Guiding cells with light: Studying cell migration in photoresponsive hydrogels

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INTRODUCTION

Hydrogels can mimic the extracellular matrix (ECM) and provide a suitable environment for 3D cell culture [1]. Given the heterogeneity of native tissue and the interrelation between ECM stiffness and cell behavior, processes that are capable of precisely manipulating material properties are necessary to study these events in vitro [2]. 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 study the influence on cell migration.

MATERIALS AND METHODS

Two photosensitive gelatin-based hydrogels, namely gelatin methacryloyl (Gel-MA) and gelatin-norbornene (Gel-NB), are used. 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. 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 system, have been utilized to process the hydrogels. Spheroids are formed using agarose molds. Three different cell types are used in the present study, namely human adipose-derived mesenchymal stem cells (hASCs/TERT1), human umbilical vein endothelial cells (HUVECs) and L929 fibroblast cells. A confocal microscope (LSM 800, Carl Zeiss AG) has been used to track the cell migration.

RESULTS AND DISCUSSION

We are investigating two different methods of altering the physico-chemical properties of photosensitive hydrogels using MPL. In the first one, a hydrophilic molecule (DSSA) is covalently bound to the C-H groups of Gel-MA in a process called photografting [3]. The presence of the grafted DSSA molecules most likely increases the hydrophilicity, which affects the pore size, the stiffness and the density of cell culture medium. By creating a 3D pattern with varying laser power directly around the cell spheroids, we were able to show that ASCs are preferentially migrating into regions that have been subjected to a higher exposure dose, as visualized in Figure 1. This indicates that a higher laser power leads to an increase in the density of covalently bound DSSA molecules.

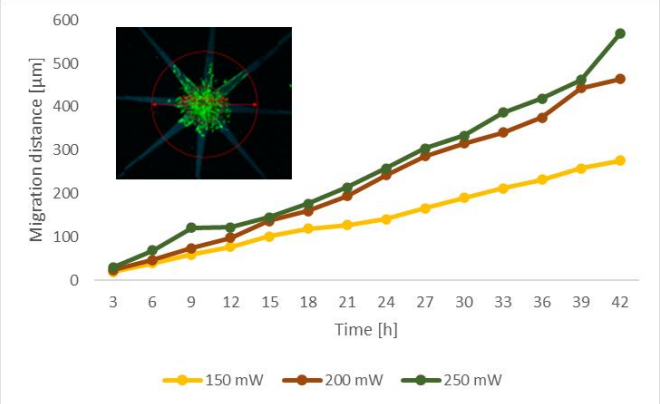

Figure 1: Quantification of ASC migration: The migration distance of GFP-ASCs into the photografted patterns is plotted over time. Each curve corresponds to a different laser power.

In the second method, Gel-NB is crosslinked upon light irradiation. By varying the exposure dose, it is possible to control the degree of crosslinking and thereby the hydrogel stiffness. This enables the fabrication of a precise 3D stiffness gradient in a cell-laden hydrogel. Broadening the laser voxel increases the throughput by a factor of more than 10, which facilitates the crosslinking of comparatively large hydrogel volumes and the creation of stiffness gradients at the required scale. Subsequently, L929 fibroblast spheroids will be encapsulated and their migration direction and speed in response to 3D stiffness gradients will be studied.

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

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