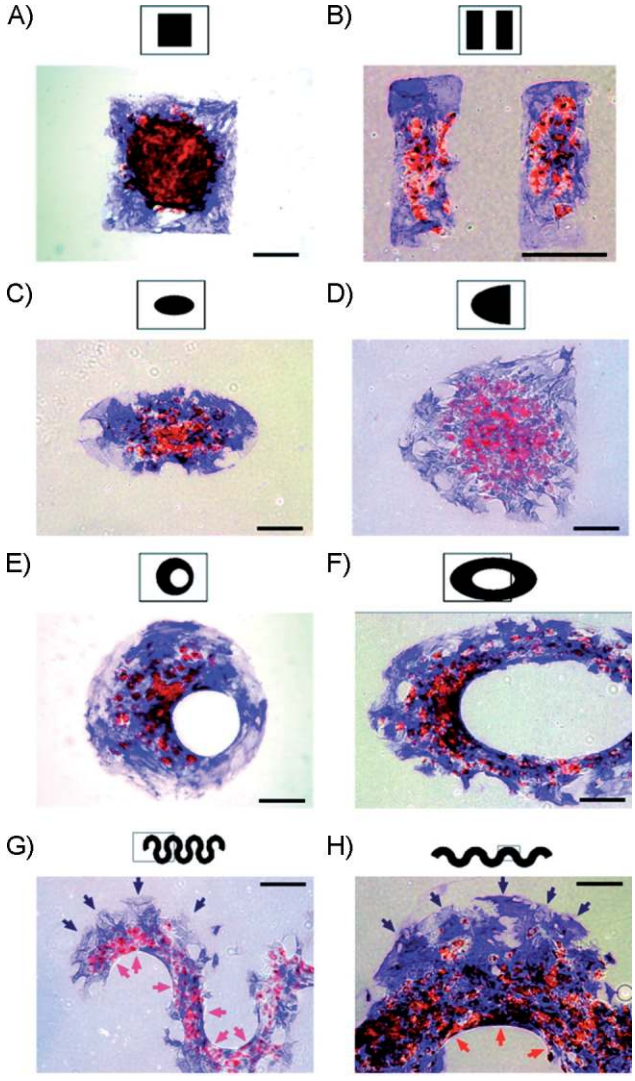

Figure 7. Patterned organization of differentiating MSC aggregates. Fat droplets (red) and ALP (blue) activity were localized to specific regions corresponding to traction forces and geometry: square **(A)**, rectangle **(B)**, ellipse **(C)**, half-ellipse **(D)**, offset annulus **(E)**, elliptical annulus **(F)**, and sinusoidal bands **(G, H)** after 14 days. Red arrows indicate adipogenesis at concave edges, and blue arrows indicate osteogenesis at convex edges. Scale bars = 250 μm. Reproduced with permission from [141]. Copyright 2008, Alpha Med Press.

experienced by cells.[141] In rounded aggregates, a radial pattern of differentiation was observed where cells in the center were committed to an adipogenic lineage and cells in the periphery were driven to an osteogenic lineage (Fig. 7). Furthermore, in more complex geometries, field strains experienced by cells resulted in patterned differentiation behavior, where cells in softer regions were driven to adipogenesis versus stiffer regions where cells were driven to osteogenesis. In this same study, MSCs exhibited a differentiation pattern similar to that of long bones (i.e., osteogenic zones on the outside and an inner adipogenic zone) when cultured in 3D tubular collagen hydrogels. The use of a constitutively active Rho-kinase gene (involved in cytoskeletal tension) resulted in a thicker osteogenic outer zone due to an increase in tractional forces and local mechanics.

