Automated, multiplexed electrical impedance spectroscopy platform for continuous monitoring of microtissue spheroids

Supporting Information

Sebastian C. Bürgel, Laurin Diener, Olivier Frey, Jin-Young Kim, and Andreas Hierlemann

Department of Biosystems Science and Engineering, ETH Zurich, Basel, Switzerland

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Chip Fabrication

The device was fabricated in PDMS and bonded onto a glass slide that featured platinum electrodes as schematically shown in Supplementary Figure 1. Microfluidic structures were patterned in PDMS to retain the spheroids in the central channel and to guide them over the electrodes upon tilting of the device. The respective PDMS piece was casted from an SU-8 mold on a 4-inch silicon wafer and was bonded onto a glass slide, which has been patterned with the platinum microelectrodes. The SU-8 mold consisted of an 80 µm bottom layer, made of SU-8 50, and a 500 µm top layer, made of SU-8 100. The mold was silanized for 2 h with trichloro(1H,1H,2H,2H-perfluoro-octyl)silane (Sigma-Aldrich, USA) in a vacuum desiccator. 25 g of PDMS (Sylgard 184, Dow Corning Corp., USA), mixed with 10% cross-linking agent, were poured over the wafer yielding a device thickness of 5 mm. After 1 h of degassing in a vacuum chamber, the mold was baked for 2 h at 80 °C, pealed from the master and cut into individual chips. After punching 0.75-mm-diameter loading ports and 3-mm-diameter medium reservoirs, the PDMS pieces and glass slides were cleaned with acetone, isopropanol and water. The blow-dried pieces were treated in oxygen plasma before bonding. Irreversible bonding was ensured by a final baking step at 80 °C for 2 h. The microfluidic channel had a width of 700 µm, the retaining pillars on each side of the channel had a width and spacing of 100 µm (Figure 1D and E).

Platinum electrodes were patterned on the glass slides by a lift-off procedure. A four-inch glass wafer of 500 µm thickness was spin-coated with the positive-tone resist S1813 on top of an LOR3B resist (both from micro resist technology GmbH, Berlin, Germany) bottom layer. Efficient lift-off without side-wall metallization was ensured by a 1.5 µm undercut of the bottom LOR3B layer. A 200 nm Pt layer was then deposited on top of a 20 nm W/Ti seed layer. The lift-off of the metal was carried out by using mr-rem-400 remover (micro resist technology GmbH, Berlin Germany). After metallization, the glass wafers were diced into individual glass slides of 32 mm x 80 mm. The electrodes extended across the entire channel and had a width and spacing of 500 µm (Figure 1D and E).

Some designs required isolation of the electrodes to prevent electrical shortcuts through the large media reservoirs on the chip. Therefore, an optional 500 nm Si_3N_4 deposition was performed after the metallization and before dicing. The areas, in which the Si_3N_4 was intended to remain, were masked using a lithographically structured S1813 layer. The nitride layer was then opened by plasma etching, and the S1813 mask layer was removed by mr-rem-400 wet etching.

The device was designed for an alignment precision of +/- 200 µm between the PDMS piece and the electrodes on the glass chips, which could be achieved by manual alignment under a stereomicroscope. Irreversible bonding was achieved by oxygen plasma treatment of both, the PDMS piece and the glass substrate followed by a baking step for 2 h at 80 °C on a hotplate.

EIS Measurements

The impedance of the 15 separate spheroid chambers was evaluated by supplying AC voltages and analyzing the resulting currents. Six frequencies were equidistantly spaced on a logarithmic scale between 10 kHz and 1.86 MHz and simultaneously applied via the microelectrode set. The output voltages of 200 mV at each frequency were supplied by an

