Published in final edited form as:

Curr Opin Biotechnol. 2013 October ; 24(5): 841–846. doi:10.1016/j.copbio.2013.03.009.

Engineering synthetic hydrogel microenvironments to instruct stem cells

Murat Guvendiren and Jason A Burdick

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

Abstract

Advances in our understanding and ability to manipulate stem cell behavior are helping to move stem cell-based therapies toward the clinic. However, much of our knowledge has been gained from standard 2-dimensional culture systems, which often misrepresent many of the signals that stem cells receive in their native 3-dimensional environments. Fortunately, the field of synthetic hydrogels is developing to better recapitulate many of these signals to guide stem cell behavior, both as *in vitro* models and as delivery vehicles for *in vivo* implantation. These include a multitude of structural and biochemical cues that can be presented on the cellular scale, such as degradation, adhesion, mechanical signals, topography, and the presentation of growth factors, often with precise spatiotemporal control.

Introduction

There is a continued and growing demand for innovative treatment alternatives in regenerative medicine, as our ability to treat patients is still limited by the available organs and tissue for transplantation. Within the field of regenerative medicine, stem cell based therapies are growing in interest, as our understanding and ability to control stem cells improves [1]. For example, adult stem cells can be isolated from tissues such as bone marrow and adipose tissue and may be a clinically relevant cellular source for regenerative medicine, particularly as these cells may be used autologously to circumvent issues with transplantation between patients. Despite advances in this area, our understanding of stem cell behavior in biomimetic 3-dimensional (3D) microenvironments is fairly immature.

Stem cells naturally reside in a 3D extracellular matrix (ECM), a complex and dynamic network that provides the physical structure, mechanical integrity and biochemical activity of their environment. However, the majority of our knowledge of stem cell function is based on conventional 2-dimensional (2D) cell culture systems, which do not mimic many aspects of this native tissue environment. The work in 2D has provided significant information on how stem cells feel and respond to their microenvironment in terms of migration, proliferation, survival and fate decision [2]; however, there is increasing evidence of differences in stem cell function when cultured in 3D [3–5]. Additionally, when stem cell based therapy is needed, native 3D ECM is often damaged and deviates from healthy matrix and stem cells can potentially sense and respond to these perturbations. Thus, advanced 3D microenvironment systems are needed to both better understand stem cells *in vitro* and to realize their potential *in vivo*.

With this motivation in mind, this article highlights recent advances and challenges to instructing stem cell differentiation within synthetic 3D microenvironments. We first define

material systems that are used to recapitulate 3D microenvironments and then focus on a range of studies where native ECM cues — adhesion, degradation, mechanics, growth factors and mechanical loading — have been introduced to control stem cell function. Our goal is to use this perspective to encourage and inspire researchers to further develop this field and design improved 3D synthetic mimics of the native ECM both for *in vitro* cell culture studies and stem cell delivery *in vivo* to promote tissue repair.

Designing a 3D microenvironment

The natural ECM is a 3D network composed of fibrous matrix proteins (e.g. collagen, fibrin, elastin) that provides structural integrity for cellular anchorage and naturally sequesters soluble signals. Hydrogels are 3D water-swollen polymer networks and are the most investigated class of materials for developing 3D ECM mimics due to their resemblance to soft tissues in many aspects [6–8], such as high water content and mechanics. Hydrogels have tunable properties and are crosslinked through either covalent (e.g. Michael-type addition, photopolymerization) or physical (e.g. ionic interactions, self-assembly) methods, often under mild conditions for cell encapsulation [9–12]. Hydrogels from natural materials (e.g. collagen, fibrin, chitosan, dextran, hyaluronic acid (HA), alginate and Matrigel™) are inherently biocompatible and bioactive, but their use may be limited due to batch-to-batch variability and difficulty in tuning features such as mechanics, degradation and bioactivity [13].