Pre-osteoblasts exposed to soft and stiff RGD-functionalized PEG gels expressed higher levels of MAPK activation and osteocalcin secretion on stiffer gels.[142] Activation of MAPK (through phosphorylation) has been associated with focal adhesions and further downstream activation of Runx2, which regulates osteocalcin and ALP expression. RhoA is another molecule involved in the generation of intracellular tension, and is influenced by matrix mechanics in both differentiated and undifferentiated cells. RhoA expression can be controlled by altering the cell morphology, as well as the stiffness of the substrate.[115,143] Changes in RhoA expression in MSCs seeded on soft and stiff polyacrylamide gels resulted in different Ca^{2+} oscillations.[144] MSC Ca^{2+} oscillations are controlled by ROCK, a downstream effector molecule of RhoA, and therefore can be modulated by the mechanics of the substrate. Cells such as pancreatic acinar cells and cardiomyocytes demonstrate spontaneous Ca^{2+} oscillations, and applications involving these tissues would likely benefit from the use of materials with tunable mechanics to direct stem cell differentiation.

Hydrogels with controlled mechanics have also been used to investigate the differences in mechanosensitivity of various cell types. Cells can possess varied degrees of mechanosensitivity, from highly sensitive cells (fibroblasts) to highly insensitive cells (neutrophils).[145] Interestingly, stem cells alter their mechanosensitivity based on their level of commitment or "differentiation stage." A clonally derived bone marrow stem cell line (D1), able to differentiate to adipo-, chondro-, and osteogenic lineages, and a more committed pre-osteoblast cell line were cultured in the presence of RGD-coupled alginate gels with varied mechanics (20, 60, 110 kPa) by changing the amount of Ca^{2+}.[146] The pre-osteoblasts showed higher mechanosensitivity (as evidenced by cell proliferation) than the undifferentiated D1 cell line. However, when the D1 cells were pre-differentiated to a pre-osteoblast-like state, their mechanosensitivity increased dramatically and was nearly identical to the MC3T3 cells. This change in mechanosensitivity may be attributed to different integrin expression patterns of the uncommitted and more committed pre-osteoblast cells. This could also explain the observed difference in mechanosensitivity for MSCs undergoing myogenesis and osteogenesis differentiation.[134]

6. Conclusions

In order to effectively control stem cell differentiation, many aspects of the microenvironment must be considered including soluble factor presentation, matrix mechanics and chemistry, and topography. Because cells in the body are exposed to highly evolved, complex environments, biomaterials that provide these cues cannot be passive or static, but should be instructive and dynamic. If a material is to be used to direct stem cell lineage commitment, it is important to consider the desired spatial and temporal context of specific cues. While many of the methods to control stem cell differentiation can be utilized individually, it is the incorporation of material control over many aspects of the 3D microenvironment that will be necessary to create fully functional tissue equivalents, particularly with complex multi-cellular tissues.

Acknowledgements

The authors would like to acknowledge funding from a National Science Foundation graduate research fellowship (RAM), a National Science Foundation CAREER award (JAB), and a Fellowship in Science and Engineering from the David and Lucille Packard Foundation (JAB).

Received: March 27, 2009
Published online: July 17, 2009

[1] R. M. Nerem, *Tissue Eng.* **2006**, *12*, 1143.
[2] E. Dawson, G. Mapili, K. Erickson, S. Taqvi, K. Roy, *Adv. Drug Deliv. Rev.* **2008**, *60*, 215.
[3] J. A. Elisseeff, A. Ferran, S. Hwang, S. Varghese, Z. Zhang, *Stem Cells Dev.* **2006**, *15*, 295.
[4] J. A. Burdick, G. Vunjak-Novakovic, *Tissue Eng. Part A* **2008**, *15*, 205.
[5] J. L. Ifkovits, J. A. Burdick, *Tissue Eng.* **2007**, *13*, 2369.
[6] M. Lutolf, J. A. Hubbell, *Nat. Biotechnol.* **2005**, *23*, 47.
[7] C. Chung, J. A. Burdick, *Tissue Eng. Part A* **2009**, *15*, 243.
[8] J. A. Xu, W. Wang, M. Ludeman, K. Cheng, T. Hayami, J. A. Lotz, S. Kapila, *Tissue Eng. Part A* **2008**, *14*, 667.
