Bowie, M.B., Kent, D.G., Dykstra, B., McKnight, K.D., McCaffrey, L., Hoodless, P.A., and Eaves, C.J. (2007). Identification of a new intrinsically timed developmental checkpoint that reprograms key hematopoietic stem cell properties. Proc. Natl. Acad. Sci. USA *104*, 5878–5882.

Copley, M.R., and Eaves, C.J. (2013). Developmental changes in hematopoietic stem cell properties. Exp. Mol. Med. *45*, e55.

Ikuta, K., Kina, T., MacNeil, I., Uchida, N., Peault, B., Chien, Y.H., and Weissman, I.L. (1990). A developmental switch in thymic lymphocyte maturation potential occurs at the level of hematopoietic stem cells. Cell *62*, 863–874.

Kikuchi, K., and Kondo, M. (2006). Developmental switch of mouse hematopoietic stem cells from fetal to adult type occurs in bone marrow after birth. Proc. Natl. Acad. Sci. USA *103*, 17852–17857.

Li, Y., Kong, W., Yang, W., Patel, R.M., Casey, E.B., Okeyo-Owuor, T., White, J.M., Porter, S.N., Morris, S.A., and Magee, J.A. (2020). Single-Cell Analysis of Neonatal HSC Ontogeny Reveals Gradual and Uncoordinated Transcriptional Reprogramming that Begins before Birth. Cell Stem Cell *27*, this issue, 732–747.

Müller, A.M., Medvinsky, A., Strouboulis, J., Grosveld, F., and Dzierzak, E. (1994). Development of hematopoietic stem cell activity in the mouse embryo. Immunity *1*, 291–301.

Pietras, E.M., Reynaud, D., Kang, Y.A., Carlin, D., Calero-Nieto, F.J., Leavitt, A.D., Stuart, J.M., Göttgens, B., and Passegué, E. (2015). Functionally Distinct Subsets of Lineage-Biased Multipotent Progenitors Control Blood Production in Normal and Regenerative Conditions. Cell Stem Cell *17*, 35–46.

Dynamic Hydrogels for Investigating Vascularization

Gordana Vunjak-Novakovic[1,*]
[1]Columbia University, New York, NY 10032, USA
*Correspondence: gv2131@columbia.edu
https://doi.org/10.1016/j.stem.2020.10.009

Extracellular matrix is known to regulate vascularization by a sequence of multiple factors including mechanical forces. In this issue of *Cell Stem Cell*, Wei et al. (2020) investigate the roles of matrix biomechanics on early stages of vasculogenesis by using hydrogels with tunable stiffness and stress relaxation.

Cell fate and function, *in vivo* and *in vitro*, are regulated by the entire context of the cellular environment, through cascades of interacting molecular, structural, and physical signals that change in space and time. The cells in turn regulate their environment as they secrete cytokines, build and degrade the extracellular matrix, influence the surrounding cells, and generate physical forces. These two-way interactions between the cells and their environment are instrumental for native tissue development, phenotypic changes associated with diseases, and the assembly and function of engineered tissues. In recent years, *in vitro* models of increasingly high biological fidelity have been developed to enable controllable studies under conditions more closely emulating the *in vivo* milieu (Paek et al., 2019; Ronaldson-Bouchard and Vunjak-Novakovic, 2018; Skylar-Scott et al., 2019).

In this issue of *Cell Stem Cell*, the Wei et al. (2020) article reports a study of vascularization, which remains one of the universal challenges in cell and tissue culture. While vascular phenotypes change with the developmental stage, parent tissue type, and the state of health or disease, achieving angiogenesis and vasculogenesis is critically important both for the survival of implanted tissues and for designing tissue models for biological research. In this study, the focus is on the effects of the mechanical environment, established by a hydrogel used to encapsulate cells, on the initiation, progression, and outcomes of early stages of vasculogenesis.

It is well known that the extracellular matrix provides not only a three-dimensional setting for the cultured cells but also serves as a major source of regulatory signals that determine vascular development and function, including physical forces (Discher et al., 2009). With this understanding, the field has gradually moved from generic, inert, and nondegradable biomaterials toward designing those with tunable properties that can be tailored to control cell differentiation and assembly. In recent years, the field has also started to incorporate another facet of control where materials that change their properties in response to cell-generated signals are being explored. The present study is an important step toward establishing and utilizing dynamic cellular environments. The authors explored if the activation of specific mechanosensing mechanisms in cultured vascular cells, in conjunction with matrix remodeling, can be utilized to induce the assembly of vascular networks. To this end, they developed a viscoelastic hydrogel with dynamic crosslinks that promoted cellular contractility and enabled formation of large focal adhesions and robust vascular networks. This sequence of events could not be initiated using otherwise identical hydrogels with static covalent crosslinks.

To decouple the effects of stiffness and matrix dynamics, the authors conducted experiments with dynamic and static matrices that had the same initial stiffness at two different levels: low (soft matrix) and high (stiff matrix). Cultivation of

endothelial cells in dynamic hydrogels resulted in remarkable effects on the length and volume of vascular tubes, both of which increased 10-fold relative to the vascular tubes formed in static hydrogels. In addition, vascular sprouting and branching were observed only in dynamic hydrogels. Importantly, these results were not due to the differences in stiffness or diffusion rates in dynamic and static hydrogels.

