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Feature Perspectives in Tissue Engineering

Tissue Mimetics: Engineered Hydrogel Matrices Provide Biomimetic Environments for Cell Growth

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Introduction

TISSUE-ENGINEERED SCAFFOLDS were initially designed for implantation with the idea of growing tissues *in vitro* before transplantation. This spawned several decades of research, which is still active today, into highly porous polymeric scaffolds in which cells are seeded and expected to develop into tissue.[1,2] Biomaterial matrices were designed to provide structured extracellular matrix (ECM) analogs for cell growth, offering a milieu in which to direct cell migration, proliferation, and fate.[3] More recently, strategies to mimic the ECM *in vitro* to study development, disease progression, or drug screening have led to a new generation of dynamic engineered scaffolds—that is, scaffolds that cells can remodel.[4–6] Hydrogels are widely used to form these three-dimensional (3D) matrices and have evolved from simple materials into dynamic scaffolds designed with multiple features that mimic the ECM. Natural, synthetic, or hybrid materials are used to form these crosslinked hydrogels, with each system possessing advantageous features. Whereas natural materials (such as collagen, fibrin, and hyaluronic acid [HA]) inherently contain biological signals, hydrogels formed from these materials tend to be mechanically weak and manipulation of their chemical and physical properties requires chemical crosslinking reactions. Matrigel™, which is derived from mouse sarcoma, has been used in many studies and also has a low modulus; however, Matrigel is chemically ill-defined, confounding the influence of the microenvironment on cell behavior. Whereas synthetic hydrogels [e.g., poly(ethylene glycol) (PEG)] are well defined, these materials are biologically inert, requiring the separate introduction of regulatory cues to guide cell function.

The development of biomimetic hydrogels has seen complex new materials that exploit inherent material strength with unique chemical and physical modifications to create physiologically relevant cell culture models. In this perspective, the current state of hydrogel design is discussed with emphasis on the engineering approaches used to recapitulate the microarchitecture (porosity, pore size, interconnectivity), mechanical properties, and biochemical cues found in native ECM, as well as recent techniques developed to introduce chemical and physical properties that can be dynamically and spatiotemporally controlled.

Current State of Hydrogel Design

To facilitate cellular infiltration and adequate nutrient transport, hydrogels must emulate the microarchitecture of native ECM.[7–9] A variety of techniques have been used in hydrogel fabrication to incorporate and control bulk porosity, including solvent casting/particle leaching, freeze-drying, gas foaming, soft lithography micromolding, electrospinning, solid freeform fabrication, and cryogelation.[8,10,11] Cryogelation has gained popularity in tissue-engineered scaffold design as it can be used to incorporate interconnected pores into a variety of hydrogel systems without the use of cytotoxic porogens[12] and advanced instrumentation. By altering thaw temperature and through the addition of carbohydrates during cryogelation, tunable pore size and gel stiffness have been achieved in HA/PEG hydrogels.[11] Cryogels also possess elastic and shape-memory properties,[13] which may be beneficial for application as cardiac patches.[14] Spatially controlling the nanoarchitectural features of a 3D hydrogel has been recently described by Anseth and coworkers, who used photosensitive crosslinkers that could be cleaved in specific regions within the 3D hydrogels using multiphoton confocal microscopy[15,16]; this resulted in erosion of structures of defined size and dimension, and allowed guided cell migration through hollowed-out architectural features.

Cells sense the local mechanical properties of their environment, converting mechanical signals into chemical signals that in turn alter gene expression.[17] Therefore, it is essential to design hydrogels with mechanical properties that can be tuned to match to those of native tissue. A major challenge when tuning hydrogel rigidity is ensuring that other hydrogel properties are not altered in the process.[18] For example, varying the concentration of hydrogel constituents commonly controls hydrogel stiffness; however, this can affect protein transport, which consequently alters cell function.[19,20] Moreover, simply varying the polymer concentration to change gel stiffness can also change the concentration of the bioactive ligands present in the gel. Recent studies have focused on decoupling the ligand concentration

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from the gel concentration.[21] With the development of cytocompatible, aqueous "click" chemistry techniques,[22] bioactive ligands can be covalently bound to synthetic hydrogels in a controlled manner, where the concentration of the bulk hydrogel can be altered independently of ligand density.[23,24] In addition, mechanical properties can also be tuned by varying the molecular weight of the macromer or by varying the number of functional groups on the polymer, which participate in the crosslinking reaction.[11,25] The Burdick laboratory has recently developed an innovative method to temporally control hydrogel mechanics.[26] A dithiothreitol crosslinked methacrylated HA hydrogel was further crosslinked through radical polymerization of the remaining methacrylate groups, thereby stiffening the gel on demand. This resulted in selective differentiation of mesenchymal stem cells based on the timing of the hydrogel's mechanical change.