[9] T. P. Kraehenbuehl, P. Zammaretti, A. J. A. Van der Vlies, R. G. Schoenmakers, M. P. Lutolf, M. E. Jaconi, J. A. Hubbell, *Biomaterials* **2008**, *29*, 2757.
[10] Y. Yeo, W. Geng, T. Ito, D. Kohane, J. A. Burdick, M. Radisic, *J. Biomed. Mater. Res.* **2007**, *81B*, 312.
[11] J. J. A. Schmidt, J. A. Rowley, H. Kong, *J. Biomed. Mater. Res.* **2008**, *87*, 1113.
[12] N. S. Hwang, S. Varghese, P. Theprungsirikul, A. Canver, J. A. Elisseeff, *Biomaterials* **2006**, *27*, 6015.
[13] K. Saha, E. F. Irwin, J. A. Kozhukh, D. V. Schaffer, K. E. Healy, *J. Biomed. Mater. Res.* **2007**, *81*, 240.
[14] X. Z. Shu, Y. Liu, F. S. Palumbo, Y. Luo, G. D. Prestwich, *Biomaterials* **2004**, *25*, 1339.
[15] J. B. Leach, K. A. Bivens, C. W. Patrick, C. E. Schmidt, *Biotechnol. Bioeng.* **2003**, *82*, 578.
[16] K. A. Smeds, A. Pfister-Serres, D. Miki, K. Dastgheib, M. Inoue, D. L. Hatchell, M. W. Grinstaff, *J. Biomed. Mater. Res.* **2001**, *54*, 115.
[17] J. A. Kim, I. S. Kim, T. H. Cho, K. B. Lee, S. J. Hwang, G. Tae, I. Noh, S. H. Lee, Y. Park, K. Sun, *Biomaterials* **2007**, *28*, 1830.
[18] C. Barnes, S. Sell, E. Boland, D. Simpson, G. Bowlin, *Adv. Drug Deliv. Rev.* **2007**, *59*, 1413.
[19] P. Janmey, J. P. Winer, J. W. Weisel, *J. R. Soc. Interface/R. Soc.* **2009**, *6*, 1.
[20] T. J. Sill, H. A. von Recum, *Biomaterials* **2008**, *29*, 1989.
[21] A. R. Tan, J. L. Ifkovits, B. M. Baker, D. M. Brey, R. L. Mauck, J. A. Burdick, *J. Biomed. Mater. Res.* **2008**, *87*, 1034.
[22] B. M. Baker, R. L. Mauck, *Biomaterials* **2007**, *28*, 1967.
[23] B. M. Baker, A. O. Gee, R. B. Metter, A. S. Nathan, R. A. Marklein, J. A. Burdick, R. L. Mauck, *Biomaterials* **2008**, *29*, 2348.
[24] X. Liu, P. Ma, *Ann. Biomed. Eng.* **2004**, *32*, 477.
[25] M. P. Linnes, B. D. Ratner, C. M. Giachelli, *Biomaterials* **2007**, *28*, 5298.
[26] C. E. Petrie Aronin, J. A. Cooper, L. S. Sefcik, S. S. Tholpady, R. C. Ogle, E. A. Botchwey, *Acta Biomater.* **2008**, *4*, 1187.
[27] M. R. Alison, S. Islam, *J. Pathol.* **2009**, *217*, 144.
[28] F. Yang, C. G. Williams, D. A. Wang, H. Lee, P. N. Manson, J. Elisseeff, *Biomaterials* **2005**, *26*, 5991.
[29] J. T. Connelly, A. J. García, M. E. Levenston, *J. Cell. Physiol.* **2008**, *217*, 145.
[30] W. A. Comisar, N. H. Kazmers, D. Mooney, J. J. Linderman, *Biomaterials* **2007**, *28*, 4409.
