CONTINUOUS AND LABEL-FREE TOXICITY SCREENING OF HUMAN HEPATOCYTES ON CHIP REVEALS FREQUENCY-DEPENDENT IMPEDANCE PROFILES

R. Meissner*, B. Eker and Ph. Renaud
Ecole Polytechnique Fédérale de Lausanne (EPFL), SWITZERLAND

ABSTRACT

We present an electrical impedance based biosensor on a microfluidic cell culture platform that enables label-free and real-time investigation of drug toxicity-induced effects on hepatocytes. The temporal evolution of the impedance at different frequencies strongly suggests distinct information on toxicity effects within the cell culture. The findings indicate that the sensor distinguishes between morphological changes and severe cell injury within a cell population owing to the combination of high-density culture, perfusion flow and whole impedance-frequency spectrum analysis. More importantly, cellular changes can be sensed long before cellular death, offering a highly sensitive technique over standard viability assays.

KEYWORDS: Toxicity, Hepatocytes, Impedance, Drugs, Acetaminophen, Cell culture

INTRODUCTION

In vitro toxicity tests have become an indispensable and complementary tool for animal testing in pharmaceutical industry. In this context, hepatocyte culture in microfluidic devices has been shown to offer many advantages in toxicity screening such as high-throughput analysis and low sample need. However, current viability screening methods are generally limited to expensive and time consuming biochemical end-point assays [1]. They do not have the capacity to screen cellular changes that precede cellular death, resulting in insensitive measurements to subtle toxic effects [2]. In addition, existing impedance sensors are mostly used only for single frequency time scan measurements, thus neglecting the dielectric nature of biological cells [3]. Here, we present an electrical impedance based biosensor on a microfluidic cell culture platform that enables label-free and real-time investigation of drug toxicity-induced effects on hepatocytes. The temporal evolution of the impedance at different frequencies strongly suggests distinct information on toxicity effects within the cell culture.

THEORY

Measuring the dielectric properties of cells is a very sensitive, label-free and real time method to screen cellular properties such as morphology and viability. A cell can be described with an equivalent circuit made of a membrane capacitor C_m (constant phase element (CPE) for a cell population [4]) with a series intracellular resistance R_{intra} and a parallel extracellular resistance R_{extra} (Fig.1). Considering such a circuit, it is possible to extract information about the circuit elements at different frequencies. Thus, drug toxicity effects were examined at low frequency (LF) and high frequency (HF). LF is defined as the frequency before membrane shortening takes place and gives information about extracellular properties. HF is defined as the frequency where the membrane capacitor is short-circuited and provides information about the cell interior.

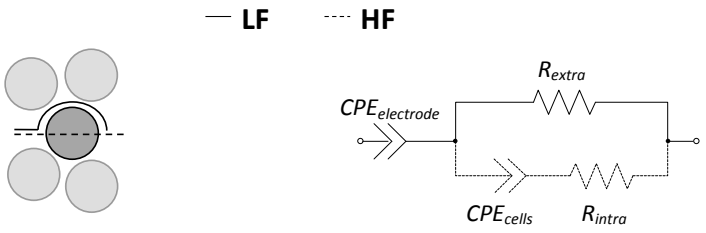


Figure 1: Left: Schematic of current pathway at low and high frequency, Right: Equivalent circuit of electrode-cell population model

EXPERIMENTAL

We designed a microfluidic chip with four arrays of PDMS pillars that immobilize HepG2/C3A cells (Fig. 2a). A continuous perfusion flow guarantees efficient nutrient supply and hinders cell proliferation outside the filters due to shear stress. Interdigitated electrodes were implemented in the device such that the sensing area only includes cells trapped in filters.Each electrode trace carries four arms (w=20) with nine fingers (w=10 µm, s=15 µm). Continuous perfusion of cell medium was performed in the microchip for 5 days in order to make sure that the cells proliferated and were healthy inside the filters (Fig.

2b-d). Acetaminophen, one of the widely used analgesic with a well-known severe hepatotoxicity, was then added to the perfusion medium at 20 mM dose and perfused for 60 h. Impedance spectra were recorded from 100 Hz to 4 MHz (10 mV).