These limitations can be overcome through either the synthetic modification of these natural materials [14–16] or the use of purely synthetic systems that provide a blank state environment with fine-tuning of mechanical and biochemical properties [17]. Common synthetic hydrogels are formed from polymers such as poly(ethylene glycol) (PEG) and poly(vinyl alcohol) (PVA) and exhibit inherent porosity with average pore size (i.e. mesh size, nm scale) on a much smaller scale than that of a cell (micron scale). This may limit cellular migration, cell–cell interactions and solute transport, but can be overcome through material design considerations, such as controlled degradation or alterations in crosslink density. Beyond their presentation as a continuous material, hydrogels can also be formed as microporous or fibrous scaffolds. Traditionally, microporous hydrogel scaffolds are used as a support for seeded stem cells since their pore size ($>100\ \mu\text{m}$) is larger than the average cell size ($\sim 10\text{--}20\ \mu\text{m}$); however, material presentation often mimics more of a 2D substrate to cells. In contrast, fibrous scaffolds can better mimic the nanofibrous structure of the native ECM with the ability to control matrix organization (topography) and presentation of anisotropic elasticity, which is important for many connective tissues [18–20]. The following sections address many design considerations in the use of hydrogels as 3D microenvironments, which are also outlined in Figure 1.

Hydrogel degradation

Network degradability is an important property that is required to create space for encapsulated cells to spread, migrate and generate cell–cell interactions, as well as to deposit matrix components toward formation of functional tissue structures [21]. Native ECM undergoes dynamic remodeling through matrix assembly and degradation, and degradation occurs locally via proteolytic enzymes (e.g. matrix metalloproteinases (MMPs)) produced by cells during migration or signaling [21]. However, many hydrogels are designed to either be stable or to undergo hydrolytic degradation, where degradation is controlled by crosslink density or number of hydrolytically unstable groups. This lack of control over local degradation led researchers to mimic the enzymatic degradation found in native ECM, by incorporating protease-cleavable peptides as crosslinks to locally support cell mediated degradation [22,23,24]. Motivated by cell instructed degradation, Anseth and colleagues

developed photodegradable PEG-based hydrogels for improved spatiotemporal control of degradation *in situ*, creating channels to direct cell migration and local release of pendant biofunctionality [25••]. Furthermore, photopolymerization can be used to spatially control degradation within hydrogels [26]. These efforts in controlled degradation will advance hydrogel use for stem cell based therapies as the temporal nature of local environments may be crucial to mimic various aspects of development and healing.

Cell adhesion

Many stem cells are anchorage dependent and require adhesion to a matrix or another cell to survive; thus, cell adhesion cues are often presented within synthetic hydrogels. In native ECM, cells bind through integrins to adhesive proteins such as fibronectin and laminin; yet, the arginine–glycine–aspartic acid (RGD) peptide found within many adhesion proteins is often incorporated into nonadhesive hydrogels rather than full-length proteins due to ease of chemical modification [27]. However, the presentation of the adhesive cues significantly affects cell function, such as hMSC survival in PEG hydrogels where the peptide flexibility and spacing influenced viability [28]. Another study reported improved MSC osteogenesis with BMP-2 within HA hydrogels modified with integrin-specific fibronectin fragments [29]. Stem cells can also interact with the surrounding matrix via surface receptors such as CD44 and CD168 that bind to HA [30]. For instance, when compared to inert PEG hydrogels, hMSCs in HA hydrogels showed improved chondrogenesis, illustrating the importance of interactions between polymer networks and cells [31]. Also, human embryonic stem cells (hESCs) maintained their undifferentiated state when encapsulated in HA hydrogels, but not within other gels or in monolayer cultures, indicating the influence of HA binding sites and receptors of hESCs [5]. The signaling that occurs through stem cell adhesion appears to be critical to the survival and function of stem cells and advanced systems will improve our knowledge of the desired timing of the presentation of these signals to cells.

