ELECTRICAL IMPEDANCE SPECTROSCOPY FOR LABEL-FREE, CONTINUOUS MONITORING OF DRUG IMPACT ON 3D TISSUE SPHEROIDS

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ABSTRACT

This contribution demonstrates that electrical impedance spectroscopy (EIS) can be used to detect dose- and time-dependent changes in 3D microtissue properties upon drug exposure. Inhibition of growth of microtissues, derived from human colon carcinoma cells (HCT-116), upon dosage of different NaN_3 concentrations could be assessed by using a simple EIS microdevice. The same device was used to investigate the impact of Diclofenac dosage on liver (HepG2) microtissues.

KEYWORDS: impedance spectroscopy, tissue spheroids, drug screening

INTRODUCTION

Microtissue spheroids are 3-dimensional tissue structures of large interest for the drug discovery process, as they feature morphologies and biochemical functionalities that closely mimic in-vivo conditions [1]. The monitoring of 3D microtissue properties upon exposure to, for example, drug candidates is however challenging, as most read-out methods are optimized for 2D cell-cultures or would imply a destruction of the tissue. In contrast, EIS is label-free and non-invasive and can provide frequency-dependent information on the dielectric properties of the tissue, which often correlates to their viability and functionality [2].

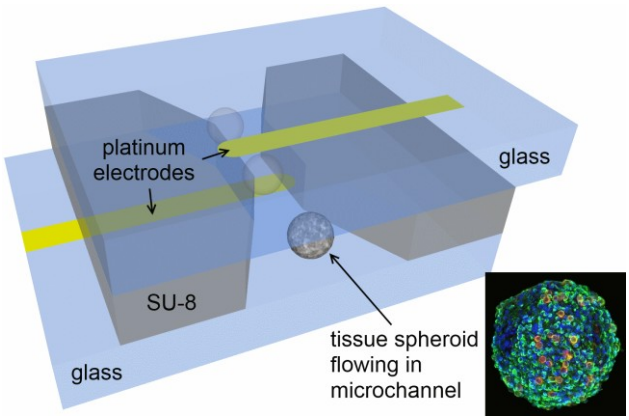

Figure 1: Schematic of an EIS microdevice: A microtissue spheroid of several hundred micron diameter is dragged by gravity through a microchannel with a cross-section of 500×500 µm² and passes the electric field of a set of opposing platinum electrodes for EIS measurements. The channel walls consist of SU-8 sealed at the top and bottom to glass. The flow-through approach leads to more consistent impedance signals in comparison to microtissue immobilization, where the signal is sensitive to minute motions of the tissue within the electric field.

RESULTS

HCT-116 microtissues of different sizes were introduced individually into a simple EIS microdevice shown in Figure 1. The impedance spectra of five different microtissues in Figure 2 show that larger tissue diameters lead to a bigger impedance signal. Tissues with diameters of, for example, 280 µm and 305 µm can be clearly discriminated.

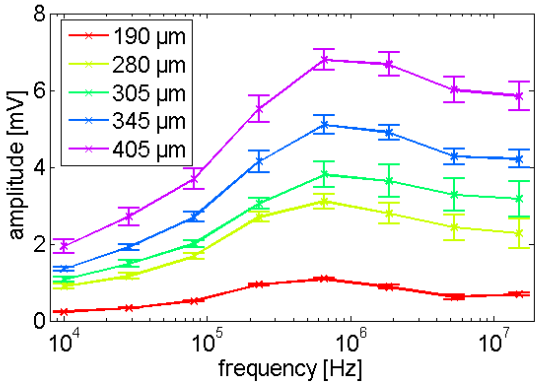

Figure 2: Impedance spectra of 5 HCT-116 microtissues of different diameters. Bigger diameters lead to larger signal amplitudes upon microtissue passage through the electrodes at frequencies between 10 kHz and 15 MHz. The signals of the 280-µm and 305-µm-diameter microtissues are more than two standard deviations apart throughout the spectrum so that the resolution is better than 25 µm. Error bars indicate the standard deviation over 5 measurements of the same tissue that have been obtained by tilting the chip back-and-forth.

Figure 3 shows the EIS data of HCT-116 microtissues treated for 2 h with different concentrations of toxic NaN_3. The impedance signal after 2 h decreases with increasing toxin concentrations. The impedance signal of the microtissue treated with 0.1% NaN_3 recovers after 2 days of subsequent culturing in standard medium not containing any toxin but featured a reduced growth rate. For higher concentrations the growth completely stops and does not recover after switching back to pure culture medium.

