MONITORING OF 3D MULTI-CELLULAR SPHEROIDS IN HANGING DROP NETWORKS THROUGH IN-SITU IMPEDANCE SPECTROSCOPY

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ABSTRACT

We present the integration of electrical impedance spectroscopy (EIS) into a microfluidic network of hanging drops, in which spherical microtissues can be formed and cultured over time. EIS offers continuous monitoring of the drop volume and media conductivity and in-situ measurements of spheroid growth for different culturing conditions. The integration has been achieved by using a hybrid fabrication method, in which electrode modules on glass substrates were embedded into a microfluidic PDMS structure. The measurement results show robust drop volume control and, for the first time, size determination of human colon carcinoma spheroids directly inside a hanging drop.

KEYWORDS: Hanging Drop, Spheroid, Impedance, 3D Tissue

INTRODUCTION

Multi-cellular spheroids constitute an important three-dimensional tissue model system in pharmaceutical compound development and are used as simple organ models in basic research. Currently, two major factors limit their application in more complex multi-tissue formats: First, adequate perfusion-based culturing systems are missing, which can host multiple spheroids without functional loss and which can interconnect them in a fluidic network for continuous nutrient supply and compound exposure protocols. Second, readout method, which are currently optimized for 2D cell layers, have to be made compatible or have to be newly developed with respect to the uprising 3D tissue formats and new culturing setups.

We recently presented an analytical platform concept, in which arrays of hanging drops are interconnected to a microfluidic network [1]. The platform combines the formation and cultivation of 3D multi-cellular spheroids. In short, the hanging drop network is a complete open microfluidic system underneath an inverted PDMS substrate. The liquid is constrained by hydrophobic PDMS rims, which prevent its spreading over the whole surface. The layout of the rims comprises circular areas, where drops are forming and narrow grooves, where interconnection channels are established. The hanging drops act as compartments for the spherical microtissues, which sediment to the liquid-air interface at bottom of the drop. In this contribution, we present the integration electrical impedance spectroscopy (EIS) providing label-free information and holds great potential for tissue monitoring.

EXPERIMENTAL

The presented hanging-drop network is a completely open microfluidic system underneath an inverted PDMS substrate. The liquid is constrained by hydrophobic PDMS rims, forms hanging drops in the circular regions and is guided through channels in the narrow regions (Figure 1a). PDMS is used due to its bio-compatibility and the possibility to easily modify its wetting properties through O_2-plasma.

Four electrodes for EIS have been implemented on small glass plug-ins also comprising rim structures, which fit to the rim structures on the PDMS to yield a leakage-free overall microfluidic network (Figure 1b). The two electrode pairs serve two different purposes: (1) & (2) are used for monitoring/control of the drop size and medium conductivity throughout an experiment.

