# Electrical Recording of Effects of Chemotherapeutic Treatment on Cancer Spheroids

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***Abstract*—Monitoring cells on substrates time-continuously without relying on optical microscopy imaging is of broad interest in biotechnological applications. We propose an approach to electrically investigate cytotoxic effects of a chemotherapeutic treatment on cancer cells using cell adhesion noise spectroscopy. Recordings are taken with high-density microelectrode arrays, data are analyzed in terms of spectral power density.**

***Keywords—CMOS-based microelectrode array, cell adhesion noise spectroscopy, colorectal cancer cells, spheroids, anti-cancer therapeutics.***

## I. Introduction

CMOS-based microelectrode arrays (CMOS MEAs) are used in biotechnological applications to record neuronal activity with high spatial (~15 µm) and high temporal resolution (~20 kHz bandwidth) using thousands of densely packed sensor sites [1-2]. A new application of CMOS MEAs is the label-free and noninvasive detection of adherent cells by studying the voltage noise from the resistive adhesion cleft [3-6]. The voltage noise is described as cell adhesion noise (CAN) of human cells and analyzed in terms of the spectral power density ($S_V$) [7].

So far, only electrical impedance spectroscopy (EIS) reported the electrical identification of nonelectrogenic cells [8-9]. In EIS, varying the stimulation amplitude offers a wider parameter range than CAN spectroscopy but is time-consuming. While CAN spectroscopy records thousands of CMOS MEA sensor sites in under a minute, a readout of the same number of sensors is not feasible with EIS. Here we aim to detect early changes in cell adhesion of colorectal cancer (CRC) cells growing in 2D and 3D cultures after treatment with the chemotherapeutic agent 5-Fluorouracil (5-FU). In contrast to optical imaging, CAN spectroscopy using CMOS MEAs offers label-free and, in principle, continuous recording capability. We therefore establish a correlation between the adhesive properties of cancer cells on the CMOS MEA and their proliferation by assuming a loss in cell adhesion caused by the 5-FU anti-cancer cytotoxic drug [10]. Furthermore, the 3D microtumors (spheroids) are of great interest since they mimic better the physiology, spatial structure, gene expression profiles, drug metabolism and drug resistance mechanisms of tumors compared to conventional 2D cell monolayers [11]. Besides, this label-free noninvasive approach is of broad interest to investigate drug efficacy in a fast and cost-effective manner in comparison with the *in vitro* viability and cytotoxicity assays used in preclinical studies, e.g., MTT and TUNEL assays. The CAN-based proliferation status is related to commercially available cell viability assay (CCK8) and light microscopic images.

## II. Methods

### A. Cell culture 2D models

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

### B. Cell culture spheroids formation

For the formation of the spheroids, we culture the CRC cell line HT-29 in Nunclon™ Sphera™ 96-well plates (Thermo Fisher Scientific Inc.) in a 5 % $CO_2$ atmosphere at 37 °C. We use Dulbecco's Modified Eagle's Medium/F12 and Ham's media (Sigma-Aldrich) in a proportion 1:1 with 3.151 g/L glucose, L-Glutamine, 20 ng/mL of Epidermal Growth Factor (EGF, Sigma-Aldrich), 10 ng/mL Fibroblast Growth Factor-Basic (bFGF, Merck KGaA), 5 µg/mL insulin (Gibco™), 10 % v/v bovine serum EU standard and Pen Strep (Sigma-Aldrich), at a working concentration of 100 units of potassium penicillin and 100 µg of streptomycin sulfate per 1 mL of culture medium. We change the medium every two days. From day 7 the spheroids are ready to use.

### C. Drug treatment

Drug treatment is conducted on the CMOS MEA and in 96-well plates (control), both in 2D and 3D cultures. For the 2D assays, we seed 10 000 cells per well in Nunc™ MicroWell™ 96-well, Nunclon Delta-Treated, Flat-Bottom Microplates (Thermo Fisher Scientific Inc.) and 20 000 cells on the CMOS MEA. For the 3D assays, we use the generated spheroids in the 96-well plates and the ones deposited on the CMOS MEA.

For both 2D and 3D assays after 24 h of conditioning, the cell culture medium is aspirated and replaced with medium containing 5-FU. We added 5-FU (Sigma-Aldrich), $c_{5\text{-}FU}$=0.0105 mg/mL to the 2D [12] and $c_{5\text{-}FU}$=0.1 mg/mL to the 3D assays. After treating the cells for 30 min, we remove the medium containing the 5-FU and add maintenance medium to the cultures and proceed with both the CCK8 and the recording of the CMOS MEA assays.

