SELECTIVE CELL CULTURING STEP USING LAMINAR CO-FLOW TO ENHANCE CELL CULTURE IN SPLENON-ON-A-CHIP BIOMIMETIC PLATFORM

L.G. Rigat-Brugarolas[1,2], A. Elizalde[3], H.A. del Portillo[3,4], A. Homs-Corbera[1,2] and J. Samitier[1,2,5]

[1]Nanobioengineering Group, Institute for Bioengineering of Catalonia (IBEC), SPAIN,
[2]Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), SPAIN,
[3]Barcelona Centre for International Health Research (CRESIB, Hospital Clínic - UB), SPAIN,
[4]Institució Catalana de Recerca i Estudis Avançats (ICREA), SPAIN and
[5]Department of Electronics, Barcelona University (UB), SPAIN

ABSTRACT

Constant evolution and improvements on areas such as tissue engineering, microfluidics and nanotechnology have made it possible to partially close the gap between conventional *in vitro* cell cultures and animal model-based studies. A step forward in this field concerns organ-on-chip technologies, capable of reproducing the most relevant physiological features of an organ in a microfluidic platform. In this work we have exploited the capabilities of laminar co-flow inside our biomimetic platform, the splenon-on-a-chip, in order to enhance cell culture inside its channels to better mimic the spleen's environment.

KEYWORDS: Cell culture, Co-flow, Laminar flow, Organ-on-a-chip, Spleen.

INTRODUCTION

Research in microfluidic devices that represents organ-on-a-chip models is still in its infancy, but offers a tantalizing glimpse into future of drug testing and biological hypotheses evaluation [1]. In that sense, in order to recreate *in vitro* a specific organ, mimicking both the physical and physiological features, correct cell culturing inside the microfluidic systems becomes a critical and fundamental step. Several platforms, in the interface between nanobio and tissue engineering, have been developed in the past years [1].

Recently the applicability of microfluidics to recreate and translate spleen red pulp's hydrodynamic properties into a microengineered splenon-on-a-chip platform was shown [2]. The spleen, similar in structure to a large lymph node, is a complex branched vasculature organ that contains two mains sections in its red pulp: the closed/rapid microcirculation, were circulates de 90% of blood flow, and the open/slow microcirculation, that holds the remaining 10% [3], condition that we translated in our previous work [2]. Analogously to that blood flow division, cell culturing suffers the same effect, so when seeding cells inside the device, slow-flow channel is poorly cultured. In this work we present the utility of laminar co-flow properties, by adding a secondary entrance to the splenon-on-a-chip, for selectively culture cells and increase its density inside the microfluidic device.

Taking advantage of this phenomena it is possible to enhance cell density in the slow-flow channel and/or co-culture different types of cells in different channels mimicking better the different parts of the splenon in the same device. In addition, multiple drug testing can be done simultaneously. This methodology could be used in any microfluidic system to obtain localized culture or functionalized regions.

MATERIALS AND METHODS

A splenon-on-a-chip biomimetic platform, upgraded for cell culturing enhancement purposes, was fabricated using customized photo-lithographic and soft-lithographic techniques as described elsewhere [2]. The device, designed to translate a simplified but accurate version of spleen's red pulp physiology, mimic the closed-fast and the open-slow microcirculations by means of two main microfluidic channels fabricated to provide a physiological flow division. The upgrade is achieved by introducing a secondary inlet, shown in figure 1 in red (inlet 2), so that the vast majority of the sample introduced in it goes directly to the lower channel, representing the slow compartment.

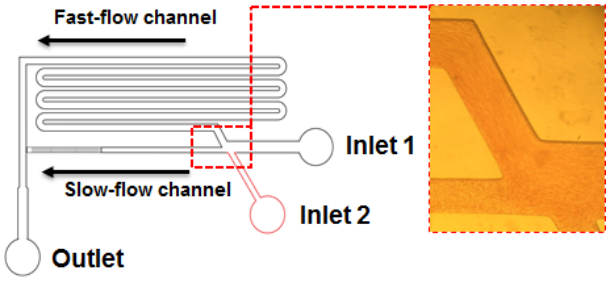

Figure 1: Layout of the splenon-on-a-chip with the secondary inlet (in red) adapted to enhance cell culturing.

The fabrication procedure for the splenon-on-a-chip consists in a multi-step process established to obtain two different height using two different photoresists (SU8 and Ordyl) by means of photo-lithographic techniques [2]. To replicate the master's microchannels, a PDMS pre-polymer mixture (curing agent-to-PDMS ratio of 1:10, Sylgard®184, Dow Corning) was placed in a desiccator and vacuum was applied in order to remove bubbles. Then the mixture was poured on top of the master to fabricate a PDMS mould, heat up at 65ºC for 3 hours and afterwards kept at room temperature for 24 hours. All works were carried out in the clean room facility of the Institute for BioEngineering of Catalonia (IBEC).

EXPERIMENTAL

Briefly, the experimental setup consists in dos syringe pumps (PHP 2000, Harvard Apparatus) used to introduce the samples into the microfluidic device at defined flow rates. The experiments were carried out at room temperature (25ºC) and the sample was kept at physiological temperature of 37ºC.