Whereas hydrogels formed from natural materials such as collagen, fibronectin, and HA are inherently bioactive, synthetic hydrogels such as PEG are biologically inert and require the addition of bioactive molecules to regulate cell function. Some of the earliest hydrogel ligand modifications occurred over 20 years ago when the fibronectin-derived peptide RGD was tethered to synthetic hydrogels such as polyacrylamide and poly(lactic acid-co-lysine) copolymer.[27,28] Since then, a large number of adhesive peptide sequences have been incorporated into both natural and synthetic hydrogels, including peptide sequences derived from fibronectin (PHSRN), collagen (GFOGER), elastin (VAPG), and laminin (YIGSR, IKVAV), providing additional biomimetic ligands available to recapitulate *in vivo* microenvironments.[29–33]

Elaborate conjugation methods through site-specific cleavage of photolabile protecting groups to selectively expose reactive functional groups for conjugating desired molecules have been developed, allowing for precise spatial control over the 3D organization and concentration of bioactive peptides. Agarose hydrogels with defined photopatterned gradients of VEGF-165 successfully guided the migration of endothelial cells, and recently, HA/PEG hydrogels were patterned with gradients of EGF, showing the versatility of the same photochemistry with multiple gel types.[11,34] Shoichet and coworkers demonstrated simultaneous spatial control of multiple biomolecules using photochemistry in combination with the orthogonal physical binding pairs, barnase–barstar and streptavidin–biotin.[35] This technique defines the 3D distribution of multiple signaling molecules and has utility in spatially guiding the fate of stem/progenitor cells. Using wavelength-selective photocleavage, Anseth and colleague developed hydrogels that are able to release molecules on-demand, creating a dynamic display of chemical cues.[36] In addition to the immobilization of bioactive peptides/proteins, stimuli responsive hydrogels have also been created in an effort to recapitulate the dynamic nature of the ECM. Enzyme cleavable sequences (e.g., matrix metalloprotease-sensitive sequences GPQGIWGQ, GPQGIAGQ and GCRDVPMSMRGGDRCG) have been incorporated into hydrogel backbones to give synthetic hydrogels the capacity to be proteolytically degraded by cell secreted enzymes, thus enabling cell migration and matrix remodeling.[6,37,38]

In addition to the chemically, physically, and mechanically defined tissue-engineered scaffolds, the ECM facilitates cell–cell interactions, often between multiple cell types. The interplay of multiple cell types in defined hydrogels has been explored. For example, the symbiotic relationship between retinal precursor cells and endothelial cells in GRGDS/VEGF-165-gradient agarose scaffolds was demonstrated, whereby the endothelial cells formed stable tubular-like structures in the presence of retinal precursor cells that migrated and extended cellular processes along the endothelial cells.[39] Similarly, Bhatia *et al.* have shown the importance of fibroblasts to hepatocyte function using micropatterning techniques, which localized cell populations, allowing control over cell–cell interactions at the coculture interface.[40]

Integrated Design

To create defined environments for cell culture, the microarchitecture, mechanical properties, and biochemical cues must be all capable of independent spatial manipulation within a single hydrogel system. The incorporation of spatiotemporally controlled chemical and physical cues into a hydrogel has been enabled with the development of multiphoton laser patterning and carefully designed photochemically labile hydrogels. These independent spatial manipulations of a hydrogel culture system allow precise control over the cell microenvironment, but still represent an overly simplified model of cell-ECM interactions. Since native ECM contains a plethora of bioactive signaling molecules, engineering strategies must control the spatiotemporal distribution of multiple chemical and physical cues. The field of tissue engineering has seen greater complexity built into the hydrogel design through the development of dynamic and tunable physical and chemical properties. The dynamic reciprocity between cell–matrix interactions may require chemical and physical cues to be displayed in a reversible manner. For example, during development, cells are exposed to chemical cues at specific times, and prolonged exposure will result in a change in cell function.[41,42] The continued development of novel conjugation chemistry that can enable stimuli responsive activation/deactivation of chemical and physical cues is a powerful method to enable the incorporation of multiple factors.