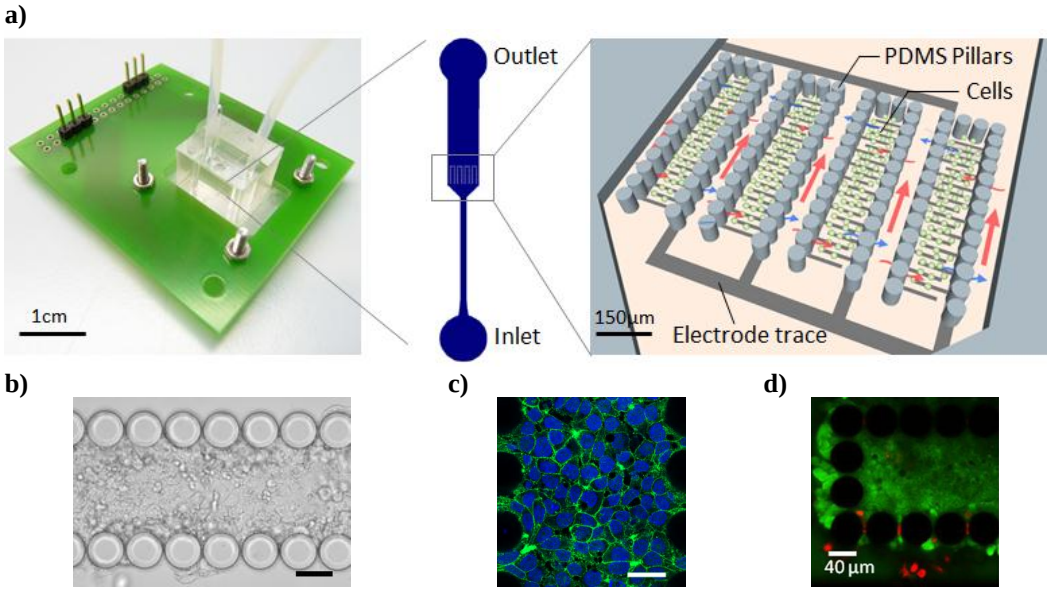


Figure 2: Microfluidic cell culture. a) Microfluidic device with electrical interface (left), perfusion channel design (middle), cell culture area involving PDMS pillars for cell immobilization and electrodes (right), b) Brightfield image of cells within a filter structure (without electrodes), c) Close cell-cell contacts are visualized through occludin staining (green=Cy-2, blue=DAPI), d) Life/dead staining (green=fluoresceine-diacetate; red=propidium-iodide). (bars=40 µm)

RESULTS AND DISCUSSION

The presence of cells in the electric field of the electrode pair induces frequency-dependent changes (Fig. 3a). The relative phase angle change provides qualitative information about the different circuit elements. Consequently, the choice of the measurement frequencies for toxicity screening was made according to the two relative phase angle maxima $\phi_{max\ 1}$ and $\phi_{max\ 2}$ (Fig. 3b), both representing sensitive frequencies to obtain quantitative impedance magnitude information about R_{extra} and R_{intra}. We observed that these maxima do not occur at the same frequency as the maximum relative magnitude change $|Z|_{rel\ max}$ that is commonly used in other studies [3].