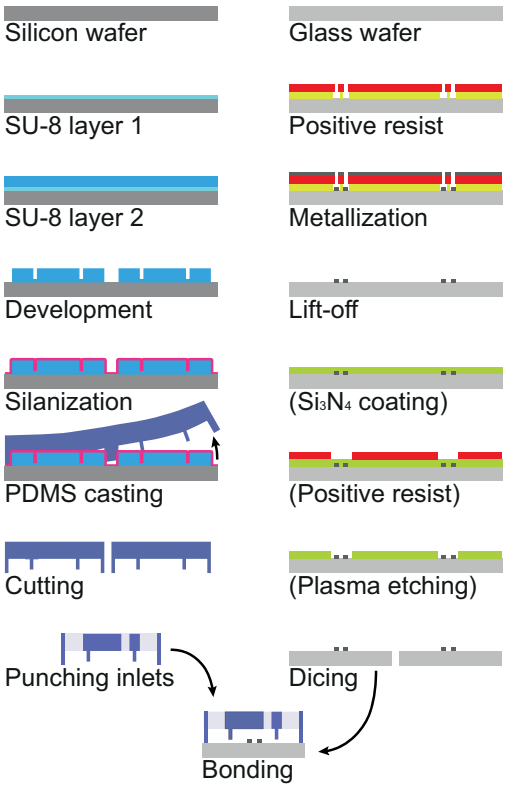
HF2 impedance spectroscope (Zurich Instruments AG, Zurich, Switzerland). The resulting currents were transformed into voltages using a trans-impedance amplifier (TA) with a feedback gain of 100 Ω and then fed back to the impedance spectroscope for recording. The impedance spectroscope featured maximally two signal outputs and inputs, but up to 15 separate chambers were to be measured. Thus, a multiplexing and switching architecture was required to supply the output of the impedance spectroscope as the input signal to each of the 15 chambers. Correspondingly, a de-multiplexing stage was used to receive the output signals from each of the 15 chambers and routing it to the input of the trans-impedance amplifier. This multiplexing/de-multiplexing and switching was facilitated via the previously described custom-made AMEIS switch board, which was directly interfaced to the microfluidic chip as shown in Figure 1B. In this way, the input and output channels of the impedance spectroscope could be connected to any of the 15 chambers in the microfluidic chip. The output current of the AMEIS switch board was fed back via the trans-impedance amplifier to the impedance spectroscope and recorded on a PC for later off-line analysis. Both, the impedance spectroscope and the trans-impedance amplifier were acquired from Zurich Instruments AG, Zurich, Switzerland.

Spheroid Preparation

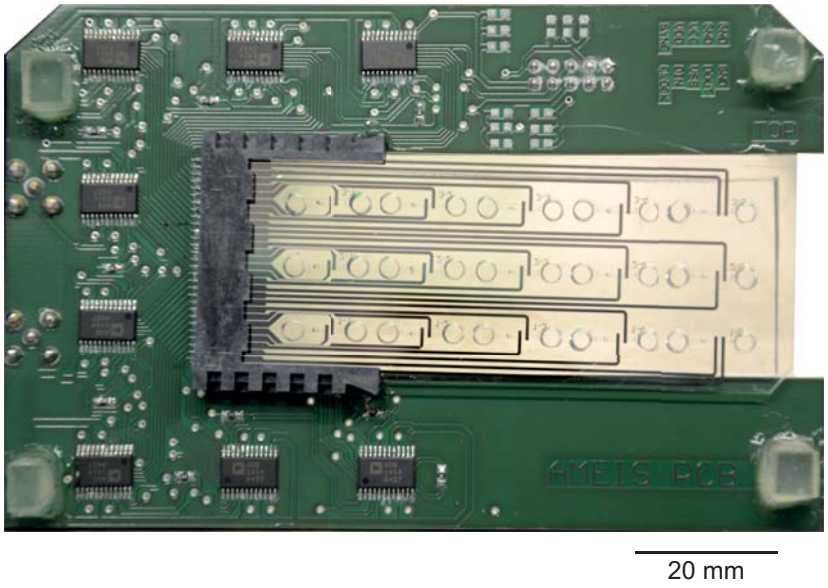
HCT116 cells were grown in RPMI 1640 medium (Chemie Brunschwig AG, Basel, Switzerland), supplemented with 10% fetal bovine serum (Sigma-Aldrich) and 100 IU/ml penicillin, 100 µg/ml streptomycin (P/S, Chemie Brunschwig AG, Basel, Switzerland) and 0.3 µg/ml puromycin. The cells were grown to 80% confluence in cell culture flasks, harvested by trypsinization and seeded into U-bottom 96-well plates. After 4 days, spheroids had formed and were ready to be transferred into the chip. For control experiments Langerhans microislets were used and kept in pancreatic microislet maintenance medium. Human cardiac microtissues were maintained in cardiac maintenance medium. The micro-islets and cardiac spheroids, as well as the respective media were provided by InSphero AG, Schlieren, Switzerland. Islets and cardiac spheroids were handled and loaded in the same way as the other cancer spheroids. All spheroids were maintained in a humidified incubator at 37 °C and 5% CO_2. Some cancer spheroids were exposed to different concentrations of the anti-cancer drug Fluorouracil (5-FU) in the microfluidic chip. 5-FU was dissolved in dimethyl sulfoxide (DMSO) before diluting it to the target concentrations (0.4 µM, 4 µM and 40 µM) in culturing medium. Two different control groups were used: One group was grown in standard RPMI medium without 5-FU, the second control group contained RPMI, supplemented with DMSO at the concentration that has also been used for delivering the highest concentration of 5-FU.