[31] M. M. Martino, M. Mochizuki, D. A. Rothenfluh, S. A. Rempel, J. A. Hubbell, T. H. Barker, *Biomaterials* **2009**, *30*, 1089.
[32] S. M. Dellatore, A. S. Garcia, W. M. Miller, *Curr. Opin. Biotechnol.* **2008**, *19*, 534.
[33] B. Valamehr, S. J. Jonas, J. Polleux, R. Qiao, S. Guo, E. H. Gschweng, B. Stiles, K. Kam, T. J. Luo, O. N. Witte, X. Liu, B. Dunn, H. Wu, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14459.
[34] D. S. Benoit, M. P. Schwartz, A. R. Durney, K. S. Anseth, *Nat. Mater.* **2008**, *7*, 816.
[35] L. Jongpaiboonkit, W. J. King, W. L. Murphy, *Tissue Eng. Part A* **2008**, *15*, 343.
[36] C. J. Flaim, S. Chien, S. N. Bhatia, *Nat. Methods* **2005**, *2*, 119.
[37] G. H. Underhill, S. N. Bhatia, *Curr. Opin. Chem. Biol.* **2007**, *11*, 357.
[38] D. G. Anderson, S. Levenberg, R. Langer, *Nat. Biotechnol.* **2004**, *22*, 863.
[39] D. G. Anderson, D. Putnam, E. B. Lavik, T. A. Mahmood, R. Langer, *Biomaterials* **2005**, *26*, 4892.
[40] A. Peters, D. M. Brey, J. A. Burdick, *Tissue Eng. Part B, Rev.* **2009**, in press.
[41] D. M. Brey, I. Erickson, J. A. Burdick, *J. Biomed. Mater. Res.* **2008**, *85*, 731.
[42] D. M. Brey, J. L. Ifkovits, R. I. Mozia, J. S. Katz, J. A. Burdick, *Acta Biomaterialia* **2008**, *4*, 207.
[43] D. G. Anderson, C. A. Tweedie, N. Hossain, S. M. Navarro, D. M. Brey, K. J. Van Vliet, R. Langer, J. A. Burdick, *Adv. Mater.* **2006**, *18*, 2614.
[44] J. C. Schense, J. Hubbell, *Bioconjug. Chem.* **1999**, *10*, 75.
[45] H. Liu, S. F. Collins, L. J. Suggs, *Biomaterials* **2006**, *27*, 6004.
[46] G. Zhang, X. Wang, Z. Wang, J. Zhang, L. Suggs, *Tissue Eng.* **2006**, *12*, 9.
[47] R. Nair, S. Shukla, T. C. McDevitt, *J. Biomed. Mater. Res.* **2008**, *87*, 1075.
[48] R. Nair, A. V. Ngangan, T. C. McDevitt, *J. Biomater. Sci. Polym. Ed.* **2008**, *19*, 801.
[49] M. A. Serban, G. D. Prestwich, *Methods* **2008**, *45*, 93.
[50] X. Z. Shu, Y. Liu, Y. Luo, M. Roberts, G. D. Prestwich, *Biomacromolecules* **2002**, *3*, 1304.
[51] I. E. Erickson, A. H. Huang, C. Chung, R. T. Li, J. A. Burdick, R. L. Mauck, *Tissue Eng. Part A* **2009**, *15*, 1041.
[52] D. L. Hern, J. A. Hubbell, *J. Biomed. Mater. Res.* **1998**, *39*, 266.
[53] S. Royce Hynes, L. M. McGregor, M. Ford Rauch, E. B. Lavik, *J. Biomater. Sci. Polym. Ed.* **2007**, *18*, 1017.
[54] C. N. Salinas, K. S. Anseth, *Biomaterials* **2008**, *29*, 2370.
[55] A. M. DeLise, L. Fischer, R. S. Tuan, *Osteoarthr. Cartil.* **2000**, *8*, 309.
[56] J. T. Connelly, A. J. García, M. E. Levenston, *Biomaterials* **2007**, *28*, 1071.