Optical measurements for microfluidic analyses were performed using an inverted optical microscope (Olympus IX71) with an integrated CCD Hamamatsu camera.

Teflon tubing was inserted into the access holes, which were slightly smaller than the outer diameter of the tubing to form a pressure seal between the tubing and the hole.

NIH 3T3 fibroblast cultures were performed at 37°C and 5% CO_2 in Dulbecco's modified Eagle's medium supplemented with 10% FBS, L-glutamine, Penicillin Streptomycin and Sodium Pyruvate, 1% each, until confluence and maintained in exponential growth phase during 48 hours and afterwards cultured inside the microfluidic device at the same conditions. All trials involving cell culture were done with this fibroblast cell line.

RESULTS

Initial fluid modelizations were done using COMSOL multiphysics software (COMSOL AB, Sweden), and results were practically translated to trials with fluorescent microbeads diluted in phosphate buffered saline and blood samples, as shown in figure 2. The length of non-focused sample is tunable under different entrance flow rate ratios (Q1:Q2).

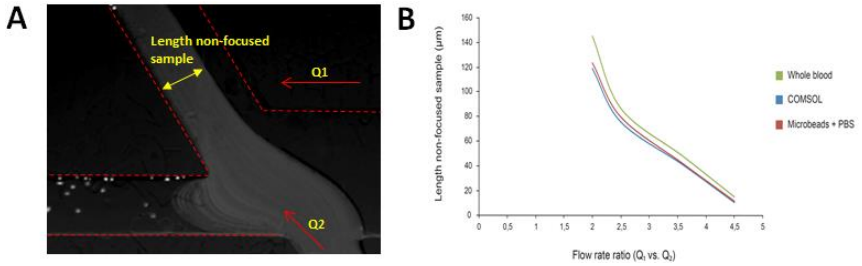

Figure 2: (A) Co-flow trials with fluorescent microbeads. (B) Table showing the length of non-focused sample obtained at different inlet flow rate ratios.

Enhancement of the cell density in the slow-flow channel has been successfully achieved, and up to a 70% of surface coverage may be obtained, where even no cells can be displayed in the fast-flow channel (figure 3A). Also it is possible to co-culture simultaneously different types of cells in the different channels (figure 3B) with the aim of mimicking better different parts of the spleen in the same device. As a proof of concept, trials were performed using 3T3 fibroblasts with different cell fluorescence staining and the results attained supports the applicability of laminar co-flow for this commitment.

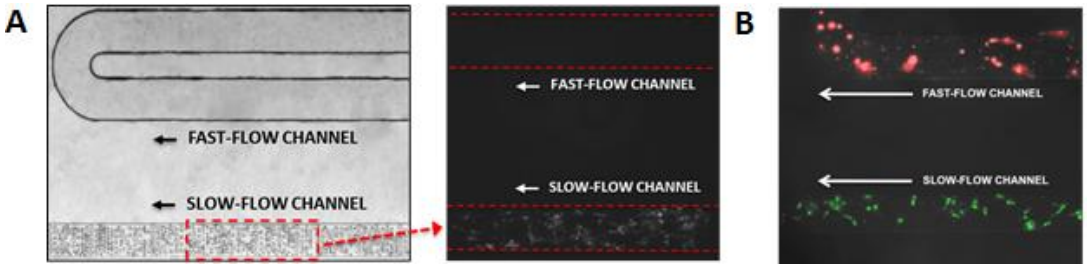

Figure 3: (A) Slow-flow channel's cell culture enhanced by laminar co-flow application. (B) Co-culture of different 3T3 fibroblast population in different channels of the biomimetic platform.

CONCLUSIONS

In this work, the utility of laminar co-flow to obtain localized cell cultures in any microfluidic system has been thoroughly investigated. As a proof of concept, 3T3 fibroblasts cell culture trials were performed in the new version of the splenon-on-a-chip, upgraded with a secondary inlet, and preliminary results highlight the fact that is possible to enhance cell culture inside the biomimetic platform. This datum clearly opens a wide range of possibilities of improving the cell culture prospects in organ-on-chip devices and therefore be able to better mimic the cellular environment of an organ.

ACKNOWLEDGEMENTS

Part of this work was financially supported by the technology transfer program of the Fundación Botín and by the Explora Program of the Ministry of Economy and Competitiveness of the Government of Spain. We thank David Izquierdo and Miriam Funes for their help in this project.

REFERENCES

[1] D. Huh *et al*. "Reconstituting Organ-Level Lung Functions on a Chip", *Science*, 328, 1662.
[2] L.G. Rigat-Brugarolas *et al*. "Functional microengineered model of the human splenon-on-a-chip", *Lab Chip*, 2014, 14, 1715-1724.
[3] A.J. Bowdler, "The complete spleen", *Humana Press*, 2nd edition.

CONTACT

*L.G. Rigat-Brugarolas, tel: +34 934 037 185; lrigat@ibecbarcelona.eu