Finite-Element Modelling

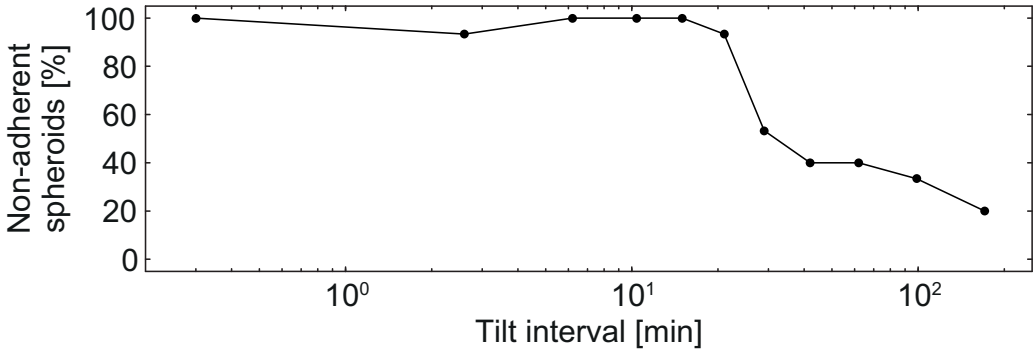
A finite-element model (FEM) was used to verify the independence of the ΔI_{norm} values from the medium conductivity (see Results section). The current through one electrode in the measurement chamber was calculated in the FEM by integrating the current density over the electrode surface using Comsol Multiphysics 4.3 (COMSOL AB, Sweden). Since a simplified cross-sectional 2D model was used, as shown in Supplementary Figure 6A, current values were given in units of [A/m]. In order to obtain the ΔI data, the position of the particle along the channel had to be obtained first. Therefore, the position of a 300-µm-diameter, non-conductive particle was swept axially through the channel, and the current density was determined for every increment to obtain the plot in Supplementary Figure 6B.



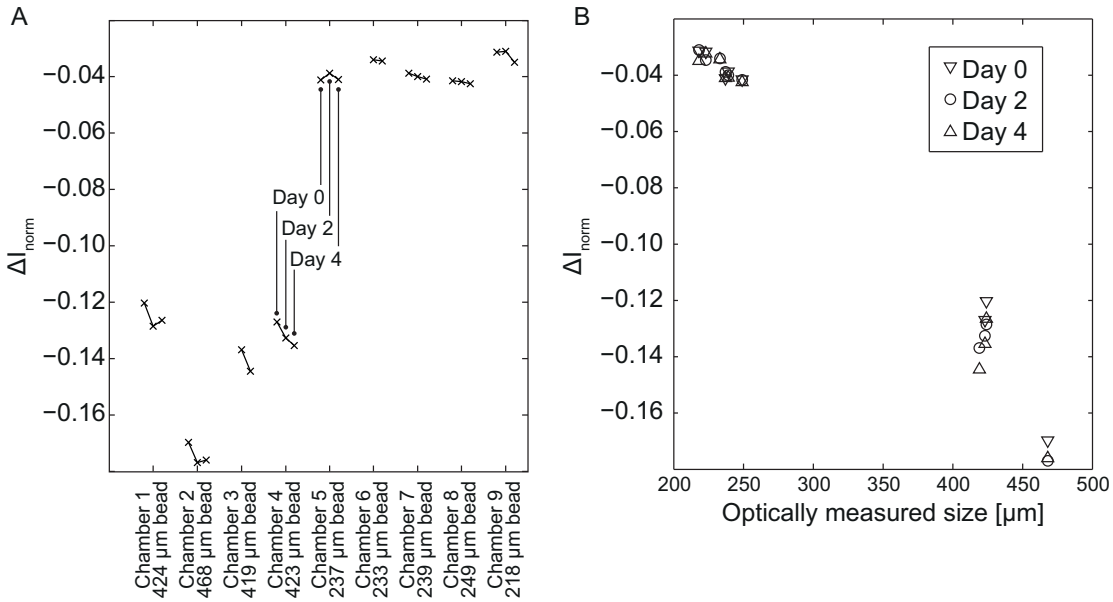
Supplementary Figure 1. *Fabrication of the microfluidic chip. The left column shows the steps required for the SU-8 mold, which was then used to cast the PDMS device. The right column shows the electrode fabrication process on the glass substrate with the optional Si_3N_4 deposition and structuring step.*



