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Guiding cells with light: Patterning of cell-laden hydrogels using multi-photon lithography

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INTRODUCTION: Hydrogels can mimic the extracellular matrix (ECM) and provide a suitable environment for 3D cell culture [1]. Multi-photon lithography (MPL) is a versatile tool that can be used to initiate photochemical reactions at a micrometer resolution. We utilize this method to alter the chemical and mechanical properties of cell-laden hydrogels and guide cell migration.
METHODS: Gelatin methacryloyl (Gel-MA), supplemented with the photoinitiator (PI) (2,4,6-trimethylbentoyl) phenylphosphinate (Li-TPO), is crosslinked using a UV curing chamber. After crosslinking, it is soaked in a 1 mM 4,4'-diazido-2,2'-stilbenedisulfonic acid (DSSA) solution. Gelatin norbornene (Gel-NB) is supplemented with dithiothreitol (DTT) as a crosslinker and a modified diazosulfonate-based (DAS) photoinitiator at an equimolar thiol-ene ratio. A custom-built, as well as a commercial (NanoOne Bio, UpNano GmbH) MPL systems, were used to pattern the hydrogels. Three different cell types are used in the present study, namely human adipose-derived mesenchymal stem cells (hASCs/TERT1, Evercyte), human umbilical vein endothelial cells (HUVECs, PELOBiotech) and L929 fibroblast cells.
RESULTS: In the first method, a hydrophilic molecule (DSSA) is covalently bound to the C-H groups of Gel-MA in a process called photografting [2]. By creating a 3D pattern with varying laser power directly around the cell spheroids, we were able to show that hASCs are preferentially migrating into regions that have been exposed to a higher laser dose. DSSA-free samples that were exposed to the laser showed no preferential migration direction. This indicates that a higher laser power leads to an increase in the density of bound DSSA molecules and that cells prefer to migrate into regions with a higher DSSA density. In the second method, Gel-NB is crosslinked upon initial laser irradiation and amide bonds of the gelatin backbone are cleaved following a subsequent laser exposure [3]. The combination of a highly efficient PI with an on-the-fly optical voxel tuning facilitates the fabrication of centimeter large structures with a micrometer resolution. A perfusable spheroid-containing hydrogel structure is printed inside of a microfluidic chip, 3D patterns are cleaved around the spheroid and the migration of L929 cells is observed.
DISCUSSION & CONCLUSIONS: We have demonstrated two different approaches to altering the chemical and mechanical characteristics of cell-laden hydrogels using MPL. Both approaches satisfy the requirements of biocompatibility, high-resolution and high complexity that are necessary to manipulate the microenvironment of cells.
REFERENCES: 1. M. Ravi et al., J Cell Physiol, 230, 2015
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