Matrix mechanics and mechanical loading

In their native microenvironments, cells experience a wide range of matrix mechanics, from soft (e.g. brain ~0.1 kPa) to stiffer (e.g. precalcified bone ~80 kPa) tissues, which direct many aspects of cellular function [2]. Hydrogel mechanics can be easily controlled by increasing crosslinking density or the relative hydrophobicity of the network; however, most investigations of stem cell mechanotransduction have been performed with 2D systems. In 3D, Mooney and colleagues fabricated physically crosslinked RGD-modified alginate hydrogels with a wide range of mechanics (2.5–110 kPa), and reported optimal osteogenic differentiation of encapsulated MSCs for intermediate stiffness values (11–30 kPa) [32•]. One of the major challenges in understanding mechanosensitivity with 3D environments is the ability to decouple matrix mechanics from inherent porosity and permeability, since increased mechanics results in decreased mesh size and solute diffusivity. To this end, increasing the overall hydrophilicity of the network by incorporating hydrophilic pendant chains or polymers (i.e. alginate) with increasing crosslinking density led to permeability independent increases in stiffness [33,34].

Beyond the ability of stem cells to sense the mechanics of their surroundings, mechanical loading can also be transmitted through the surrounding environment and may play a crucial role in the development and maintenance of many tissues (e.g. cartilage). Thus, 3D mimetic environments may require dynamic mechanical signals. For instance, chondrogenic differentiation of MSCs encapsulated in a variety of scaffolds (e.g. agarose, alginate, fibrin, HA, PEG) was enhanced under dynamic compression [35–39]. Mechanical loading can also play a role in both the distribution and organization of ECM within engineered tissues. Thus,

the development of bioreactors that mimic *in vivo* loading may help to recapitulate these environments to understand stem cells and produce functional tissues.

Topography

Native ECM is composed of nanofibers that provide topographical signals that direct cellular migration, development and structural organization of tissues in microscale. Cellular and matrix organization are very important for proper tissue function and are often lost during disease. To this end, electrospinning is the most common technique to fabricate nanofibrous scaffolds to mimic the nanofibrous structure of the ECM and control matrix organization and mechanical properties [40]. For instance, Mauck and colleagues developed anisotropic nanofibrous scaffolds that mimic the multi-scale structure hierarchy of annulus fibrosus, which directed deposition of highly organized ECM [41]. One of the limitations of fibrous scaffolds is cellular infiltration, and some degree of improvement is shown by spinning fibers with dual size scale (micro and nano), incorporating sacrificial fibers, photopatterning channels, spinning with cells or creating depth-wise adhesive cues [18]. Only recently has this technology been transferred into hydrogel fibers, which act as soft structures when compared to the typical semicrystalline polymers used in electrospun scaffolds [42]. Here, hydrogel precursors are electrospun and then crosslinked in the dry state, which then swell when placed in an aqueous environment [43], adding another feature into hydrogels to mimic the ECM.

Growth factor presentation

Natural ECM hosts soluble signaling molecules, including growth factors (GFs), which play a significant role in tissue development by triggering a wide range of cellular responses. Therefore, it is important to introduce well-controlled GF presentation into hydrogels to instruct encapsulated stem cell behavior, for local morphogenesis, and even for recruitment of endogenous cells (cell homing) [44–46]. Direct encapsulation is the traditional method of GF presentation in hydrogels; yet, limitations in this approach (e.g. lack of control over delivery profiles) led to the development of micro/nano delivery vehicles within hydrogels for GF delivery [47–48]. Amsden and colleagues reported rapid induction and enhancement of chondrogenesis of encapsulated adipose-derived stem cells (ASCs) in chitosan-based hydrogels with coencapsulation of microspheres containing either BMP-6 or TGF- β 3 [49]. In contrast to physical encapsulation techniques, covalent tethering of GFs to the hydrogel provides long-term control over GF availability; however, this approach may affect the activity of GFs due to possible changes in protein conformations or hindrance of active binding sites [50–52]. An alternative approach is to harness endogenous GF activity by mimicking noncovalent interactions of proteoglycans (PGs) and glycosaminoglycans (GAGs) with GFs [53], such as with the covalent or noncovalent incorporation of chondroitin sulfate or heparin sulfates into hydrogels to sequester GFs [54,55]. For instance, the stimulation and sequestering of BMP2 within heparin-functionalized PEG hydrogels promoted hMSC osteogenesis [56]. In some occasions, heparin binding GFs can exhibit direct reversible binding to the hydrogels, such as in alginate hydrogels, enabling sustained and localized release [15,57]. These approaches represent another step toward controlling stem cell signaling with synthetic hydrogels.

