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Stability of cell adhesion noise analysis for the detection of cancer cell lines

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Abstract: Detecting cells on substrates time-continuously without the need of optical microscopy is broad interest in biotechnological applications. We demonstrate the stability and repeatability of a method to detect cancer cells using cell adhesion noise spectroscopy across different CMOS-based microelectrode arrays. We analyze the recordings in terms of spectral power density.

Keywords: CMOS-based microelectrode array, electrical imaging, cell adhesion noise spectroscopy, colorectal cancer cells.

1 Introduction

Electrical impedance spectroscopy (EIS) is an established method to characterize cell properties [1]. However, it suffers from a relatively low spatial resolution, usually in the range of 0.1 – 1 mm. A related technique, noise spectroscopy, benefits from the high spatial resolution offered by CMOS technology without the need to perturb the cells under test by external stimulation currents [2-3]. Cell adhesion noise (CAN) spectroscopy takes advantage of the increased resistance due to the tight interface between cells or tissue and sensor sites of a CMOS-based high-density microelectrode array (MEA). These CMOS-based MEAs are widely employed in electrogenic cell sensing and actuating [4-5]. Also, they can be used to identify adherent non-electrogenic cells, as recently shown by us [6]. An essential requirement for CAN spectroscopy to detect cancer cell lines and changes therein is the stability across sensors, time, and different sensor chips employed. We address this stability using the cancer cell line HT-29 cultured on CMOS MEAs with 98 304 sensor sites. The identified stable conditions pave the way for future studies investigating the effect of chemotherapeutic agents on cancer cell lines or cancer spheroids using CAN spectroscopy.

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2 Methods

2.1 Cell culture

We cultivated the colorectal cancer cell line HT-29 (ATCC) in Dulbecco's Modified Eagle's Medium (DMEM (1X), Thermo Fisher Scientific Inc., Germany) supplemented with 10 % v/v Fetal Bovine Serum (FBS, heat-inactivated, Thermo Fisher Scientific Inc., Brazil), 1 % v/v Penicillin Streptomycin (Pen Strep, Thermo Fisher Scientific Inc., Germany) and 1 % v/v L-Glutamine 200 mM (Thermo Fisher Scientific Inc., Germany). We changed the cell culture medium every two days and incubated the cells at 37 °C in a 5 % CO_2 atmosphere.

2.2 Electrical Recording and cell-chip interface

The CMOS MEA (obtained from formerly Venneos GmbH, Stuttgart, Germany) offers 256 x 384 capacitive recording sites with a pitch of 5.6 µm x 6.5 µm covering an active area of 1.6 mm x 2.5 mm as described in [7]. The sensor array is coated with a 30 nm ALD-TiO_2 top oxide layer. A Perspex culture chamber was glued on the chip to provide a reservoir for cell cultivation. Thus, only the recording sites of the MEA are exposed to cell culture and medium [3]. We employ an external Ag/AgCl electrode as a reference electrode to calibrate the MEA using the CAN-Q Acquisition software (Venneos GmbH) with default settings. The CAN-Q Acquisition software estimates the spectral power density S_V of the extracellular voltage at frequencies between 1 kHz and 450 kHz. We record Phosphate-Buffered Saline (PBS, Thermo Fisher Scientific Inc., Germany) as control and adherent cells in cell culture medium as cells under test.

We cleaned the chip surface with Tickopur R60 (5 % v/v, 80 °C, Dr. H. Stamm GmbH Chemische Fabrik, Berlin, Germany), sterilized it with UV light, and rinsed the chip with

distilled water. Next, we coated the recording sites with collagen (10 % v/v, Sigma Aldrich GmbH, Vienna, Austria) to ensure tight cell adhesion to the MEA surface, incubated it at room temperature, and washed it with distilled water to remove the excess coating solution. To seed the cells, we pipetted 100 µL of a cell-culture-medium suspension (20 000 cells/chip) on top of the sensor array. For proper cell sedimentation, we incubated the chips for one hour at 37 °C in a 5 % CO_2 atmosphere and filled the culture chamber with cell culture medium to ensure cell viability. We placed the chips and recording hardware in a safety cabinet at room temperature without additional shielding during the recordings.