[57] Y. J. Li, E. H. Chung, R. T. Rodriguez, M. T. Firpo, K. E. Healy, *J. Biomed. Mater. Res.* **2006**, *79*, 1.
[58] M. Lutolf, J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields, J. Hubbell, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 5413.
[59] L. Urech, A. G. Bittermann, J. A. Hubbell, H. Hall, *Biomaterials* **2005**, *26*, 1369.
[60] S. Hong, J. K. Kang, J. J. Park, E. S. Ryu, S. S. Choi, S. H. Lee, J. H. Lee, J. S. Seo, *Int. J. Cardiol.* **2009**, in press.
[61] B. Z. Barkho, A. E. Munoz, X. Li, L. Li, L. A. Cunningham, X. Zhao, *Stem Cells* **2008**, *26*, 3139.
[62] S. Khetan, J. S. Katz, J. A. Burdick, *Soft Matter* **2009**, *5*, 1601.
[63] S. Sahoo, C. Chung, S. Khetan, J. A. Burdick, *Biomacromolecules* **2008**, *9*, 1088.
[64] M. Mahoney, K. Anseth, *Biomaterials* **2006**, *27*, 2265.
[65] G. A. Hudalla, T. S. Eng, W. L. Murphy, *Biomacromolecules* **2008**, *9*, 842.
[66] A. Khademhosseini, R. Langer, J. Borenstein, J. P. Vacanti, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 2480.
[67] C. M. Metallo, J. C. Mohr, C. J. Detzel, J. J. de Pablo, B. J. Van Wie, S. P. Palecek, *Biotechnol. Prog.* **2007**, *23*, 18.
[68] B. Shen, A. Wei, H. Tao, A. D. Diwan, D. D. Ma, *Tissue Eng. Part A* **2008**, *15*, 1311.
[69] H. J. Kim, Y. J. Kim, G. I. Im, *Cells Tissues Organs (Print)* **2008**, *190*, 1.
[70] A. H. Huang, N. A. Motlekar, A. Stein, S. L. Diamond, E. M. Shore, R. L. Mauck, *Ann. Biomed. Eng.* **2008**, *36*, 1909.

[71] G. Cetinkaya, H. Türkoğlu, S. Arat, H. Odaman, M. A. Onur, M. Gümüşderelioğlu, A. Tümer, *J. Biomed. Mater. Res.* **2007**, *81*, 911.
[72] K. Alberti, R. E. Davey, K. Onishi, S. George, K. Salchert, F. P. Seib, M. Bornhäuser, T. Pompe, A. Nagy, C. Werner, P. W. Zandstra, *Nat. Methods* **2008**, *5*, 645.
[73] C. H. Chou, W. T. Cheng, C. C. Lin, C. H. Chang, C. C. Tsai, F. H. Lin, *J. Biomed. Mater. Res. Part B Appl. Biomater.* **2006**, *77*, 338.
[74] M. C. Degat, L. Dahri-Correia, F. Lavigne, A. Meunier, L. Sedel, J. Correia, H. Petite, D. Logeart-Avramoglou, *J. Biomed. Mater. Res.* **2009**, in press.
[75] J. S. Park, D. G. Woo, H. N. Yang, H. J. Lim, H. M. Chung, K. H. Park, *Transplantation* **2008**, *85*, 589.
[76] K. Park, K. J. Cho, J. J. Kim, I. H. Kim, D. K. Han, *Macromol. Biosci.* **2008**, *9*, 221.
[77] C. L. Casper, W. Yang, M. C. Farach-Carson, J. F. Rabolt, *Biomacromolecules* **2007**, *8*, 1116.
[78] N. Takehara, Y. Tsutsumi, K. Tateishi, T. Ogata, H. Tanaka, T. Ueyama, T. Takahashi, T. Takamatsu, M. Fukushima, M. Komeda, M. Yamagishi, H. Yaku, Y. Tabata, H. Matsubara, H. Oh, *J. Am. Coll. Cardiol.* **2008**, *52*, 1858.