Future outlook

As stem cells reside in natural ECM, our progress in designing synthetic materials that mimic ECM will help advance material-based strategies in stem cell therapies. It is very important to be able to manipulate ECM mimetic cues independently, particularly when probing the influence of specific signals on cellular behavior. However, it is very challenging to decouple features such as hydrogel degradation from mechanics since

degradation (either hydrolytically or proteolytically) leads to a gradual decrease in hydrogel mechanics either in the bulk or locally to cells.

Native EMC is dynamic in nature and presents spatial and temporal heterogeneity, which is now becoming possible with advances in material synthesis and processing. In terms of spatial heterogeneity, photopatterning, UV triggered polymerization or cleavage, can be used to spatially control mechanics, degradation and bioactivity in microscale [26,58,59••]. Recently a sacrificial template via 3D printing was used to create channels in hydrogel scaffolds for vascularization, enabling progress toward large tissue constructs [60••]. Because of advances in material design, hydrogel mechanics can now also be temporally manipulated *in situ*, in an attempt to mimic dynamically changing matrix mechanics [25,61•]. To sum up, it is now possible to robustly control mechanics and bioactivity (i.e. cell adhesion and growth factor presentation) independently in space and time. These examples of enhanced hydrogel complexity will only grow further in the future and aid in our ability to mimic ECM in both healthy and diseased tissues, allowing us to better understand stem cell responses to their surroundings and guide the development of future therapies.

Acknowledgments

The authors acknowledge funding from the David and Lucile Packard Foundation, a National Science Foundation CAREER award, and the National Institutes of Health (EB008722, AR056624 and HL107938).

References and recommended reading

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

- of special interest
- of outstanding interest

1. Marklein RA, Burdick JA. Controlling stem cell fate with material design. *Adv Mater.* 2010; 22:175–189. [PubMed: 20217683]
2. Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. *Science.* 2009; 324:1673–1677. [PubMed: 19556500]
3. Haycock JW. 3D cell culture: a review of current approaches and techniques. *3D Cell Culture: Methods and Protocols.* Methods in Molecular Biology. 2011:1–15. [PubMed: 21042962]
4. Tanaka H, Murphy CL, Murphy C, Kimura M, Kawai S, Polak JM. Chondrogenic differentiation of murine embryonic stem cells: effects of culture conditions and dexamethasone. *J Cell Biochem.* 2004; 93:454–462. [PubMed: 15372628]
5. Gerecht S, Burdick JA, Ferreira LS, Townsend SA, Langer R, Vunjak-Novakovic G. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. *Proc Natl Acad Sci U S A.* 2007; 104:11298–11303. [PubMed: 17581871]
6. Seliktar D. Designing cell-compatible hydrogels for biomedical applications. *Science.* 2012; 336:1124–1128. [PubMed: 22654050]
7. Tibbitt MW, Anseth KS. Hydrogels as extracellular matrix mimics for 3D cell culture. *Biotechnol Bioeng.* 2009; 103:655–663. [PubMed: 19472329]
8. Slaughter BV, Khurshid SS, Fisher OZ, Khademhosseini A, Peppas NA. Hydrogels in regenerative medicine. *Adv Mater.* 2009; 21:3307–3329. [PubMed: 20882499]
9. Griffin DR, Kasko AM. Photodegradable macromers and hydrogels for live cell encapsulation and release. *J Am Chem Soc.* 2012; 134:13103–13107.
10. Li YL, Rodrigues J, Tomas H. Injectable and biodegradable hydrogels: gelation, biodegradation and biomedical applications. *Chem Soc Rev.* 2012; 41:2193–2221. [PubMed: 22116474]