Tissue-engineered hydrogels require integrated design strategies, including the interplay of multiple cell types. Although there are currently no hydrogels that contain all the necessary cues needed to emulate native ECM, the goal is to produce microenvironments that contain the essential signals needed to recapitulate *in vivo* cell migration, proliferation, and fate. This will enable better systems with which to study and understand development, disease progression, and drug screening and ultimately lead to tissue mimetics with greater opportunity for successful transplantation.

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References

1. Mikos, A.G., Bao, Y., Cima, L.G., Ingber, D.E., Vacanti, J.P., and Langer, R. Preparation of poly(glycolic acid) bonded fiber structures for cell attachment and transplantation. J Biomed Mater Res **27,** 183, 1993.
2. Lee, K.Y., and Mooney, D.J. Hydrogels for tissue engineering. Chem Rev **101,** 1869, 2001.
3. Owen, S.C., and Shoichet, M.S. Design of three-dimensional biomimetic scaffolds. J Biomed Mater Res A **94,** 1321, 2010.
4. Kim, S., Chung, E.H., Gilbert, M., and Healy, K.E. Synthetic MMP-13 degradable ECMs based on poly(N-isopropylacrylamide-co-acrylic acid) semi-interpenetrating polymer networks. I. Degradation and cell migration. J Biomed Mater Res A **75,** 73, 2005.
5. Levesque, S.G., and Shoichet, M.S. Synthesis of enzyme-degradable, peptide-cross-linked dextran hydrogels. Bioconjug Chem **18,** 874, 2007.
6. Raeber, G.P., Lutolf, M.P., and Hubbell, J.A. Molecularly engineered PEG hydrogels: a novel model system for proteolytically mediated cell migration. Biophys J **89,** 1374, 2005.
7. Shoichet, M.S. Polymer scaffolds for biomaterials applications. Macromolecules **43,** 581, 2010.
8. Annabi, N., Nichol, J.W., Zhong, X., *et al.* Controlling the porosity and microarchitecture of hydrogels for tissue engineering. Tissue Eng Part B Rev **16,** 371, 2010.
9. Harley, B.A., Kim, H.D., Zaman, M.H., Yannas, I.V., Lauffenburger, D.A., and Gibson, L.J. Microarchitecture of three-dimensional scaffolds influences cell migration behavior via junction interactions. Biophys J **95,** 4013, 2008.
10. Hollister, S.J. Porous scaffold design for tissue engineering. Nat Mater **4,** 518, 2005.
11. Owen, S.C., Fisher, S.A., Tam, R.Y., Nimmo, C.M., and Shoichet, M.S. Hyaluronic acid click hydrogels emulate the extracellular matrix. Langmuir **29,** 7393, 2013.
12. Henderson, T.M.A., Ladewig, K., Haylock, D.N., McLean, K.M., and O'Connor, A.J. Cryogels for biomedical applications. J Mater Chem B **1,** 2682, 2013.
13. Bencherif, S.A., Sands, R.W., Bhatta, D., *et al.* Injectable preformed scaffolds with shape-memory properties. Proc Natl Acad Sci U S A **109,** 19590, 2012.
14. Leor, J., Amsalem, Y., and Cohen, S. Cells, scaffolds, and molecules for myocardial tissue engineering. Pharmacol Ther **105,** 151, 2005.
15. Kloxin, A.M., Kasko, A.M., Salinas, C.N., and Anseth, K.S. Photodegradable hydrogels for dynamic tuning of physical and chemical properties. Science **324,** 59, 2009.
16. DeForest, C.A., and Anseth, K.S. Cytocompatible click-based hydrogels with dynamically tunable properties through orthogonal photoconjugation and photocleavage reactions. Nat Chem **3,** 925, 2011.
17. Miller, C.J., and Davidson, L.A. The interplay between cell signalling and mechanics in developmental processes. Nat Rev Genet **14,** 733, 2013.
18. Trappmann, B., Gautrot, J.E., Connelly, J.T., *et al.* Extracellular-matrix tethering regulates stem-cell fate. Nat Mater **11,** 642, 2012.
19. Serpooshan, V., Julien, M., Nguyen, O., *et al.* Reduced hydraulic permeability of three-dimensional collagen scaffolds attenuates gel contraction and promotes the growth and differentiation of mesenchymal stem cells. Acta Biomater **6,** 3978, 2010.
20. Drury, J.L., and Mooney, D.J. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials **24,** 4337, 2003.
21. Zaman, M.H., Trapani, L.M., Sieminski, A.L., *et al.* Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. Proc Natl Acad Sci U S A **103,** 10889, 2006.