### D. CCK8 assay

After drug treatment at indicated concentrations, wells are washed with 100 µL Phosphate-Buffered Saline (PBS, Thermo Fisher Scientific Inc.) and incubated in 100 µL medium and 10 µL CCK8 (MedChemExpress) for 2 h following manufacturer's instruction. In case of a time course, wells are washed with PBS after CCK8 incubation before adding 100 µL fresh medium. For the control group, cells are grown in medium supplemented with PBS in the same volume as the drug. The optical density is read at 450 nm with a microplate reader (Absorbance 96, byonoy) and cell viability is calculated with (treatment signal/control signal) · 100. Cells growing solely in culture medium determine the CCK8 control sample.

### E. Recording with CMOS MEA

The CMOS MEA (obtained from formerly Venneos GmbH) provides 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 [13]. A top oxide layer (30 nm ALD-$TiO_2$) covers the entire sensor array. The spectral power density $S_V$ is evaluated at frequencies between 1 kHz and 450 kHz using the CAN-Q Acquisition software (Venneos GmbH). The chip calibration is performed by the CAN-Q Acquisition software with default settings, an Ag/AgCl electrode is used as external reference electrode. A Perspex culture chamber is glued on the chip such that the recording site arrays are exposed to cell culture and medium [6]. The measurements are taken either with PBS or with adherent cells in cell culture medium.

The cells are measured before the drug treatment (0 h) and 24 h, 48 h and 72 h after treatment in compliance with the recovery time following [12].

### F. Cell–CMOS MEA interface

The surface of the CMOS MEA chips is cleaned with Tickopur R60 (5 % v/v, 80 °C, Dr. H. Stamm GmbH Chemische Fabrik), followed by sterilization with 70 % v/v ethanol for 15 min and rinsing with distilled water. To promote tight cell adhesion, the recording sites are coated with 50 µL of collagen (10 % v/v, Sigma-Aldrich) and incubated at room temperature for 2 h. The excessive coating solution is removed by rinsing the chips with distilled water. Next, 100 µL of a cell-culture-medium suspension (200 cells/µL) is pipetted on top of the sensor array. To ensure proper cell sedimentation, the chips are first incubated for 1 h at 37 °C in a 5 % $CO_2$ atmosphere and then the chips' chambers are filled with cell culture medium to guarantee the cells' viability. The chips and recording hardware are placed in a safety cabinet at room temperature without additional shielding during recordings.

### G. Data processing and statistics

The data are analyzed with customized Python scripts (Python version 3.9.5). The estimated $S_V$ is filtered with a spatial positive Gaussian filter [14] for contrast enhancement and single-pixel errors are removed via Gaussian blur technique using the OpenCV Library [15]. Statistical analysis is performed with Origin (version 10.0.0.154) using the mean and the standard error of the mean for the error bars.

### H. Microscopy

Brightfield images captured with an upright light microscope (Zeiss Axioplan, 10x objective) relate the estimated cell positions to ground truth.

## III. Results

The goal of the presented work is to make a correlation between the effects of a chemotherapeutic drug on cell proliferation and the cell adhesion noise of cancer cells adhered to the CMOS-based MEA. The electrical detection of adherent cells follows the method described previously [7]. A high correspondence (90 %) is shown between adhesion noise-based identification and microscopically identified cells (Fig. 1A). Undetected small structures on the sensor surface indicate poor cell attachment or dead cells. According to prior studies, the cleft resistance between the