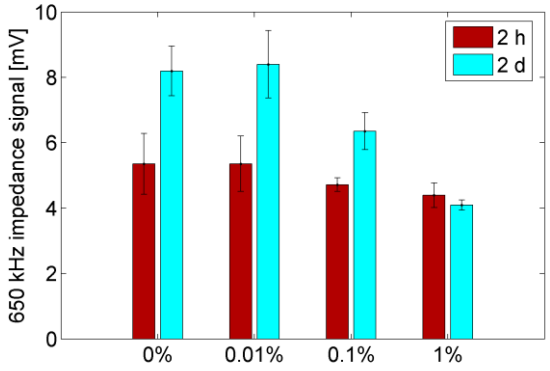

Figure 3: EIS data of HCT-116 tissues treated with different concentrations of toxic NaN_3 for 2h. The untreated tissue and the tissue treated with 0.01% NaN_3 are growing and show an increased impedance signal after 2 days of culturing in toxin-free medium. The tissue treated with 0.1% NaN_3 shows a smaller impedance signal after 2 h before it recovers and grows at a reduced rate as evident from the smaller signals in comparison to the first two tissues. The tissue treated with 1% NaN_3 shows a smaller signal after 2h and does not recover or grow within the next 2 days in toxin-free solution. Error bars indicate the standard deviation over 5 measurements of the same tissue.

Figure 4 shows another application of EIS to monitor the effect of the drug Diclofenac on microtissues of human liver carcinoma cells, HepG2. Microtissues treated with Diclofenac concentrations below 0.4 mM do not show significant changes in the impedance signal. Higher concentrations lead to swelling, which starts earlier for larger concentrations; the microtissue treated with the highest concentration of 5 mM Diclofenac even dissociated.

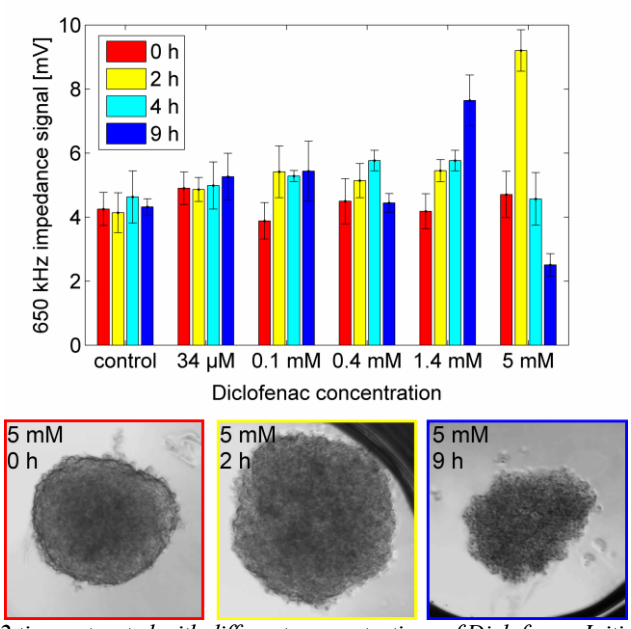

Figure 4: EIS data of 6 HepG2 tissues treated with different concentrations of Diclofenac. Initially, the seven untreated tissues show similar signals at t = 0 h. After 2 h, the tissues treated with the highest concentration (5 mM) start to swell, which entails an increased signal; afterwards they dissociate, which leads to a decrease in the impedance signal at t = 4 h and t = 9 h. The second highest concentration of the drug (1.4 mM) leads to a tissue swelling after 9 h. The control microtissue does not show any significant change over the duration of 9 h. Error bars indicate the standard deviation over 5 measurements of the same tissue.

DISCUSSION AND CONCLUSION

A resolution of better than 25 µm diameter in tissue volume measurements renders EIS suitable for monitoring growth of spheroids and for studying the effects of growth-inhibiting drugs on microtissues. The effects of NaN_3 treatment on HCT-116 microtissue and Diclofenac on HepG2 microtissue were evaluated. We observed growth inhibition of HCT-116 microtissue for concentrations higher than 0.1% NaN_3. The temporal difference between the occurrence of the effects for 5 mM and 1.4 mM Diclofenac on HepG2 microtissues is in agreement with reported EC50 values within a range of >1 mM to 240 mM over 4 h to 48 h. A concentration of 0.4 mM – 1.4 mM for affecting microtissues is in agreement with literature values: EC50 between few 100 µM to more than 1 mM over 16 h, depending on the assay [3]. These results demonstrate the potential of EIS as a complementary tool for non-invasive and label-free monitoring of microtissue spheroids during in vitro drug testing.

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