[79] K. H. Park, K. Na, *J. Biosci. Bioeng.* **2008**, *106*, 74.
[80] D. S. Benoit, C. R. Nuttelman, S. D. Collins, K. S. Anseth, *Biomaterials* **2006**, *27*, 6102.
[81] J. Kettenbach, A. Stadler, I. V. Katzler, R. Schernthaner, M. Blum, J. Lammer, T. Rand, *Cardiovasc. Intervent. Radiol.* **2008**, *31*, 468.
[82] A. L. Doiron, K. A. Homan, S. Emelianov, L. Brannon-Peppas, *Pharm. Res.* **2009**, *26*, 674.
[83] M. K. Nkansah, S. Y. Tzeng, A. M. Holdt, E. B. Lavik, *Biotechnol. Bioeng.* **2008**, *100*, 1010.
[84] M. J. Seiler, B. B. Thomas, Z. Chen, S. Arai, S. Chadalavada, M. J. Mahoney, S. R. Sadda, R. B. Aramant, *Exp. Eye Res.* **2008**, *86*, 92.
[85] K. Park, J. S. Park, D. G. Woo, H. N. Yang, H. M. Chung, K. H. Park, *Biomaterials* **2008**, *29*, 2490.
[86] A. M. Bratt-Leal, R. L. Carpenedo, T. C. McDevitt, *Biotechnol. Prog.* **2009**, *25*, 43.
[87] R. L. Carpenedo, C. Y. Sargent, T. C. McDevitt, *Stem Cells* **2007**, *25*, 2224.
[88] A. Mogi, H. Ichikawa, C. Matsumoto, T. Hieda, D. Tomotsune, S. Sakaki, S. Yamada, K. Sasaki, *Tissue Cell* **2009**, *41*, 79.
[89] R. L. Carpenedo, A. M. Bratt-Leal, R. A. Marklein, S. A. Seaman, N. J. Bowen, J. F. McDonald, T. C. McDevitt, *Biomaterials* **2009**, *30*, 2507.
[90] E. Sachlos, D. T. Auguste, *Biomaterials* **2008**, *29*, 4471.
[91] M. W. Laschke, Y. Harder, M. Amon, I. Martin, J. Farhadi, A. Ring, N. Torio-Padron, R. Schramm, M. Rücker, D. Junker, J. M. Häufel, C. Carvalho, M. Heberer, G. Germann, B. Vollmar, M. D. Menger, *Tissue Eng.* **2006**, *12*, 2093.
[92] T. P. Richardson, M. C. Peters, A. B. Ennett, D. J. Mooney, *Nat. Biotechnol.* **2001**, *19*, 1029.
[93] F. B. Basmanav, G. T. Kose, V. Hasirci, *Biomaterials* **2008**, *29*, 4195.
[94] Y. Qin, G. X. Pei, D. M. Xie, D. Jin, K. H. Wei, *Di Yi Jun Yi Da Xue Xue Bao (Academic Journal of the first medical college of PLA)* **2003**, *23*, 1021.
[95] X. Niu, Q. Feng, M. Wang, X. Guo, Q. Zheng, *J. Microencapsul.* **2008**, 1. *26*, 297.
[96] H. Fan, C. Zhang, J. Li, L. Bi, L. Qin, H. Wu, Y. Hu, *Biomacromolecules* **2008**, *9*, 927.
[97] E. Cukierman, R. Pankov, D. R. Stevens, K. M. Yamada, *Science* **2001**, *294*, 1708.
[98] M. Kato, M. Mrksich, *Biochemistry* **2004**, *43*, 2699.
[99] A. L. Zajac, D. E. Discher, *Curr. Opin. Cell Biol.* **2008**, *20*, 609.
[100] F. Rehfeldt, A. Engler, A. Eckhardt, F. Ahmed, D. Discher, *Adv. Drug Deliv. Rev.* **2007**, *59*, 1329.