22. Nimmo, C.M., Owen, S.C., and Shoichet, M.S. Diels-Alder Click cross-linked hyaluronic acid hydrogels for tissue engineering. Biomacromolecules **12,** 824, 2011.
23. Gill, B.J., Gibbons, D.L., Roudsari, L.C., *et al.* A synthetic matrix with independently tunable biochemistry and mechanical properties to study epithelial morphogenesis and EMT in a lung adenocarcinoma model. Cancer Res **72,** 6013, 2012.
24. Kyburz, K.A., and Anseth, K.S. Three-dimensional hMSC motility within peptide-functionalized PEG-based hydrogels of varying adhesivity and crosslinking density. Acta Biomater **9,** 6381, 2013.
25. Xin, X., Borzacchiello, A., Netti, P.A., Ambrosio, L., and Nicolais, L. Hyaluronic-acid-based semi-interpenetrating materials. J Biomater Sci Polym Ed **15,** 1223, 2004.
26. Guvendiren, M., and Burdick, J.A. Stiffening hydrogels to probe short- and long-term cellular responses to dynamic mechanics. Nat Commun **3,** 792, 2012.
27. Brandley, B.K., and Schnaar, R.L. Covalent attachment of an Arg-Gly-Asp sequence peptide to derivatizable polyacrylamide surfaces: support of fibroblast adhesion and long-term growth. Anal Biochem **172,** 270, 1988.
28. Barrera, D.A., Zylstra, E., Lansbury, P.T., and Langer, R. Synthesis and Rgd peptide modification of a new biodegradable copolymer—poly(lactic acid-co-lysine). J Am Chem Soc **115,** 11010, 1993.
29. Gobin, A.S., and West, J.L. Val-ala-pro-gly, an elastin-derived non-integrin ligand: smooth muscle cell adhesion and specificity. J Biomed Mater Res A **67,** 255, 2003.
30. Gunn, J.W., Turner, S.D., and Mann, B.K. Adhesive and mechanical properties of hydrogels influence neurite extension. J Biomed Mater Res A **72,** 91, 2005.
31. Liu, S.Q., Tian, Q.A., Hedrick, J.L., Hui, J.H.P., Ee, P.L.R., and Yang, Y.Y. Biomimetic hydrogels for chondrogenic differentiation of human mesenchymal stem cells to neocartilage. Biomaterials **31,** 7298, 2010.
32. Schmidt, D.R., and Kao, W.J. Monocyte activation in response to polyethylene glycol hydrogels grafted with RGD and PHSRN separated by interpositional spacers of various lengths. J Biomed Mater Res A **83,** 617, 2007.
33. Zhu, J., and Marchant, R.E. Design properties of hydrogel tissue-engineering scaffolds. Expert Rev Med Devices **8,** 607, 2011.
34. Aizawa, Y., Wylie, R., and Shoichet, M. Endothelial cell guidance in 3D patterned scaffolds. Adv Mater **22,** 4831, 2010.
35. Wylie, R.G., Ahsan, S., Aizawa, Y., Maxwell, K.L., Morshead, C.M., and Shoichet, M.S. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nat Mater **10,** 799, 2011.
36. Azagarsamy, M.A., and Anseth, K.S. Wavelength-controlled photocleavage for the orthogonal and sequential release of multiple proteins. Angew Chem Int Ed Engl **52,** 13803, 2013.
37. Lutolf, M.P., Lauer-Fields, J.L., Schmoekel, H.G., *et al.* Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: engineering

cell-invasion characteristics. Proc Natl Acad Sci U S A **100,** 5413, 2003.
38. Khetan, S., Guvendiren, M., Legant, W.R., Cohen, D.M., Chen, C.S., and Burdick, J.A. Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. Nat Mater **12,** 458, 2013.
39. Aizawa, Y., and Shoichet, M.S. The role of endothelial cells in the retinal stem and progenitor cell niche within a 3D engineered hydrogel matrix. Biomaterials **33,** 5198, 2012.
40. Bhatia, S.N., Balis, U.J., Yarmush, M.L., and Toner, M. Microfabrication of hepatocyte/fibroblast co-cultures: role of homotypic cell interactions. Biotechnol Prog **14,** 378, 1998.
41. Discher, D.E., Mooney, D.J., and Zandstra, P.W. Growth factors, matrices, and forces combine and control stem cells. Science **324,** 1673, 2009.
42. Purpura, K.A., Morin, J., and Zandstra, P.W. Analysis of the temporal and concentration-dependent effects of BMP-4, VEGF, and TPO on development of embryonic stem cell-derived mesoderm and blood progenitors in a defined, serum-free media. Exp Hematol **36,** 1186, 2008.

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