Novel Fabrication Technique to Confine Hydrogels with Different Patterns inside Microfluidic Devices without Pillars

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Abstract

In the field of microtechnologies applied to the simulation of controlled biological environments are the so-called organ-on-a-chip, microfluidic cell culture devices. The gradient model plays an indispensable role in this technology. Here, we present a novel microfabrication process for pillarless microfluidic platforms which enables the creation of gradients inside them.

Introduction

Human tissues are highly complex systems where numerous cell types and microenvironmental factors are intertwined. Microfluidic devices for cell culture applications have recently allowed to resemble that complex microenvironment. Among the different designs described, one is recurrent by their versatility to simulate multiple pathophysiological conditions.

This chip design (also known as gradient chip) is composed by a central chamber flanked by one or two lateral channels to confine a hydrogel. Apart from the mechanical stimuli simulation, this chip design can promote multiple gradient formations.

Different types of gradients, such as chemical or oxygen gradients, have an essential role in several biological activities in vivo. Gradient devices, for organ-on-chip applications, made of PDMS can be widely found in the literature [1][2]. Gradient devices made of thermoplastic materials [3] can be also found in literature [4], but most of them include micropillars. This inert hydrogel alteration can create a physical barrier between the central chamber and side channels, hindering the gradient formation and cell interactions. Recently, some pillarless devices have been described in the literature [5] but it was limited to parallel geometries where the shear stress could not be variable along the chip. Here, a novel microfluidic fabrication process for thermoplastic chips without pillars is presented, which can achieve a wide range of geometries and variable shear stress.

Theory and experimental procedure

Our approach has been to obtain the hydrogel confinement by local surface modification creating hydrophilic/hydrophobic interfaces in thermoplastic-based chips.

The fabrication process consists in 4 phases: design, fabrication, treatment, and bonding. The first one, chip design, is performed using AutoCAD and Solid Edge to establish the 3D model (Figure 1). The second step is the cutting of different layers of thermoplastic materials with a plotter. Third phase consist of the pattern of the desired hydrophilic chamber designs through a surface treatment based on O2 plasma activation and later permanent hydrophilic surface activation with aminosilane compounds. Finally, all the layers and the injection piece are aligned and sealed with a pressure and thermal process.

The biological validation consisted in a static biocompatibility test to measure cell viability over time using U-251 cell line seeded in the central chamber. Computational simulation of different geometries was performed to study the shear profile.

Conclusions

A novel fabrication technique to create pillarless microfluidic platforms with different geometries is presented. This approach can create devices within different shear stress profiles along the chip and non-altered gradients by inert materials. We have demonstrated that the fabrication process ends with biocompatible chips and works perfectly both in static and flow modes.

Acknowledgments

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References

[1] J. S. LIU *et al.*, "Design and Validation of a Microfluidic Chip with Micropillar Arrays for Three-dimensional Cell Culture," *Chinese J. Anal. Chem.*, vol. 45, no. 8, pp. 1109–1114, 2017, doi: 10.1016/S1872-2040(17)61029-6.

[2] H. Uwamori, T. Higuchi, K. Arai, and R. Sudo, "Integration of neurogenesis and angiogenesis models for constructing a neurovascular tissue," *Sci. Rep.*, vol. 7, no. 1, pp. 1–11, 2017, doi: 10.1038/s41598-017-17411-0.

[3] M. Bernard, E. Jubeli, J. Bakar, J. Saunier, and N. Yagoubi, "Impact of simulated biological aging on physicochemical and biocompatibility properties of cyclic olefin copolymers," *Mater. Sci. Eng. C*, vol. 97, no. December 2018, pp. 377–387, 2019, doi: 10.1016/j.msec.2018.12.032.

[4] M. Virumbrales-Muñoz *et al.*, "Enabling cell recovery from 3D cell culture microfluidic devices for tumour microenvironment biomarker profiling," *Sci. Rep.*, vol. 9, no. 1, pp. 1–14, 2019, doi: 10.1038/s41598-019-42529-8.

[5] J. Loessberg-Zahl, J. Beumer, A. van den Berg, J. C. T. Eijkel, and A. D. van der Meer, "Patterning biological gels for 3d cell culture inside microfluidic devices by local surface modification through laminar flow patterning," *Micromachines*, vol. 11, no. 12, pp. 1–11, 2020, doi: 10.3390/mi11121112.

Figures

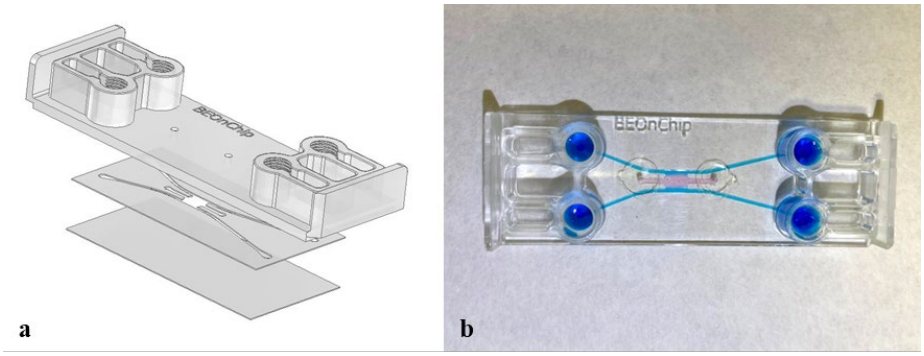

Figure 1: a) 3D design of the gradient chip without pillars and b) fabricated and seeded final device.

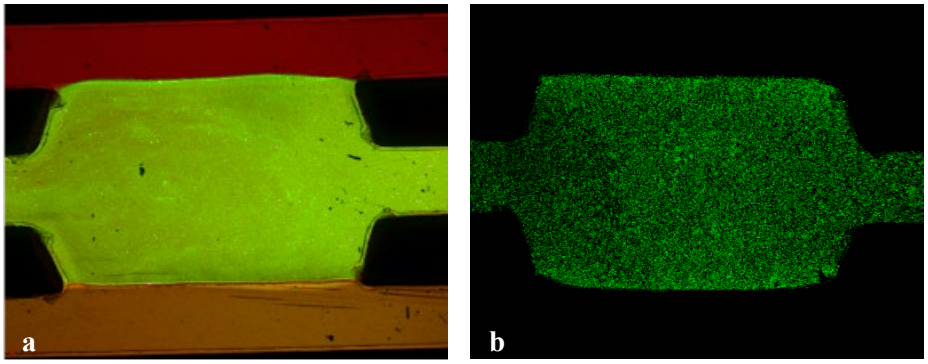

Figure 1: Nikon Eclipse Ti confocal images**.** a) Seeded device: collagen gel with green fluorospheres (central chamber) and PBS with red fluorospheres (lateral channels). b) Biological validation (4 million cells/ml of U-251 after 24 h of culture).