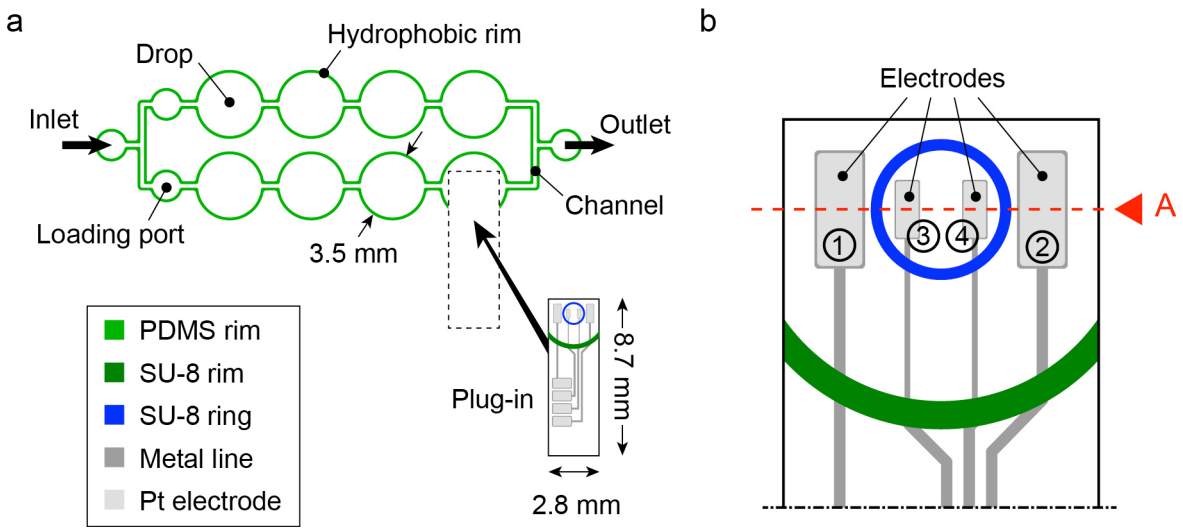

Figure 1: (a) Schematic top view of the 2x4-drop microfluidic structure including hydrophobic PDMS rims, which guide the liquid. The glass plug-in with 4 electrodes is placed into a recess and connects to the microfluidic network. (b) Close-up of the electrode-pairs in one of the two rows of drops. Two pairs of impedance electrodes are integrated: one for drop-size monitoring (1-2), one for spheroid-size monitoring (3-4).

Figure 2a presents two cross-sections of drops with different volumes and shows the respective electric field lines; Electrodes (3) & (4) are used to monitor the size of the spheroid. An integrated SU-8 ring enables precise positioning of the spheroid between the electrodes when the drop size is reduced and guides the electric field for improved sensitivity (Figure 2b).

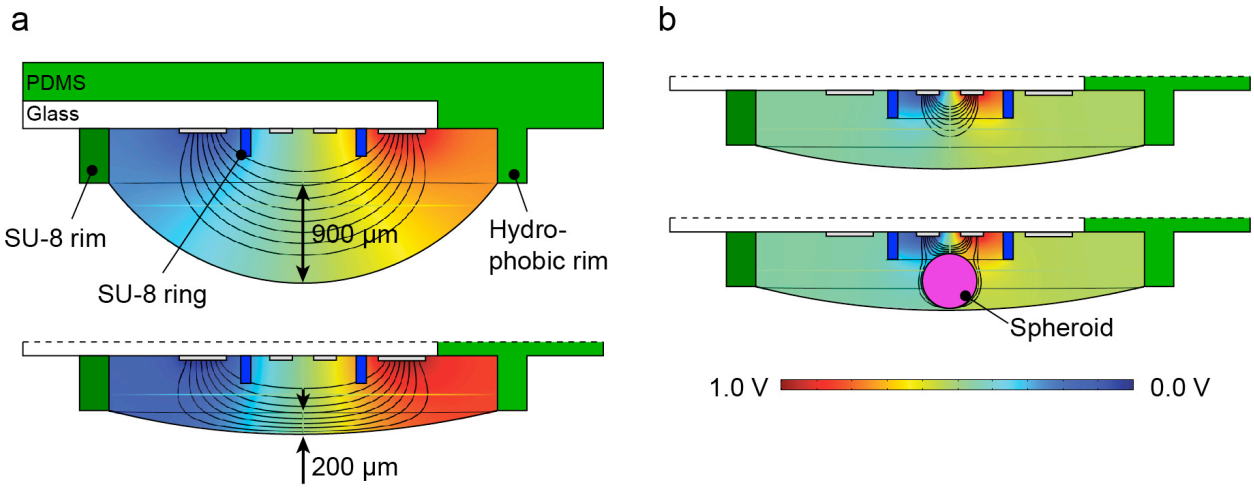

Figure 2: Simulation of the change of the electric field (at 10 MHz) as a result of (a) different drop heights, and (b) the presence of a spheroid in the hanging drop at reduced drop height. The simulations show cross-sections along "A" in Fig. 1b.

RESULTS AND DISCUSSION

Figure 3a shows the PDMS chip with integrated glass plugin. Figure 3b presents the electrical impedance amplitudes in dependence of the drop height recorded at 50 kHz. They stay in good accordance with the simulation results. Low noise allows determining the drop height at an accuracy of <50 µm. As visible, the recorded signal amplitude is also heavily influenced by the liquid conductivity represented by

different PBS dilutions. Different liquid conductivities, however, showed a drop-size-independent amplitude drop in the impedance spectrum (not shown here) at characteristic frequencies (Figure 3c), which can be used to determine the drop height with EIS independent of the liquid conductivity.