11. Guvendiren M, Lu HD, Burdick JA. Shear-thinning hydrogels for biomedical applications. *Soft Matter*. 2012; 8:260–272.
12. Wang CM, Varshney RR, Wang DA. Therapeutic cell delivery and fate control in hydrogels and hydrogel hybrids. *Adv Drug Deliv Rev*. 2010; 62:699–710. [PubMed: 20138940]
13. Monzack EL, Rodriguez KJ, McCoy CM, Gu XX, Masters KS. Natural materials in tissue engineering applications. *Biomaterials for Tissue Engineering Applications: A Review of the Past and Future Trends*. 2011:209–241.
14. Burdick JA, Prestwich GD. Hyaluronic acid hydrogels for biomedical applications. *Adv Mater*. 2011; 23:H41–H56. [PubMed: 21394792]
15. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. *Prog Polym Sci*. 2012; 37:106–126. [PubMed: 22125349]
16. Hutson CB, Nichol JW, Aubin H, Bae H, Yamanlar S, Al-Haque S, Koshy ST, Khademhosseini A. Synthesis and characterization of tunable poly(ethylene glycol): gelatin methacrylate composite hydrogels. *Tissue Eng A*. 2011; 17:1713–1723.
17. Wade RJ, Burdick JA. Engineering ECM signals into biomaterials. *Mater Today*. 2012; 15:454–459.
18. Kim IL, Mauck RL, Burdick JA. Hydrogel design for cartilage tissue engineering: a case study with hyaluronic acid. *Biomaterials*. 2012; 32:8771–8782. [PubMed: 21903262]
19. Lim SH, Mao HQ. Electrospun scaffolds for stem cell engineering. *Adv Drug Deliv Rev*. 2009; 61:1084–1096. [PubMed: 19647024]
20. Cao HQ, Liu T, Chew SY. The application of nanofibrous scaffolds in neural tissue engineering. *Adv Drug Deliv Rev*. 2009; 61:1055–1064. [PubMed: 19643156]
21. Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. *J Cell Sci*. 2008; 121:255–264. [PubMed: 18216330]
22. Sawhney AS, Pathak CP, Hubbell JA. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-co-poly(alpha-hydroxy acid) diacrylate macromers. *Macromolecules*. 1993; 26:581–587.
23. Lutolf MP, Lauer-Fields JL, Schmoekel HG, Metters AT, Weber FE, Fields GB, Hubbell JA. Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering cell-invasion characteristics. *Proc Natl Acad Sci U S A*. 2003; 100:5413–5418. [PubMed: 12686696] Incorporation of MMP-cleavable peptides into hydrogels.
24. Galler KM, Aulisa L, Regan KR, D'Souza RN, Hartgerink JD. Self-assembling multidomain peptide hydrogels: designed susceptibility to enzymatic cleavage allows enhanced cell migration and spreading. *J Am Chem Soc*. 2010; 132:3217–3223. [PubMed: 20158218]
25. Kloxin AM, Kasko AM, Salinas CN, Anseth KS. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. *Science*. 2009; 324:59–63. [PubMed: 19342581] Temporal control of degradability via UV cleavable links within PEG hydrogels.
26. Khetan S, Burdick JA. Patterning hydrogels in three dimensions towards controlling cellular interactions. *Soft Matter*. 2011; 7:830–838.
27. Hern DL, Hubbell JA. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. *J Biomed Mater Res*. 1998; 39:266–276. [PubMed: 9457557]
28. Salinas CN, Anseth KS. The influence of the RGD peptide motif and its contextual presentation in PEG gels on human mesenchymal stem cell viability. *J Tissue Eng Regen Med*. 2008; 2:296–304. [PubMed: 18512265]
29. Kisiel M, Martino MM, Ventura M, Hubbell JA, Hilborn J, Ossipov DA. Improving the osteogenic potential of BMP-2 with hyaluronic acid hydrogel modified with integrin-specific fibronectin fragment. *Biomaterials*. 2012; 34:704–712. [PubMed: 23103154]
30. Zhu H, Mitsuhashi N, Klein A, Barsky LW, Weinberg K, Barr ML, Demetriou A, Wu GD. The role of the hyaluronan receptor CD44 in mesenchymal stem cell migration in the extracellular matrix. *Stem Cells*. 2006; 24:928–935. [PubMed: 16306150]
31. Chung C, Burdick JA. Influence of three-dimensional hyaluronic acid microenvironments on mesenchymal stem cell chondrogenesis. *Tissue Eng A*. 2009; 15:243–254.
32. Huebsch N, Arany PR, Mao AS, Shvartsman D, Ali OA, Bencherif SA, Rivera-Feliciano J, Mooney DJ. Harnessing traction-mediated manipulation of the cell/matrix interface to control

