Guiding New Generations for Decades

Worthington is supporting life science research and discovery, and we have done so for over 70 years.

Authoring technical manuals and guides both in print and digital formats. And, we are cited in thousands of respected scientific journals across the globe.

Sharing Our Enzyme Expertise

Order your free copy of the *NEW* Tissue Dissociation Guide, 18th Edition. Simply go to:

Worthington-Biochem.com/TissueDissociation/default.html

Come see us at ISSCR '19 Booth 438

Worthington Biochemical Corporation

800.445.9603 • 732.942.1660

BIOMATERIALS

Nascent proteins impact cell fate

Metabolic labeling enables researchers to spatiotemporally assess nascent protein deposition in 3D hydrogels and then study how nascent proteins influence cell behaviors.

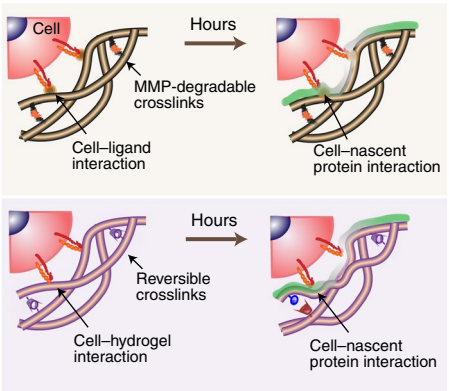
Hydrogel-based cell-culturing platforms provide an engineered three-dimensional (3D) microenvironment for mimicking native extracellular matrix (ECM), as well as for investigating how physical and chemical cues influence cell behaviors and fate.

Previous studies have demonstrated that hydrogel cues such as stiffness and proteolytic degradation have substantial influences on cell spreading and tissue morphogenesis. Aside from these cues, cells also produce and deposit proteins within their 3D environment, thereby actively mediating cell–hydrogel and cell–cell interactions over time and space. The bidirectional signaling between cells and hydrogels plays a critical role in the regulation of cell growth and ECM assembly.

Yet, little is known about the importance of the cell–hydrogel interactions triggered by these deposited proteins at the initial stage after cell encapsulation. Jason Burdick from the University of Pennsylvania notes that "while tissue engineers routinely study matrix deposition in 3D hydrogels, it has largely been ignored in the field of mechanobiology, which prompted us to consider how this newly formed matrix may contribute to the transduction of signals from these engineered environments."

To this end, Burdick and colleagues adapted a metabolic-labeling approach to visualize nascent proteins that were deposited by human mesenchymal stromal cells (hMSCs) within a day of culturing. They cultured the cell–hydrogel constructs in media containing methionine analogues (i.e., azidohomoalanine (AHA)) that were subsequently incorporated into newly synthesized proteins such as fibronectin and collagens. The azide group on AHA enables conjugation with DBCO-modified fluorophores, thus allowing visualization of the nascent proteins. The strength of azide–DBCO cyclo-addition is the capability for imaging of living cells, which offers a direct measure of how protein deposition and remodeling influence cell behaviors.

Hydrogels can accommodate cell spreading through protease-dependent degradation or protease-independent physical arrangements. "We aimed to include both of these mechanisms to illustrate the broad importance of nascent proteins in modulating cell behavior," Claudia Loebel comments. They thus engineered two types of hydrogels: covalently crosslinked and dynamic viscoelastic hydrogels.



Nascent protein adhesions influence cell behavior. Adapted with permission from Loebel et al. (2019), Springer Nature.

They found that cellular adhesion emerged from focal adhesions that interacted with nascent proteins in degradable and dynamic hydrogels. The disruption of cellular adhesion to nascent proteins or protein remodeling consequently reduced hMSC spreading and nuclear translocation of YAP/TAZ, suggesting mechanoregulatory roles of nascent proteins in cell spreading.

Looking forward, Burdick remarks, "We are currently exploring whether specific ligands, such as cell-adhesive RGD or ECM-mimetic peptides, influence the content and properties of the nascent matrix and whether we can employ this information towards redefining hydrogel design criteria for specific applications."

Lei Tang

Published online: 30 April 2019
https://doi.org/10.1038/s41592-019-0409-8

Research papers

Loebel, C. et al. Local nascent protein deposition and remodeling guide mesenchymal stromal cell mechanosensing and fate in three-dimensional hydrogels. *Nat. Mater.* https://doi.org/10.1038/s41563-019-0307-6 (2019).