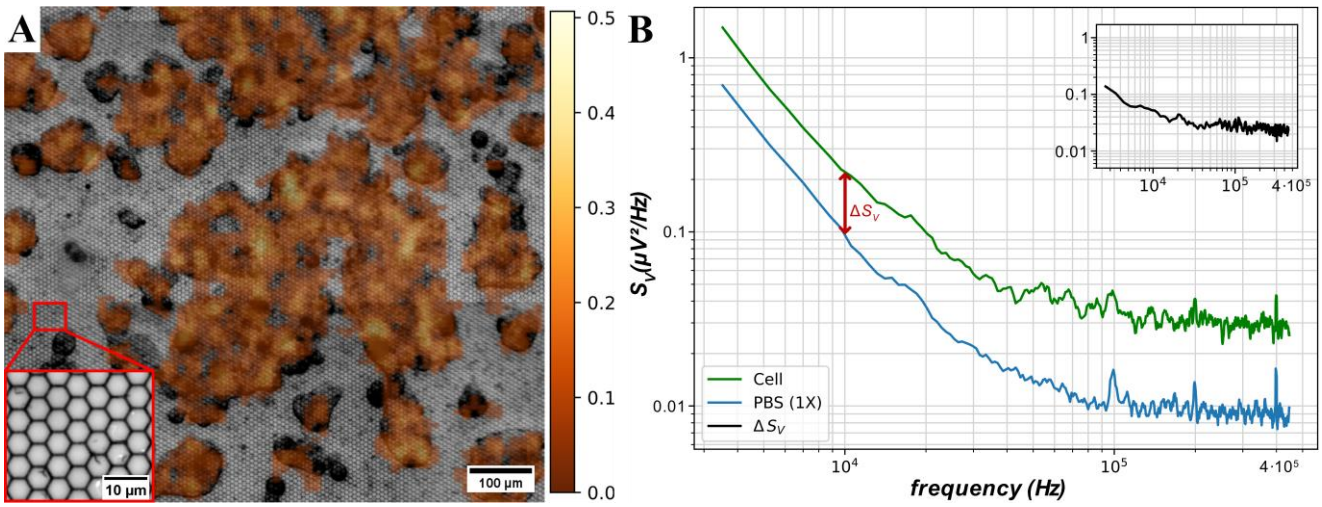


Fig. 1: Identification of adherent cells on CMOS MEA by adhesion voltage noise in terms of spectral power density ($S_V$ evaluated at 10 kHz bandwidth). (A) Overlay of electrical imaging of HT-29 cells with brightfield microscopy imaging. Inset: 50x magnification of sensor sites on the CMOS MEA. (B) Determination of the cell identification threshold via cell adhesion noise spectroscopy at 10 kHz. To identify an adherent cell, a sensor must exceed the sum of $\Delta S_V$ and the values of $S_V$ of bare sensors. Inset: A flat cell adhesion noise (i.e., difference $\Delta S_V$ between total $S_V$ (green trace), and $S_V$ of the bare sensors (blue trace)) spectrum accounts for a resistive cleft determining the bandwidth for the identification threshold.

cell membrane and a planar recording site contributes significantly to the voltage noise [4-6]. Hence, the resistive cleft is estimated from the spectral power density $S_V$ of the voltage noise. Neurons as well as nonelectrogenic cells, such as the cancer cell line HT-29, likely add to the adhesion noise with their resistive cleft. Fig. 1B demonstrates that an adherent cell shows an elevated $S_V$ of the total spectrum across the entire frequency range. The cell adhesion noise is extracted from the difference $\Delta S_V$ between total $S_V$ and $S_V$ of the bare sensors (i.e., sensors exposed to the electrolyte) and evaluated at a bandwidth of 10 kHz [7]. Sensor sites that detect adherent cells are defined as "positive sensors" based on their value $S_V$, which must exceed the sum of $\Delta S_V$ and the values of $S_V$ of bare sensors. With this threshold, sensors with cells adhered are identified (Fig. 1A).

Based on recent studies, 5-FU has a strong cytotoxic effect on HT-29 cells [12] leading to cell apoptosis [16]. It is assumed that apoptotic cells detach from the CMOS-MEA [17] resulting in a decrease in cell adhesion noise. $S_V$ below the cell identification threshold (i.e., the sum of $\Delta S_V$ and $S_V$ of bare sensors) indicates an apoptotic cell. To correlate between cell apoptosis and electrical recording, the mean coverage per chip is estimated by the number of positive sensors divided by the total number of sensor sites per chip (i.e., 256 x 384 sensors).

Cells are cultivated in a monolayer on the CMOS MEA and the cell adhesion noise identification threshold to detect adhered cells is used to calculate the mean coverage per chip to quantitatively evaluate cell proliferation with and without drug treatment. The mean coverage continuously increases to 8.35 % (Fig. 2A, black trace with white triangles) as the cells grow on the chip (optical proof via microscopy). The cell proliferation is in accordance with the CCK8 assay with cell viability rising to 147.25 % (Fig. 2A, blue trace with white squares).