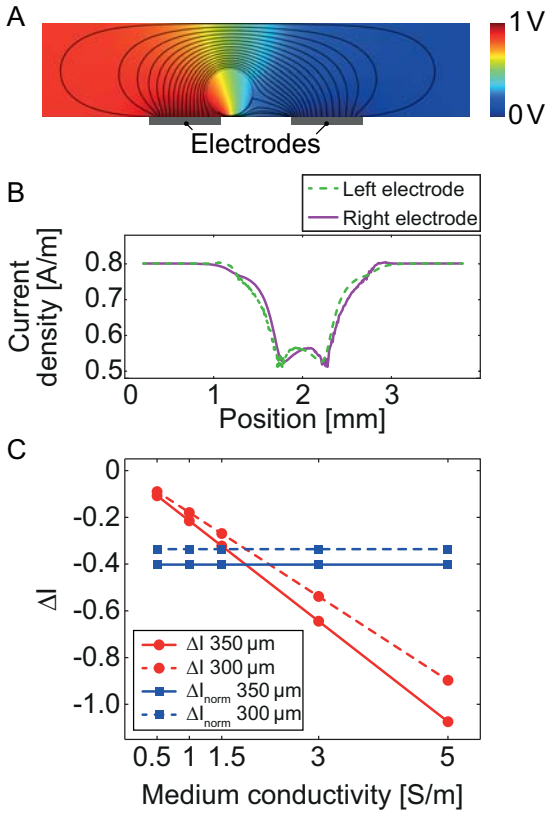
***Supplementary Figure 2**. The back side of the AMEIS switch board with a PDMS/glass chip inserted is showing the arrangements of switching ICs and the edge card connector for electrical interfacing with the microfluidic chip.*



***Supplementary Figure 3**. Adhesion of microtissue spheroids with increasing tilting interval. The percentage of spheroids that was still moving and not adhering to the channel walls and bottom was decreasing, as the tilting interval exceeded 20 minutes.*



***Supplementary Figure 4**. Control experiment using 200-µm and 500-µm diameter beads. A: The ΔI_{norm} signal was evaluated at day 0, 2 and 4 for each size and shows that the signal was stable over multiple days with relative impedance variations below 10%. B: Different visualization of the same dataset shows that ΔI_{norm} scaled with the size of the beads.*



Supplementary Figure 5. *Finite-element model of EIS measurements in the microchannel with electrodes. A: The electrical potential (rainbow surface) and electric field lines are shown for a non-conductive particle of 300 µm diameter. B: The shown particle position leads to a minimum in the plot of current density vs. position. Here, the center of a 300-µm-diameter non-conductive particle (cf. A) was swept axially through the channel. The resulting current densities for each position were integrated over the electrode surfaces and plotted versus the center coordinate of the particle. C: The peak-to-baseline (ΔI, in A/m) and baseline-normalized peak-to-baseline (ΔI_{norm}, no unit) have been simulated for different values of medium conductivity and two different spheroid sizes. The normalized magnitudes do not depend on the medium conductivity but primarily on the spheroid size.*