DIELECTROPHORETIC CELL ASSEMBLY OF ARTIFICIAL LIVER SINUSOIDS IN A MICROFLUIDIC SYSTEM FOR USE IN SUBSTANCE SCREENING

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ABSTRACT

We have developed a microfluidic system with 3D-microstructures and integrated electrodes to assemble cells in an organ like fashion by superpositioning dielectrophoretic and hydrodynamic forces. To further mimic the *in-vivo* situation, cells are allowed to adhere on liver specific extracellular matrix proteins. Numerical simulations as well as experimental results show a successful co-assembly of endothelial cells as well as hepatocytes in the chip.

KEYWORDS: cell assembly, dielectrophoresis, polymer chip

INTRODUCTION

In-vitro toxicity tests used in drug development mostly rely on 2D-cell cultures which lack predictability with regard to the *in-vivo* situation [1-3]. A problem traditionally encountered with primary hepatocyte cultures is their rapid dedifferentiation, which is reflected in a deterioration of liver-specific functions. Thus, not all effects of drugs can be discovered in early drug development. Animal models lack predictability with respect to toxicity and should be avoided for ethical reasons whenever possible. Consequentially, the pharmaceutical industry faces considerable risks regarding the safety of patients during clinical trials and the loss of large R&D investments in case of failure of a drug candidate in a late state of drug development.

EXPERIMENTAL

The devices (see Fig. 1) with external size of microscopy slides (25 mm by 75 mm) were injection molded from a precision machined brass mold and are made out of cyclo-olefin-polymer (COP) which was successfully tested for biocompatibility.

Each device contains eight cell assembly chamber with three cell assembly features in order to increase the numbers of cells available for experiments. These assembly features have a bone-like shape, with a post at both ends to create a hydrodynamic focusing effect and an elevated assembly ridge where the cells can assemble under the influence of the dielectrophoretic (DEP) force, as at this position the field strength is maximized due to the reduced channel cross-section.

Figure 2 shows the numerical calculation of the DEP forces in such an assembly structure. Maximum forces are displayed as bright areas whereas small DEP forces are found in areas displayed at low brightness. Obviously, the DEP force is confined to the region close to the assembly ridges while in a relatively large fraction of the channel cross-section particles will experience only small DEP forces. Figure 3 shows the calculated particle trajectories under the combined effects of the DEP and the hydrodynamic focusing. A significant fraction of the trajectories terminates at the assembly ridge, indicating cell trapping.

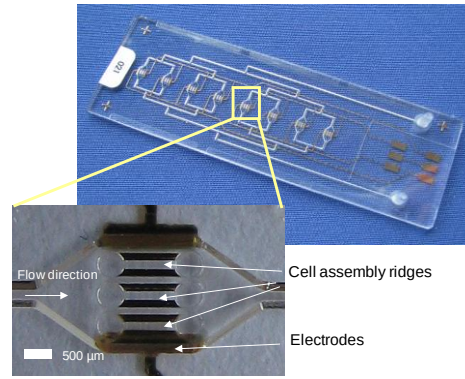

Fig. 1: Injection molded COP chip and close-up of one cell assembly chamber.

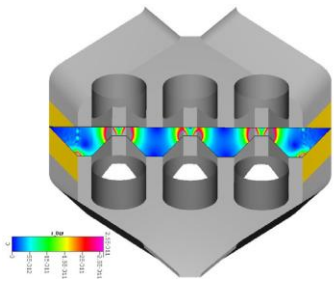

Figure 2: DEP-force distribution in cell assembly chamber Bright (red) areas denote high field strength.

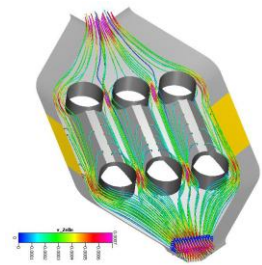

Figure 3: Particle trajectories derived from hydrodynamic and dielectro-phoretic forces.

RESULTS AND DISCUSSION

Figure 4 shows the time sequence of the assembly of primary human hepatocytes in an assembly chamber. Voltage amplitude was 280 V_{pp} @ 350 kHz; flow rate was 50 µl/min. The white arrow indicates the direction of flow. Cells adhere selectively on plateaus previously modified by extracellular matrix proteins. Figure 5 shows the co-assembly of human endothelial cells (red) on the periphery of the hepatocyte cultures. Flow rate was 10 µl/min and applied voltage 200 V_{pp} @ 350 kHz.

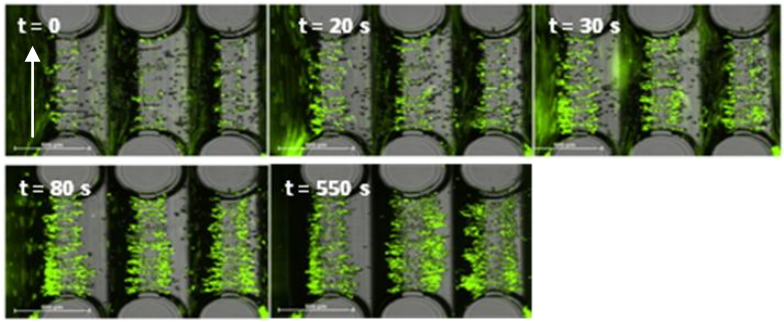

Figure 4. Assembly of human hepatocyte cells (green) in one assembly chamber.

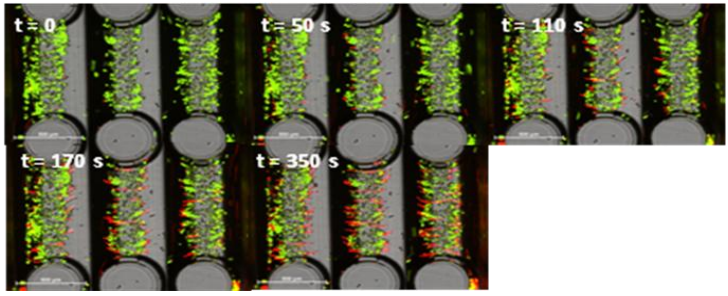

Figure 5. Co-assembly of human endothelial cells (red).

CONCLUSIONS

We have successfully co-assembled human hepatocytes and endothelial cells in a polymer microfluidic chip. Future work will be directed towards metabolic and fluorescence readout of cellular functions under the influence of test compounds.

ACKNOWLEDGEMENTS

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