2.3 Data analysis

We analyzed the recordings using a custom script (Python version 3.9.5). To enhance the contrast of the electrical image, the estimated spectral power density S_V is filtered with a spatial Gaussian filter [8], and single-pixel errors are removed with the Gaussian blur technique using the OpenCV Library [9].

2.4 Microscopy

We related the estimated cell positions to ground truth using brightfield images of the CMOS MEA's sensor array taken with an upright light microscope (Zeiss Axioplan, 10x objective).

3 Results

This study examines the reproducibility and stability of the electrical imaging of non-electrogenic cancer cells using adhesion noise spectroscopy.

We electrically imaged adherent cells following the method described previously [6] with a high correspondence (90 %) between the adhesion noise-based identification and microscopically identified cells (Fig. 1A). Undetected small structures on the sensor surface indicate poor cell attachment or dead cells.

Previous studies showed that the voltage noise is significantly increased by the resistive cleft between the cell membrane and the planar recording site [2-3,10]. Both neurons and non-electrogenic cells, such as the cancer cell line HT-29, likely contribute with their resistive cleft to the adhesion noise. We analyze the resistive cleft from the spectral power density S_V of the voltage noise at a bandwidth of 60 kHz. Sensor sites with adherent cells show an increased S_V of the total spectrum across the entire frequency range (Fig. 1B). The difference (ΔS_V) between the total S_V and the S_V of bare sensors (i.e., sensors exposed to the electrolyte) is attributed to the cell adhesion noise. Sensors that detect adherent cells are defined as "positive sensors" based on their S_V value, which must exceed the sum of ΔS_V and the S_V of bare sensors. We identify sensors with adherent cells using this threshold.

We analyzed the variability of CAN spectra by calculating the mean spectrum averaged over ~1000 sensors and the standard deviation (std) within a single recording. These sensors were selected based on high values of S_V of adherent cell networks. This mean CAN spectrum was compared to the mean spectrum for sensors without adherent cells. The CAN