[101] P. Patwari, R. T. Lee, *Circ. Res.* **2008**, *103*, 234.
[102] N. T. Swailes, M. Colegrave, P. J. Knight, M. Peckham, *J. Cell Sci.* **2006**, *119*, 3561.
[103] L. Ghasemi-Mobarakeh, M. P. Prabhakaran, M. Morshed, M. H. Nasr-Esfahani, S. Ramakrishna, *Biomaterials* **2008**, *29*, 4532.
[104] I. Sridharan, T. Kim, R. Wang, *Biochem. Biophys. Res. Commun.* **2009**, *381*, 508.
[105] J. B. Recknor, D. S. Sakaguchi, S. K. Mallapragada, *Biomaterials* **2006**, *27*, 4098.
[106] D. A. Pijnappels, M. J. Schalij, A. A. Ramkisoensing, J. van Tuyn, A. A. de Vries, A. van der Laarse, D. L. Ypey, D. E. Atsma, *Circ. Res.* **2008**, *103*, 167.
[107] A. Buxton, J. Zhu, R. Marchant, J. West, J. Yoo, B. Johnstone, *Tissue Eng.* **2007**, *13*, 2549.
[108] H. Park, X. Guo, J. S. Temenoff, Y. Tabata, A. I. Caplan, F. K. Kasper, A. G. Mikos, *Biomacromolecules* **2009**, *10*, 541.
[109] S. Varghese, N. S. Hwang, A. C. Canver, P. Theprungsirikul, D. W. Lin, J. Elisseeff, *Matrix Biol.* **2008**, *27*, 12.
[110] S. Kaihara, S. Matsumura, J. P. Fisher, *J. Biomed. Mater. Res.* **2008**, in press.
[111] J. Kim, Y. Park, G. Tae, K. B. Lee, S. J. Hwang, I. S. Kim, I. Noh, K. Sun, *J. Mater. Sci. Mater. Med.* **2008**, *19*, 3311.
[112] C. Adelöw, T. Segura, J. A. Hubbell, P. Frey, *Biomaterials* **2008**, *29*, 314.
[113] J. M. Karp, J. Yeh, G. Eng, J. Fukuda, J. Blumling, K. Y. Suh, J. Cheng, A. Mahdavi, J. Borenstein, R. Langer, A. Khademhosseini, *Lab on a chip* **2007**, *7*, 786.
[114] S. Battista, D. Guarnieri, C. Borselli, S. Zeppetelli, A. Borzacchiello, L. Mayol, D. Gerbasio, D. R. Keene, L. Ambrosio, P. A. Netti, *Biomaterials* **2005**, *26*, 6194.
[115] R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, C. S. Chen, *Dev. Cell* **2004**, *6*, 483.
[116] J. K. Wise, A. L. Yarin, C. M. Megaridis, M. Cho, *Tissue Eng. Part A* **2008**. *15*, 913.
[117] F. Yang, R. Murugan, S. Wang, S. Ramakrishna, *Biomaterials* **2005**, *26*, 2603.
[118] M. J. Biggs, R. G. Richards, S. McFarlane, C. D. Wilkinson, R. O. Oreffo, M. J. Dalby, *J. R. Soc, Interface/R. Soc.* **2008**, *5*, 1231.
[119] E. K. Yim, S. W. Pang, K. W. Leong, *Exp. Cell Res.* **2007**, *313*, 1820.
[120] K. H. Carpizo, M. J. Saran, W. Huang, K. Ishida, J. Roostaeian, D. Bischoff, C. K. Huang, G. H. Rudkin, D. T. Yamaguchi, T. A. Miller, *Plast. Reconstr. Surg.* **2008**, *121*, 424.
[121] G. Marletta, G. Ciapetti, C. Satriano, F. Perut, M. Salerno, N. Baldini, *Biomaterials* **2007**, *28*, 1132.
[122] Y. Liu, M. B. Chan-Park, *Biomaterials* **2009**, *30*, 196.