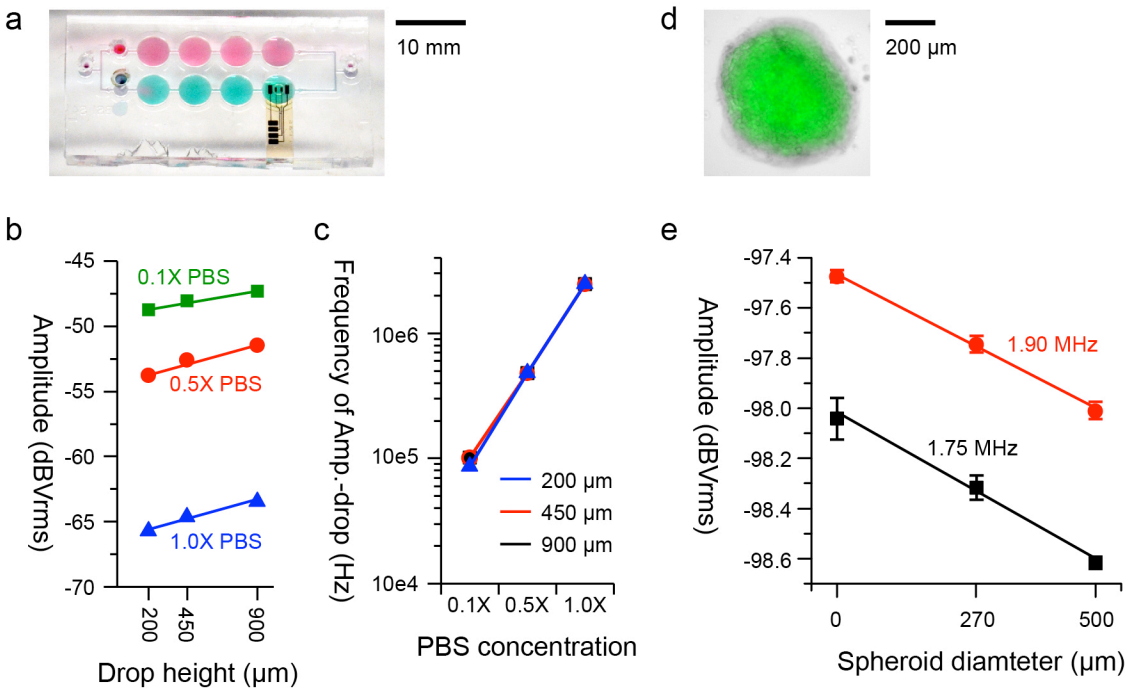

Figure 3: (a) Picture of the chip. (b) Signal amplitude (at 50 kHz) in dependency of drop height & liquid conductivity. (c) Frequency of amplitude drop in the impedance spectrum for different drop heights and conductivities. (d) Impedance amplitude signals at two different frequencies (1.75 and 1.9 MHz) for two different spheroid diameters and control. (e) Micrograph of the 500-µm spheroid (bright field and fluorescence overlay).

Spheroids were monitored after controlled reduction of the drop size, which brings the spheroid closer to the electrodes into the region of the strong electric field (Figure 3d). Spheroids of different sizes showed different impedance amplitude signals over a wide frequency range. The highest sensitivity (–1.07 dBmV/µm) and resolution (~70 µm) was achieved at 1.9 MHz (Figure 3e).

CONCLUSION

With the proposed hybrid device approach we are able to integrate micro electrodes directly into a hanging drop network and are able to perform in-situ measurement of the drop size for perfusion control and spheroid size for growth monitoring.

ACKNOWLEDGEMENTS

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REFERENCES

[1] O. Frey, P.M. Misun, D.A. Fluri, J.G. Hengstler, A. Hierlemann, Reconfigurable microfluidic hanging drop network for multi-tissue interaction and analysis, *Nature Communications*, 5:4250, 2014.

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