- stem-cell fate. *Nat Mater.* 2010; 9:518–526. [PubMed: 20418863] Investigates the role of 3D hydrogel stiffness on stem cell fate.
33. Cha C, Jeong JH, Shim J, Kong H. Tuning the dependency between stiffness and permeability of a cell encapsulating hydrogel with hydrophilic pendant chains. *Acta Biomater.* 2011; 7:3719–3728. [PubMed: 21704737]
 34. Cha C, Kim SY, Cao L, Kong H. Decoupled control of stiffness and permeability with a cell-encapsulating poly(ethylene glycol) dimethacrylate hydrogel. *Biomaterials.* 2010; 31:4864–4871. [PubMed: 20347136]
 35. Bian LM, Zhai DY, Zhang EC, Mauck RL, Burdick JA. Dynamic compressive loading enhances cartilage matrix synthesis and distribution and suppresses hypertrophy in hMSC-laden hyaluronic acid hydrogels. *Tissue Eng A.* 2012; 18:715–724.
 36. Campbell JJ, Lee DA, Bader DL. Dynamic compressive strain influences chondrogenic gene expression in human mesenchymal stem cells. *Biorheology.* 2006; 43:455–470. [PubMed: 16912417]
 37. Mauck RL, Byers BA, Yuan X, Tuan RS. Regulation of cartilaginous ECM gene transcription by chondrocytes and MSCs in 3D culture in response to dynamic loading. *Biomech Model Mechanobiol.* 2007; 6:113–125. [PubMed: 16691412]
 38. Pelaez D, Huang CYC, Cheung HS. Cyclic compression maintains viability and induces chondrogenesis of human mesenchymal stem cells in fibrin gel scaffolds. *Stem Cells Dev.* 2009; 18:93–102. [PubMed: 18399763]
 39. Steinmetz NJ, Bryant SJ. Chondroitin sulfate and dynamic loading alter chondrogenesis of human MSCs in PEG hydrogels. *Biotechnol Bioeng.* 2012; 109:2671–2682. [PubMed: 22511184]
 40. Mauck RL, Baker BM, Nerurkar NL, Burdick JA, Li WJ, Tuan RS, Elliott DM. Engineering on the straight and narrow: the mechanics of nanofibrous assemblies for fiber-reinforced tissue regeneration. *Tissue Eng Rev.* 2009; 15:171–193.
 41. Nerurkar NL, Baker BM, Sen S, Wible EE, Elliott DM, Mauck RL. Nanofibrous biologic laminates replicate the form and function of the annulus fibrosus. *Nat Mater.* 2009; 8:986–992. [PubMed: 19855383]
 42. Sundararaghavan HG, Metter RB, Burdick JA. Electrospun fibrous scaffolds with multiscale and photopatterned porosity. *Macromol Biosci.* 2010; 10:265–270. [PubMed: 20014198]
 43. Sundararaghavan HG, Burdick JA. Gradients with depth in electrospun fibrous scaffolds for directed cell behavior. *Biomacromolecules.* 2011; 12:2344–2350. [PubMed: 21528921]
 44. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface.* 2010; 8:153–170. [PubMed: 20719768]
 45. Vo TN, Kasper FK, Mikos AG. Strategies for controlled delivery of growth factors and cells for bone regeneration. *Adv Drug Deliv Rev.* 2012; 64:1292–1309. [PubMed: 22342771]
 46. Purcell BP, Elser JA, Mu AB, Margulies KB, Burdick JA. Synergistic effects of SDF-1 alpha chemokine and hyaluronic acid release from degradable hydrogels on directing bone marrow derived cell homing to the myocardium. *Biomaterials.* 2012; 33:7849–7857. [PubMed: 22835643]
 47. Basmanav FB, Kose GT, Hasirci V. Sequential growth factor delivery from complexed microspheres for bone tissue engineering. *Biomaterials.* 2008; 29:4195–4204. [PubMed: 18691753]
 48. Bian LM, Zhai DY, Tous E, Rai R, Mauck RL, Burdick JA. Enhanced MSC chondrogenesis following delivery of TGF-beta 3 from alginate microspheres within hyaluronic acid hydrogels in vitro and in vivo. *Biomaterials.* 2011; 32:6425–6434. [PubMed: 21652067]
 49. Sukarto A, Yu C, Flynn LE, Amsden BG. Co-delivery of adipose-derived stem cells and growth factor-loaded microspheres in RGD-grafted N-methacrylate glycol chitosan gels for focal chondral repair. *Biomacromolecules.* 2012; 13:2490–2502. [PubMed: 22746668]
 50. Jabbari E. Bioconjugation of hydrogels for tissue engineering. *Curr Opin Biotechnol.* 2011; 22:655–660. [PubMed: 21306888]
 51. Elbert DL, Hubbell JA. Conjugate addition reactions combined with free-radical cross-linking for the design of materials for tissue engineering. *Biomacromolecules.* 2001; 2:430–441. [PubMed: 11749203]