[123] G. Forte, F. Carotenuto, F. Pagliari, S. Pagliari, P. Cossa, R. Fiaccavento, A. Ahluwalia, G. Vozzi, B. Vinci, A. Serafino, A. Rinaldi, E. Traversa, L. Carosella, M. Minieri, P. Di Nardo, *Stem Cells* **2008**. *26*, 2093.
[124] D. L. Cohen, E. Malone, H. Lipson, L. J. Bonassar, *Tissue Eng.* **2006**, *12*, 1325.
[125] V. Tsang, A. Chen, L. Cho, K. Jadin, R. Sah, S. Delong, J. West, S. Bhatia, *FASEB J.* **2007**, *21*, 790.
[126] G. Papavasiliou, P. Songprawat, V. Pérez-Luna, E. Hammes, M. Morris, Y. Chiu, E. Brey, *Tissue Eng. Part C: Methods* **2008**, *14*, 129.
[127] S. A. Zawko, S. Suri, Q. Truong, C. E. Schmidt, *Acta Biomater.* **2009**, *5*, 14.
[128] S. Lee, J. Moon, J. West, *Biomaterials* **2008**, *29*, 2962.
[129] S. Obi, K. Yamamoto, N. Shimizu, S. Kumagaya, T. Masumura, T. Sokabe, T. Asahara, J. Ando, *J. Appl. Physiol.* **2009**, *106*, 203.
[130] D. F. Ward, R. M. Salasznyk, R. F. Klees, J. Backiel, P. Agius, K. Bennett, A. Boskey, G. E. Plopper, *Stem Cells Dev.* **2007**, *16*, 467.
[131] G. H. van Lenthe, J. P. van den Bergh, A. R. Hermus, R. Huiskes, *J. Bone Miner. Res.* **2001**, *16*, 550.
[132] A. J. Engler, M. A. Griffin, S. Sen, C. G. Bönnemann, H. L. Sweeney, D. E. Discher, *J. Cell Biol.* **2004**, *166*, 877.
[133] P. Georges, *J. Appl. Physiol.* **2005**, *98*, 1547.
[134] A. S. Rowlands, P. A. George, J. J. Cooper-White, *Am. J. Physiol, Cell Physiol.* **2008**, *295*, 1637.
[135] R. J. Pelham, Y. Wang, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13661.

[136] D. Discher, *Science* **2005**, *310*, 1139.
[137] A. Engler, S. Sen, H. Sweeney, D. Discher, *Cell* **2006**, *126*, 677.
[138] K. Saha, A. J. Keung, E. F. Irwin, Y. Li, L. Little, D. V. Schaffer, K. E. Healy, *Biophys. J.* **2008**, *95*, 4426.
[139] P. Georges, W. J. Miller, D. F. Meaney, E. S. Sawyer, P. Janmey, *Biophys. J.* **2006**, *90*, 3012.
[140] J. Leach, X. Brown, J. Jacot, P. Dimilla, J. Wong, *J. Neural Eng.* **2007**, *4*, 26.
[141] S. A. Ruiz, C. S. Chen, *Stem Cells* **2008**, *26*, 2921.
[142] C. Khatiwala, S. Peyton, M. Metzke, A. Putnam, *J. Cell. Physiol.* **2007**, *211*, 661.
[143] S. Peyton, P. Kim, C. Ghajar, D. Seliktar, A. Putnam, *Biomaterials* **2008**, *29*, 2597.
[144] T. J. Kim, J. Seong, M. Ouyang, J. Sun, S. Lu, J. P. Hong, N. Wang, Y. Wang, *J. Cell. Physiol.* **2009**, *218*, 285.
[145] R. Wells, *Hepatology* **2008**, *47*, 1394.
[146] S. X. Hsiong, P. Carampin, H. J. Kong, K. Y. Lee, D. J. Mooney, *J. Biomed. Mater. Res.* **2008**, *85*, 145.