MICROFLUIDIC IMPEDANCE SPECTROSCOPY SCANNER FOR SPHERICAL MICROTISSUES

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ABSTRACT

A microfluidic device is presented that enables the retention, cultivation and continuous analysis of spherical microtissues (MT) at a defined location in a microfluidic channel. During culturing and controlled exposure to analytical solutions, microtissues can be observed optically and analyzed using impedance spectroscopy (IS). To increase the resolution of the IS, hydrodynamic focusing is used to generate a vertical conductive liquid-sheet that can be scanned from left to right and generates independent "slices" of the microtissue. Operation principle and fabrication of the PDMS device are shown. First IS results with biological samples are presented.

KEYWORDS: 3D Microtissue, Impedance Spectroscopy, Sheath-Flow, Cell Analysis

INTRODUCTION

Multicellular spheroids are a very promising tool for tumor research and drug development, since they offer various advantages over 2D cell cultures [1]. *InSphero AG* (www.insphero.com, Zurich, Switzerland) developed a method for scaffold-free and automated high-throughput production of highly functional organotypic 3D microtissues [2]. However, fast and cost-efficient non-invasive analysis methods for such microtissues are still missing and urgently needed for application of spheroids in drug development and pharmacological research. Additionally, the analysis of the inner section of intact, larger 3-dimensional tissues is challenging with optical methods and requires new approaches. For continuous analysis under defined conditions we developed a microfluidic device, where spheroids with a diameter of 100 to 400 µm can be retained and analyzed using label-free impedance spectroscopy. The principle is shown in Figure 1.

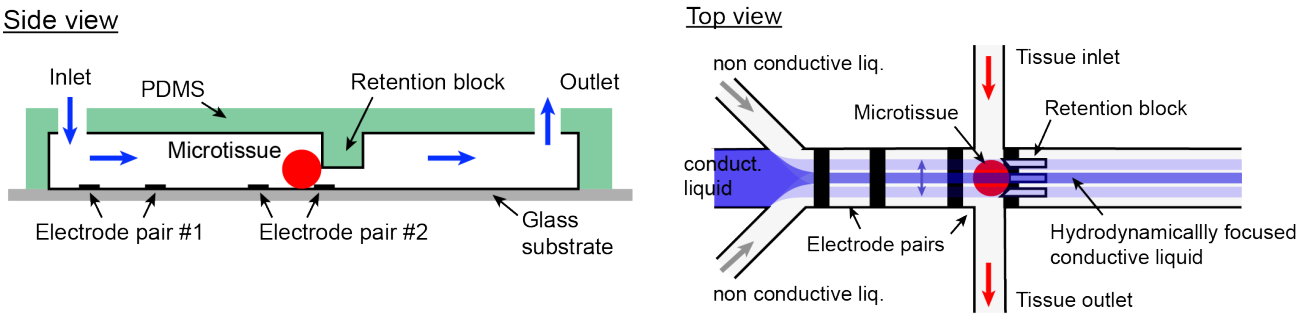

Figure 1: Schematic side and top view of the microfluidic chip. The microtissue is trapped by hanging PDMS blocks in the main channel. Hydrodynamic focusing is used to generate a vertical sheet of conductive liquid that can be scanned across the tissue in order to generate "tissue slices" during the recording.

The microtissue is loaded from the side and trapped in the main channel with a cross-section of 750 × 310 µm^2. PDMS blocks hanging from the ceiling prevent the microtissue from flowing further downstream. Two electrodes for IS surround the trap (pair #2) and two identical, additional electrodes (pair #1) are located further upstream used in a differential measurement mode. Three independent addressable inlets upstream in the mainchannel are used for hydrodynamic focusing. A conductive liquid is "squeezed" in between two non-conductive liquids entering from both sides. The width of the first can be a fraction of the diameter of the microtissue. The focusing on the one hand reduces leakage current through minimizing the measurement area, which increases the sensitivity. On the other hand–and more importantly–the liquid sheet in the form of a vertical plane can be scanned across the microtissue by precisely controlling the three inlet flow-rates. It therefore allows to record the impedance spectrograms of defined fractions or cross-sectional areas of the microtissue. After a measurement the microtissue can be removed and collected from the tissue outlet for further cultivation or additional analysis.