52. DeLong SA, Moon JJ, West JL. Covalently immobilized gradients of bFGF on hydrogel scaffolds for directed cell migration. *Biomaterials*. 2005; 26:3227–3234. [PubMed: 15603817]
53. Hudalla GA, Murphy WL. Biomaterials that regulate growth factor activity via bioinspired interactions. *Adv Funct Mater*. 2011; 21:1754–1768. [PubMed: 21921999]
54. Choi YJ, Lee JY, Park JH, Park JB, Suh JS, Choi YS, Lee SJ, Chung CP, Park YJ. The identification of a heparin binding domain peptide from bone morphogenetic protein-4 and its role on osteogenesis. *Biomaterials*. 2010; 31:7226–7238. [PubMed: 20621352]
55. Cai SS, Liu YC, Shu XZ, Prestwich GD. Injectable glycosaminoglycan hydrogels for controlled release of human basic fibroblast growth factor. *Biomaterials*. 2005; 26:6054–6067. [PubMed: 15958243]
56. Benoit DSW, Durney AR, Anseth KS. The effect of heparin-functionalized PEG hydrogels on three-dimensional human mesenchymal stem cell osteogenic differentiation. *Biomaterials*. 2007; 28:66–77. [PubMed: 16963119]
57. Kolambkar YM, Dupont KM, Boerckel JD, Huebsch N, Mooney DJ, Huttmacher DW, Guldberg RE. An alginate-based hybrid system for growth factor delivery in the functional repair of large bone defects. *Biomaterials*. 2011; 32:65–74. [PubMed: 20864165]
58. Kloxin AM, Tibbitt MW, Kasko AM, Fairbairn JA, Anseth KS. Tunable hydrogels for external manipulation of cellular microenvironments through controlled photodegradation. *Adv Mater*. 2010; 22:61–66. [PubMed: 20217698]
- 59•. Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Shoichet MS. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. *Nat Mater*. 2011; 10:799–806. [PubMed: 21874004] User defined patterning of biochemical cues in 3D hydrogels.
- 60•. Miller JS, Stevens KR, Yang MT, Baker BM, Nguyen DHT, Cohen DM, Toro E, Chen AA, Galie PA, Yu X, et al. Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nat Mater*. 2012; 11:768–774. [PubMed: 22751181] Use of a 3D sacrificial template to fabricate hydrogels with controlled channels for vascularization.
- 61•. Guvendiren M, Burdick JA. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. *Nat Commun*. 2012; 3:792. [PubMed: 22531177] Use of UV light to induce user controlled matrix stiffening in the presence of stem cells.

Figure 1.

Stem cells receive cues from numerous properties and features of their culture environment, which provide instruction on outcomes such as proliferation and differentiation. Within synthetic materials, these cues may include material degradation, topography, adhesion, growth factor presentation and mechanics, as well as the application of external loading.