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Abstract

Sensitivity Characterization of an Impedance-Based Platform for Viability Analysis of 3D Spheroids †

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Abstract: Electrical impedance spectroscopy (EIS) is a promising label-free tool for high-throughput analysis of 3D cellular constructs, also called spheroids. Here, we used an EIS platform featuring facing electrodes to characterize the viability of hepatic spheroids, which are used for bioprinting applications. By using principal component analysis (PCA), we show that this simple impedance sensor enables us to successfully distinguish healthy spheroids from spheroids exposed to toxic conditions. The sensitivity of the impedance sensor will be further characterized by using spheroids exposed to varying stress conditions like different drug concentrations and temperatures.

Keywords: impedance sensor; label-free; 3D cellular constructs; spectroscopy; bioprinting

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1. Introduction

The use of 3D cellular constructs, such as spheroids, for regenerative medicine has led to an increased interest in label-free methods that enable high-throughput characterization of spheroids for quality assessment during production [1]. Electrical impedance spectroscopy (EIS) has proven to have a large potential for high-throughput analysis of microtissue viability [2]. However, previous studies have relied on coplanar electrode configurations due to the simplicity of fabrication, which drastically limits the sensitivity of the detection method [3]. Here, we evaluate the use of an EIS platform with facing electrodes for the analysis of the viability of individual spheroids under different stress conditions.

2. Materials and Methods

The EIS platform was fabricated by embedding two 300 µm-diameter platinum wires in a poly(methyl methacrylate) (PMMA) chip, followed by milling through the wires to create a pair of facing electrodes in a 350 µm-wide microfluidic channel (Figure 1A). Impedance measurements were performed by using a multifrequency lock-in amplifier (HF2LI, Zurich Instruments) (Figure 1B) and by recording impedance variations at 20 kHz, 490 kHz, 3 MHz, and 9 MHz.

Hepatic spheroids were formed using HepG2 cells seeded in Kugelmeier SP5D culture plates and cultured for 7 days. Two populations of spheroids were analyzed: spheroids under normal medium conditions and spheroids exposed to 10% DMSO for 30 min. Live/dead fluorescent staining was carried out using Fluorescein Diacetate to stain live cells and Propidium Iodide to stain dead cells. PCA was performed with L2 normalization on 8 parameters from EIS measurements.

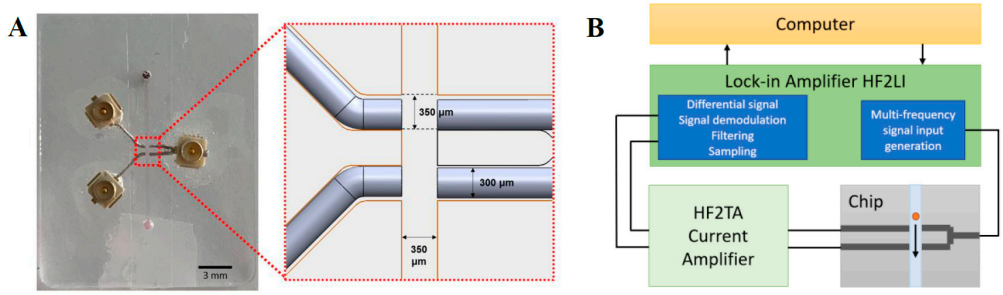

Figure 1. (**A**) Top view of the microfluidic EIS chip and close-up schematic detailing its critical dimensions. (**B**) Schematic of the measurement setup.

3. Results and Discussion

Using live/dead staining, we confirmed the impact of low concentrations of DMSO on the viability of spheroids: DMSO-exposed spheroids featured slightly increased numbers of dead cells, while morphological characteristics, such as size and shape, remained comparable to those of healthy spheroids (Figure 2A). Similarly, impedance measurements with the EIS platform enabled us to distinguish the two similar populations. PCA performed on EIS data showed that the first two principal components (PCs) explained 95% of the total variance and could, thus, be used for accurate pattern detection, displaying two identifiable clusters for two populations (Figure 2B). These results show that the EIS platform enables the characterization of spheroid viability even in case of subtle differences; so it holds great potential as a label-free alternative to imaging to achieve high-throughput analysis of spheroid viability. Upcoming experiments aim to further assess the platform sensitivity by exposing spheroids to different toxic compounds and temperature conditions.

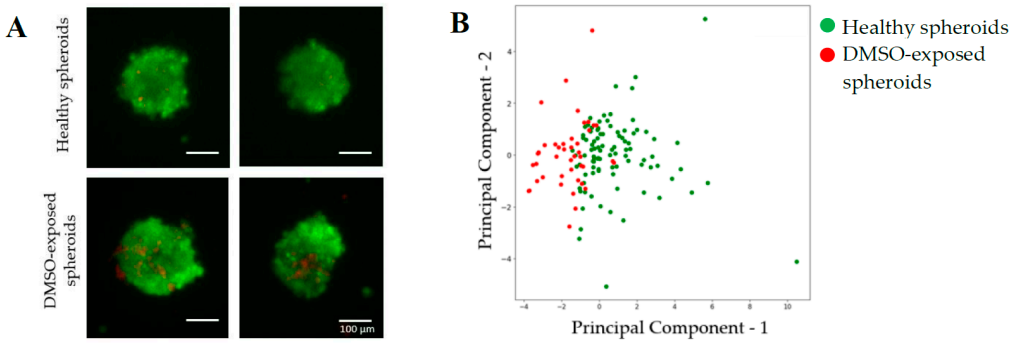

Figure 2. (**A**) Fluorescence images of the two populations, healthy spheroids and spheroids exposed to DMSO, showing fractions of dead (red) and live (green) cells. Scale bar: 100 µm. (**B**) Plot of the first two PCs retrieved after performing PCA on multifrequency EIS data for healthy (green) and DMSO-exposed (red) spheroids.

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References

1. OrganTrans Project. Available online: https://organtrans.eu/ (accessed on 15 March 2023).
2. Gong, L.; Petchakup, C.; Shi, P.; Tan, P.L.; Tan, L.T.; Tay, C.Y.; Hou, H. Direct and Label-Free Cell Status Monitoring of Spheroids and Microcarriers Using Microfluidic Impedance Cytometry. *Small* **2021**, *17*, 2007500. [CrossRef] [PubMed]
3. Zhu, S.; Zhang, X.; Zhou, Z.; Han, Y.; Xiang, N. Microfluidic impedance cytometry for single-cell sensing: Review on electrode configurations. *Talanta* **2021**, *233*, 122571. [CrossRef] [PubMed]