After exposing the cells to 5-FU and a recovery time of 48 h, cells' adhesion noise levels decline which is linked to detachment of cells indicating apoptotic behavior. The mean coverage drops from 30.48 % to 18.09 % (Fig. 2A, black trace and triangles). The cell viability from the CCK8 assay drops to 44.86 % (Fig. 2A, blue trace and squares). Two features are extracted from the proliferation studies: (i) proliferative as well as apoptotic cell behavior can be evaluated in terms of viability with the mean coverage per chip via cell adhesion noise spectroscopy and (ii) drug treatment causes a loss of the cell adhesion noise within densely packed structures. Consequently, the approach is applied to 3D cell culture (i.e., spheroids). Further studies are ongoing to check for reproducibility and stability of the approach.

3D cell cultures represent more closely the cell–cell interactions and the microenvironment conditions to better meet the physiological relevance of cell-based investigations *in vitro*. After successful initial attachment to the CMOS MEA, the mean coverage per chip rises to 12.53 % within 72 h (Fig. 2B, black trace and triangles), whereas the viability with drug treatment drops after 72 h to 4.69 % (Fig. 2B, red trace with white triangles).

## IV. Discussion

We demonstrated how to use CMOS MEAs for cell adhesion noise spectroscopy-based electrical identification of cells to investigate the health status of colorectal cancer cells after anti-cancer therapeutics treatment in 2D as well as in 3D structures. Without the need of any external stimulation or perturbation of cells, e.g., staining with a dye or contacting with a pipette, our label-free noninvasive approach qualitatively matches standardized molecular biology techniques.

Future work aims to extend the adhesion noise-based identification of cells to electrically determine the health status of cells and to elucidate cell metabolism.

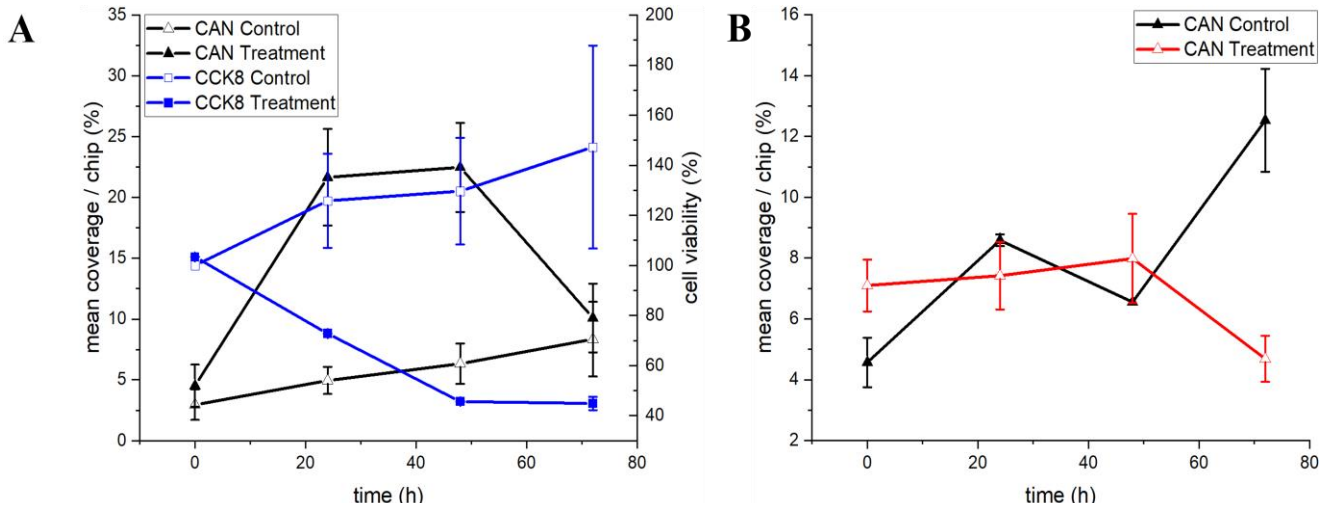


Fig. 2: Mean viability of 2D (monolayer) and 3D (spheroid) culture on CMOS MEA evaluated in terms of mean coverage per chip with cell adhesion noise (CAN) spectroscopy and cell viability via CCK8 assay. (A) Untreated cells on CMOS MEA proliferate and give rise to the coverage rate (black triangles) whereas 5-FU causes a drop in mean coverage after 48 h recovery time (white triangles, -8 % offset for better visibility). Accordingly, CCK8 assay shows an increasing viability for untreated cells (white squares) and a decreasing viability for treated cells (blue squares). (B) Spheroids cultivated on CMOS MEA proliferate without 5-FU application (black trace and triangles) and lose their adhesive properties after 5-FU treatment (red trace, white triangles). Error bars show the standard error of the mean.

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