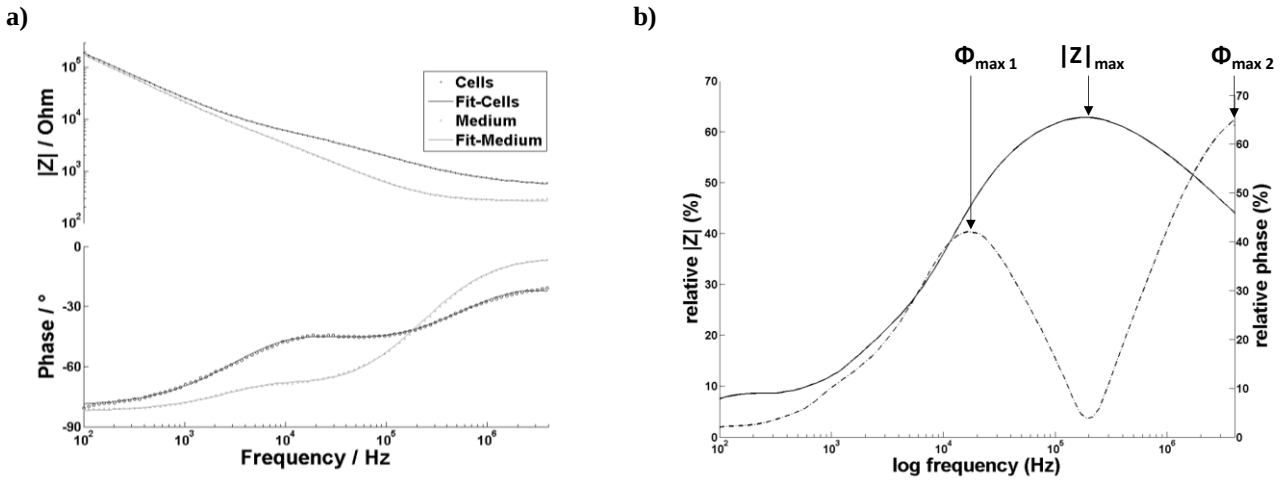
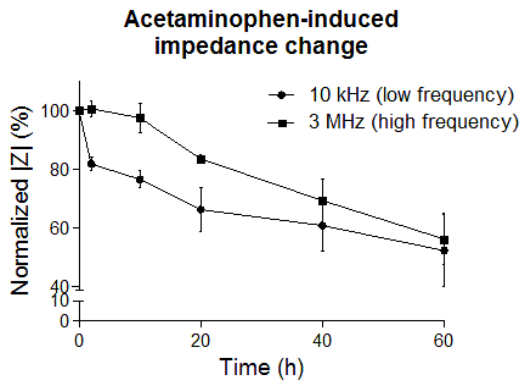


Figure 3: Determination of measurement frequencies for further toxicity screening. a) Impedance spectrum in the presence and absence of cells. b) Corresponding relative impedance magnitude and relative phase angle change in the presence of cells. The first phase maximum $\phi_{max\ 1}$ indicates maximal extracellular changes at that frequency, whereas $\phi_{max\ 2}$ points out changes at high frequency. It is observed that both phase angle maxima are not matching with the relative magnitude maximum.

We found that the long-term impedance magnitude after acetaminophen application is similar for LF and HF measurements, but its temporal evolution has different profiles for both frequencies (Fig. 4a). LF signals drop as early as 2 h of drug exposure, whereas HF signal does not change significantly. LF is defined as the frequency before membrane shortening takes place and gives information about extracellular properties. The decrease in the LF signal was correlated to the extracellular events such as loosened cell-cell contacts as indicated by the decrease in actin stress fibers (Fig. 4b). On the other hand, cell death is linked to the intracellular conductivity change and can be detected at HF. This was confirmed by the noticeable increase of dead cells observed at 24 h of drug exposure.

a)



b)

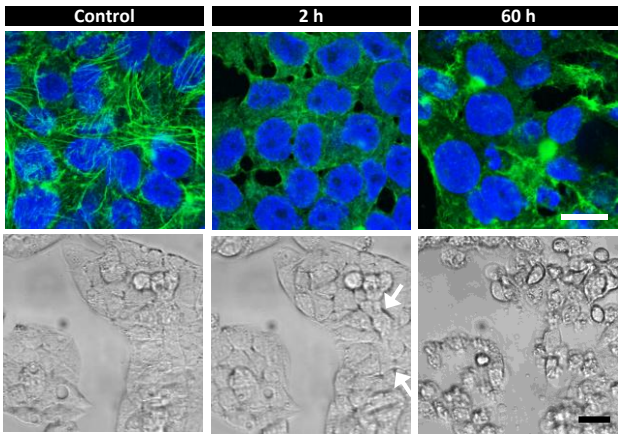


Figure 4: a) Acetaminophen (20 mM) induced impedance magnitude change with time at 10 kHz and 3 MHz (normalized). Different kinetics is apparent comparing low and high frequency. b) Transmission light and confocal images of cells before (control) and after 20mM acetaminophen treatment (2h, 60h). Immunocytochemical staining of actin cytoskeleton revealed structural changes as early as 2 h after 20 mM AP treatment. After 60h, the presence of dead cells is observed (arrows). green=FITC, blue=DAPI. (bars=10 μm)

CONCLUSION

We have developed a novel impedimetric biosensor that distinguishes between morphological changes and severe cell injury within a cell population owing to the combination of high-density culture, perfusion flow and whole impedance-frequency spectrum analysis. Cellular changes can be sensed long before cellular death, offering a highly sensitive technique over standard viability assays.

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CONTACT

*R. Meissner, tel: +41-21-693-6797; robert.meissner@epfl.ch