EXPERIMENTAL

The device consists of a PDMS mold bonded on a glass substrate, which is 2 × 5 cm^2 in size. Four Pt electrodes, 100 µm wide and separated by 400 µm are patterned by a lift-off process. The PDMS cover containing channels and the trap is molded from a 2-layer SU-8 master. The first SU-8 layer defines the bottom part of the channel, the second layer the upper

part including the traps. This way, liquid can flow underneath the trap in order to have contact of the conductive liquid with the electrode along the whole area. The PDMS retention blocks are formed from cavities introduced in the second SU-8 layer. To ensure that the microtissue cannot pass underneath the trap, the first layer never exceeds a height of 100 µm. By varying the height of the second layer, the total height of the microfluidic channel can be adapted to different-size microtissues. The fabricated chip is shown in Figure 2. Fluidic tubes are directly plugged in the PDMS cover and connected to syringes for flow control. Electrical wires are soldered to the metal pads to establish electrical connections. To precisely control the flow profile neMESYS syringe pumps form *Cetoni GmbH*, Germany, were used. For impedance measurements a HF2IS Impedance Spectroscope from *Zurich Instruments*, Switzerland, was used.

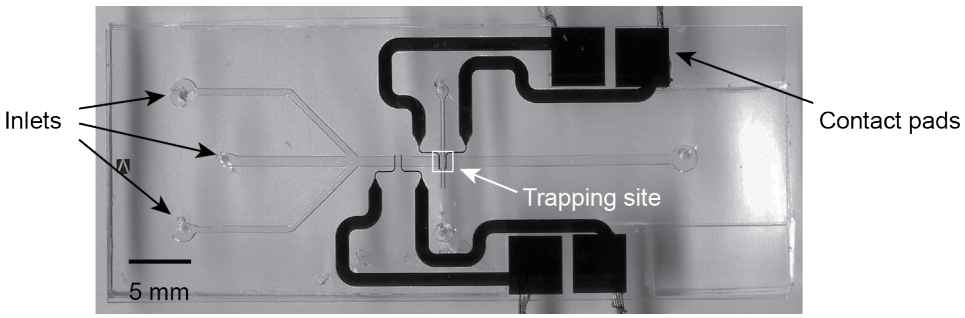

Figure 2: Microfluidic device with three fluidic inlets located on the left side. The left electrode pair is used as reference in the differential measurement mode. The trap for microtissues is flanked by the second electrode pair (white square, see Figure 3).

RESULTS AND DISCUSSION

Different types of microtissues were used in the experiments (SNB-19 and NIH-3T3), with 500 and 2000 cells per MT and corresponding diameters of 200 - 300 µm. The microtissues could be loaded and trapped reproducibly in the chip. Loading of microtissues is achieved by placing one single microtissue into a vertically positioned tissue inlet tube containing culture media and by subsequently applying a small suction force at the tissue outlet. The inlet tube is afterwards connected to a conventional syringe to close the system and unload the tissue at the end. An image of a trapped microtissue in the device can be seen in Figure 3(a). Figure 3 (b) shows a similar microtissue with applied sheath-flow, produced by hydrodynamic focusing of a conductive liquid in between non-conductive liquids. For better visibility, we used colored liquid in this image. It is clearly visible how the measurement area is reduced (brighter area), however, there is still a fraction of conductive liquid that goes around the MT and thereby reduces the spatial resolution. An adapted design will help to reduce this effect.

Impedance measurements on several days with different equal-sized microtissues showed little variation in the impedance signal. A significant difference of empty trapping sites versus a trapped microtissue was observed (Figure 4). In both cases the channel was completely filled with PBS, and a differential signal was measured between electrode pair #1 and pair #2. The non-zero impedance value of an empty trapping site is probably due to the PDMS trapping block close to the second electrode pair and due to slight differences in the electronic cables used for connection. This zero-impedance value remained constant for different devices for usage of the same electric connections.

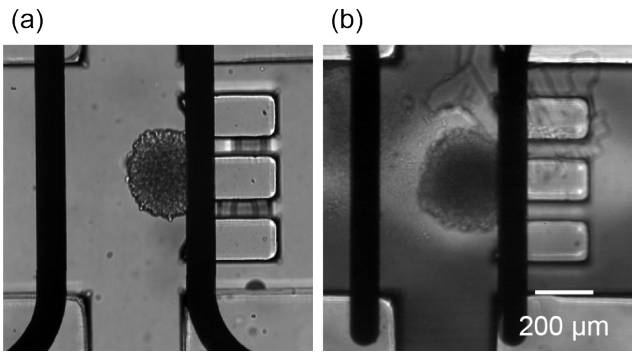

Figure 3: (a) NIH-3T3 brain tumor microtissue containing ~2000 cells trapped in the microfluidic device. The two flanking electrodes are visible. (b) Picture of the channel-centered focusing sheet. Colored liquids have been used for better visibility.