This study also showed that the inhibition of cell contraction did not decrease integrin intensity and number, but it did affect the integrin clustering, which is critical for the cellular protrusions and sprouting that lead to the formation of the vascular bed. Collectively, the data suggest that integrin clustering serves as a checkpoint for vasculogenesis in an intensity- and timing-dependent manner. These findings are consistent with previous studies that demonstrated that the formation of focal adhesions in endothelial cells is initiated by activation of FAK and contractility-mediated integrin clustering (Yu et al., 2011). The hydrogel dynamics also enhanced the remodeling of extracellular matrix by increasing both the degradation of the provisional matrix by Matrix metallopeptidases (MMPs) and the deposition of collagen IV and laminin, the two components of the basement membrane. Another previous finding supported by this study is that the dynamic remodeling of the matrix is necessary for the initiation and progression of vasculogenesis by allowing endothelial cell (EC) motility (Iruela-Arispe and Davis, 2009).

The authors integrated the experimental data and observations into a mechanistic model that describes regulation of the initiation, progression, and outcomes of early stages of vascular network formation in the dynamic and static hydrogels used to encapsulate vascular cells (Figure 7G in Wei et al., 2020). The fundamental difference between the two types of hydrogels is that the dynamic hydrogels have the ability for stress relaxation, by virtue of their elastic bonds, such that the cells are relieved of external forces and allowed to elongate, contract, and migrate inside the hydrogel and to form focal adhesions. In contrast, the covalent bonds in static hydrogels maintain the initial levels of stress in the hydrogel surrounding the cells. Importantly, this difference is maintained in both the soft and stiff hydrogels, suggesting that the observed behaviors are independent of stiffness.

The model proposes that the interactions of integrins with the Arg-Gly-Asp (RGD)binding sites in dynamic hydrogels help recruit focal adhesion proteins, including FAK. The resulting clustering of integrins, mediated by actin contractility, and the recruitment of vinculin then lead to the formation of large and stable focal adhesions. Finally, the upregulation of MT1-MMP, MMP-1, and MMP-9 promote matrix degradation, further facilitating cell sprouting and the formation of nascent vascular networks. In support of the model, the rigidity of covalent bonds in static hydrogels suppressed the recruitment of focal adhesion proteins and thereby inhibited cell contractility and the subsequent series of events leading to the formation of microvessels. Vacuole and lumen formation were also observed in static hydrogels, but they were suppressed by the lack of stress relaxation around the cells. The delayed integrin clustering and focal adhesion formation could not be compensated by prolonging the time in culture.

This study provides a conceptual approach and also engineers an important tool: hydrogels capable of providing stress relaxation in a broad and physiologically relevant range of stiffnesses. Fast degradation of hydrogels limited this study to early stages of vasculogenesis, i.e., within the first 7 days. Future studies would benefit from hydrogels designed to allow studies of the phenotypic stability and function of vascular networks over longer periods of time. Investigating the other sources of human cells, including stem cells, that give rise to vascular networks would also be of interest. Finally, this approach could be extended to vascularization of developing and regenerating tissues, as well as tumor tissues, which display a broad range of biomechanical environments. One could envision that the use of reporters designed to mark some of the key steps in vasculogenesis would lead to dynamic and noninvasive longitudinal studies of the events involved in vascularization under normal and pathological conditions.

REFERENCES

Discher, D., Mooney, D.J., and Zandstra, P.W. (2009). Growth factors, matrices, and forces combine and control stem cells. Science *324*, 1673–1677.

Iruela-Arispe, M.L., and Davis, G.E. (2009). Cellular and molecular mechanisms of vascular lumen formation. Dev. Cell *16*, 222–231.

Paek, J., Park, S.E., Lu, Q., Park, K.T., Cho, M., Oh, J.M., Kwon, K.W., Yi, Y.S., Song, J.W., Edelstein, H.I., et al. (2019). Microphysiological Engineering of Self-Assembled and Perfusable Microvascular Beds for the Production of Vascularized Three-Dimensional Human Microtissues. ACS Nano *13*, 7627–7643.

Ronaldson-Bouchard, K., and Vunjak-Novakovic, G. (2018). Organs-on-a-Chip: A Fast Track for Engineered Human Tissues in Drug Development. Cell Stem Cell *22*, 310–324.

Skylar-Scott, M.A., Uzel, S.G.M., Nam, L.L., Ahrens, J.H., Truby, R.L., Damaraju, S., and Lewis, J.A. (2019). Biomanufacturing of organ-specific tissues with high cellular density and embedded vascular channels. Sci. Adv. *5*, eaaw2459.

Wei, Z., Schnellmann, R., Pruitt, H.C., and Gerecht, S. (2020). Hydrogel Network Dynamics Regulate Vascular Morphogenesis. Cell Stem Cell *27*, this issue, 798–812.

Yu, C.H., Law, J.B., Suryana, M., Low, H.Y., and Sheetz, M.P. (2011). Early integrin binding to Arg-Gly-Asp peptide activates actin polymerization and contractile movement that stimulates outward translocation. Proc. Natl. Acad. Sci. USA *108*, 20585–20590.