
Study of cell sensitivity to stiffness in 3D environment with controlled geometry

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Abstract

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Cell culture is an important tool that enables the study of physiological and pathological cell activity in vitro with applications in fields such as cell biology, drug discovery, cancer research, tissue engineering, and stem cell studies (Serban et al., 2008). In traditional cell culture, cells adhere to rigid two-dimensional (2D) surfaces, typically made of polystyrene or glass. However, cells in the body are usually supported by a complex three-dimensional (3D) extracellular matrix (ECM) (Langhans, 2018).

There is a complex feedback relationship between cells and this matrix, as cells exert mechanical forces to remodel the extracellular matrix according to their requirements. In turn, mechanical signals from the extracellular matrix, like stiffness, create stimuli that cells can sense and transduce to regulate cellular behavior including adhesion, growth, migration, proliferation and differentiation (Wang et al., 2019). Since this dynamic environment cannot be accurately represented by the static nature of traditional 2D cell culture surfaces, data obtained from in vitro studies with these models can be non-predictive of in vivo behavior, often resulting in disparities with animal and clinical tests (Prestwich, 2007).

Given the high impact of the extra cellular matrix on cell behavior, several attempts have been made to better recreate its properties in vitro by creating artificial extra cellular matrix models. However, most current models are in 2D, including hydrogels with patterned material properties like protein composition and stiffness. Although these models represent a step forward by providing a more dynamic and relevant microenvironment that can influence aspects like cell polarity and spatial distribution, they still fall short in recreating the full dimensionality of many in vivo systems (Wang et al., 2019)

In consequence, there has been a transition towards the creation of 3D scaffolds. Various classical techniques such as solvent casting, salt leaching, freeze-drying, and phase separation, have been used for the fabrication of 3D scaffolds with high porosity and large surface

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area that stimulate cell adhesion. However, precise control over the scaffold architecture is limited, and there can be high variability from sample to sample (Bourdon et al., 2018).

New 3D printing or additive manufacturing (AM) techniques have been developed, that allow the production of structures with controllable and complex architecture based directly on Computer Aided Design (CAD) models, achieving higher repeatability. A particularly promising technique for the fabrication of 3D scaffolds with high resolution is two-photon polymerization (TPP), as it enables the control of features down to the submicrometer scale (Tayalia et al., 2008).

The objective of this project is, therefore, to develop 3D scaffolds with defined mechanical properties in the μm scale, employing the high-resolution capability of two-photon polymerization, to customize the cell environment and investigate the effect of specific mechanical cues such as scaffold stiffness and pore size in cell behavior.

These properties are highly dependent on the fabrication parameters and material selection. For this reason, first, a commercial photoresist called OrmoComp that is compatible with cell culture and has been highly reported in literature (Bonabi et al., 2019), was used to determine the attainable resolution as well as to understand and optimize the key fabrication parameters that allow to control scaffold mechanical properties.

This process resulted in the fabrication of two mesh structures with the same fiber thickness ($10\ \mu\text{m}$), but different cavity sizes (20 and $40\ \mu\text{m}$), that were used in preliminary cell culture studies with adenocarcinoma (A549) cells to assess the effect of pore size on cell behavior. Fluorescence microscopy images showed that both models displayed 3D cell distribution and cell proliferation. Differences in cell morphology and distribution were observed in function of the pore size, as cells appeared to adhere to the edges and surround the cavity in the model with a larger cavity size, while they showed a tendency to stretch out across the cavities in the case of smaller cavity size.

To further advance this technology and create models with tunable stiffness in addition to tunable pore size, initial experiments were conducted using an acrylamide-based photoresist formulation. This hydrogel, in contrast to OrmoComp, has high-swelling capabilities that allow tuning the scaffold stiffness in the range of biological tissues. However, the high solvent content poses a challenge for the creation of structures with high definition and mechanical stability (Zaari et al., 2004). Nevertheless, a proof of concept on the fabrication of 3D scaffolds was achieved.

The results from this study collectively show that two-photon polymerization combined with material technology provides a promising path for the creation of artificial 3D scaffold models that better emulate in vitro the intricate natural environment of cells within the body, towards the advancement in basic cell behavior understanding as well as application in tissue engineering and drug development.

References

Bonabi, A., Tähkä, S., Ollikainen, E., Jokinen, V., & Sikanen, T. (2019). Metallization of Organically Modified Ceramics for Microfluidic Electrochemical Assays. *Micromachines*, *10*(9), 605. <https://doi.org/10.3390/mi10090605>

Bourdon, L., Maurin, J.-C., Gritsch, K., Brioude, A., & Salles, V. (2018). Improvements in Resolution of Additive Manufacturing: Advances in Two-Photon Polymerization and Direct-Writing Electrospinning Techniques. *ACS Biomaterials Science & Engineering*, *4*(12), 3927-3938. <https://doi.org/10.1021/acsbmaterials.8b00810>

Langhans, S. A. (2018). Three-Dimensional in Vitro Cell Culture Models in Drug Discovery and Drug Repositioning. *Frontiers in Pharmacology*, *9*. <https://www.frontiersin.org/articles/10.3389/fphar.2018.00>

Prestwich, G. D. (2007). Simplifying the extracellular matrix for 3-D cell culture and tissue engineering: A pragmatic approach. *Journal of Cellular Biochemistry*, 101(6), 1370-1383. <https://doi.org/10.1002/jcb.21386>

Serban, M. A., Liu, Y., & Prestwich, G. D. (2008). Effects of extracellular matrix analogues on primary human fibroblast behavior. *Acta Biomaterialia*, 4(1), 67-75. <https://doi.org/10.1016/j.actbio.2007.09.001>

Tayalia, P., Mendonca, C. R., Baldacchini, T., Mooney, D. J., & Mazur, E. (2008). 3D Cell-Migration Studies using Two-Photon Engineered Polymer Scaffolds. *Advanced Materials*, 20(23), 4494-4498. <https://doi.org/10.1002/adma.200801319>

Wang, M., Cui, C., Ibrahim, M. M., Han, B., Li, Q., Pacifici, M., Lawrence, J. T. R., Han, L., & Han, L.-H. (2019). Regulating Mechanotransduction in Three Dimensions using Sub-Cellular Scale, Crosslinkable Fibers of Controlled Diameter, Stiffness, and Alignment. *Advanced Functional Materials*, 29(18), 1808967. <https://doi.org/10.1002/adfm.201808967>

Zaari, N. & Rajagopalan, P. & Kim, S.K & Engler, AJ & Wong, Joyce. (2004). Photopolymerization in Microfluidic Gradient Generators: Microscale Control of Substrate Compliance to Manipulate Cell Response. *Advanced Materials*. 16. 2133 - 2137. [10.1002/adma.200400883](https://doi.org/10.1002/adma.200400883).