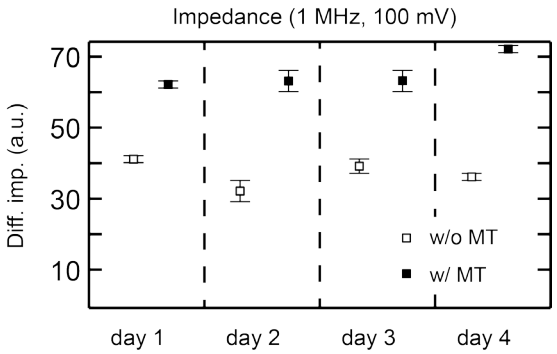

Figure 4: Impedance values with and without MT at different days: a significant difference is reproducibly measured.

In Figure 5 impedance values are shown, while scanning across a microtissue by stepwise introducing non-conductive DI-water from one side into the channel during perfusion with PBS. The flow-rate of PBS was held constant, whereas the flow-rate of DI-water was increased to twice the flow-rate of PBS. When focusing conditions are applied (2/1), the impedance difference (tissue/no-tissue) increases from 20 to 30 (a. u.) with respect to measurement without flow focussing. This demonstrates the increase in sensitivity through the focusing method even though, here, we only focused from one side. At equal flow-rates (2/2) the difference decreases due to partial coverage of the microtissue with non-conductive liquid. When the flow-rate reaches twice that of PBS (1/2), the microtissue is fully surrounded by non-conductive liquid, and the difference in impedance signals vanishes. Raw data of the change of the impedance signal during the stepwise flow increase of the non-conductive liquid are displayed in Figure 6.

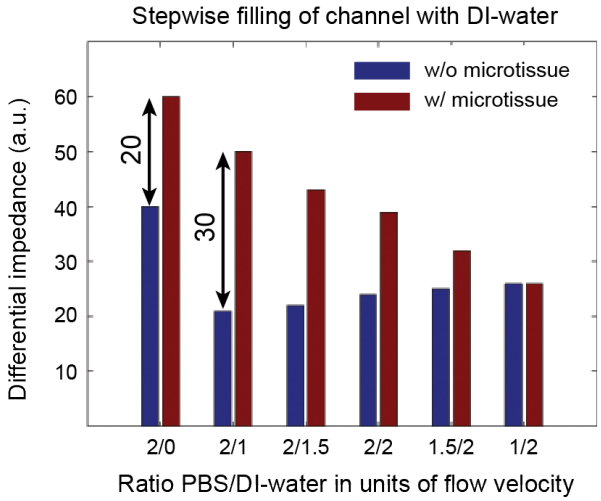

Figure 5: Impedance values during scanning across a trapped microtissue. DI-water is used as non-conductive liquid, the flow-rate of which is continuously increased (see Figure 6 for raw data). Focusing yields a higher impedance difference (=30) compared without (=20).

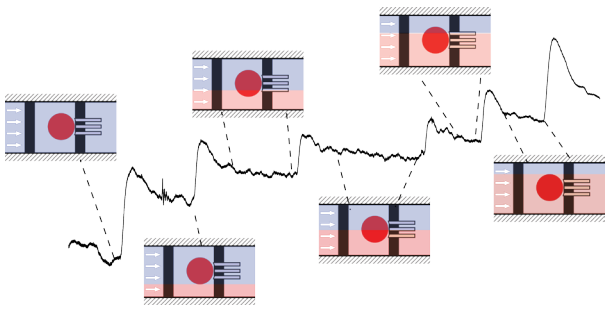

Figure 6: Change of the diff. impedance during a sheath-flow scan across a trapped microtissue (cf. Figure 5).

CONCLUSION

We were able to reproducibly trap spherical microtissues in a simple device that can be rapidly fabricated. Base-line impedance measurements are reproducible, and the intact tissues can be collected after every measurement from the tissue exit. This is essential for further analysis and allows serial investigation of multiple microtissues. Focusing of a conductive flow has two advantages: (a) It reduces the leakage current around the microtissue resulting in a sensitivity gain, and (b) it allows to analyze a fraction of the rather large cell cluster without destroying tissue. The first presented experiments are encouraging for further optimization and potential use in toxicology and efficacy testing.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Jan Lichtenberg and Dr. Wolfgang Moritz from *InSphero AG* for providing microtissues (types SNB-19 and NIH-3T3) for the experiments.

REFERENCES

[1] F. Hirschhaeuser, H. Menne, C. Dittfeld, J. West, W. Mueller-Klieser and L. A. Kunz-Schughart, "Multicellular tumor spheroids: An underestimated tool is catching up again," *Journal of Biotechnology,* vol. 148, pp. 3-15, 2010.

[2] J. M. Kelm, N. E. Timmins, C. J. Brown, M. Fussenegger and L. K. Nielsen, "Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types," *Biotechnology and Bioengineering*, vol. 83, pp. 173–180, 2003.

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