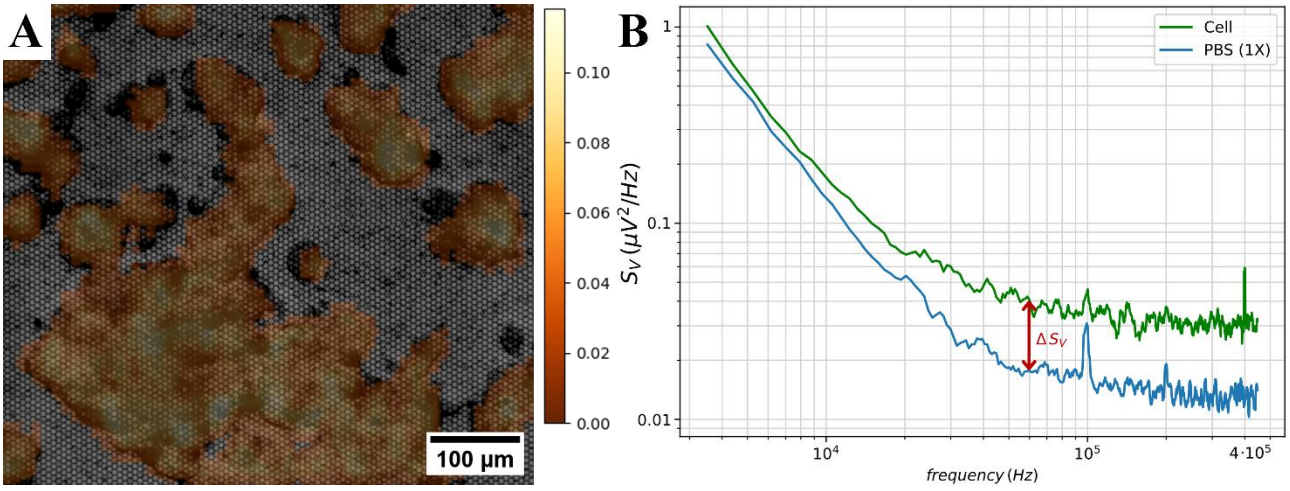

Figure 1: Identification of adherent cells on CMOS MEA by adhesion voltage noise in terms of spectral power density (S_V evaluated at 60 kHz bandwidth). **A)** Overlay of electrical imaging of HT-29 cells with brightfield microscopy imaging. **B)** Determination of the cell identification threshold via cell adhesion noise spectroscopy at 60 kHz. To identify an adherent cell, a sensor must exceed the sum of ΔS_V and the values of S_V of bare sensors.

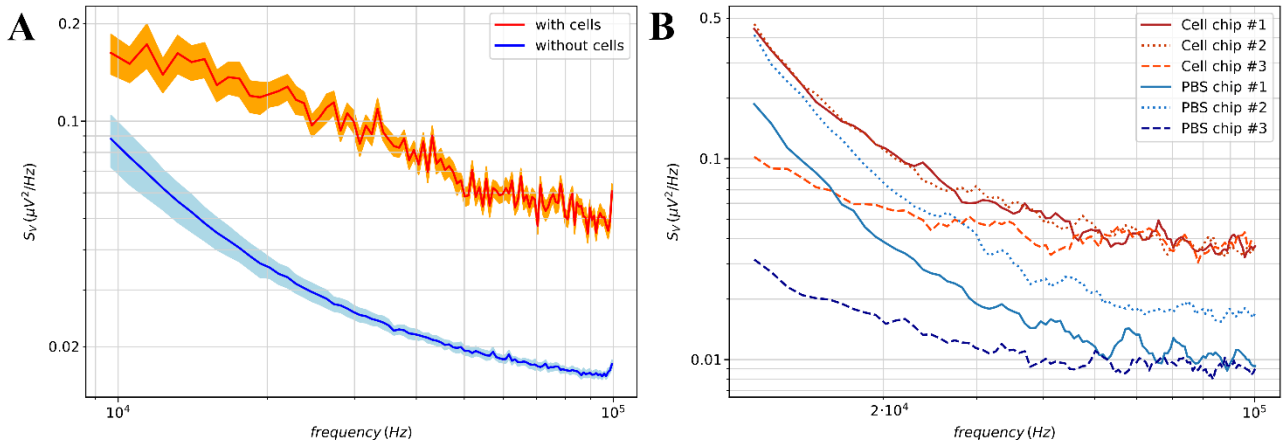

Figure 2: Variability of cell adhesion noise spectra. **A)** Mean spectrum of ~1000 sensors and standard deviation of sensors with adherent cells (red-orange trace) and without cells (blue trace) within a single recording. **B)** Mean spectra of three different CMOS MEAs with adherent cells (red traces) and without (blue traces).

spectrum did not overlap with the control spectrum in the frequency range between 10 kHz and 100 kHz (Fig. 2A). Both conditions (cell adhesion and control) showed a comparable standard deviation of 0.0047 $\mu V^2/Hz$.

In a second approach, we compared the spectra across different CMOS MEAs of the same type. We found a high overlap for different chips for both conditions, either with adherent cells on top of the sensors or control sensors (Fig. 2B).

In a third experiment, we investigated the stability of CAN for the same sensor in multiple recordings during the cultivation for three days. The adhesion noise varies for different cultivation times, which has to be investigated in further studies, but the cell detection was still reproducibly accomplished (data not shown here).

4 Conclusion

The robust recordings presented here across sensors, time, and devices are essential factors in applying MEA-based CAN spectroscopy for preclinical applications. As a test case, we demonstrated the reliable detection of adherent cancer cell lines. Ongoing experiments investigate the effect of chemotherapeutic drugs on cell adhesion and cell viability.

Author Statement

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