SUPPORTING INFORMATION

Interdependence of initial cell density, drug concentration and exposure time revealed by real-time impedance spectroscopic cytotoxicity assay

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Content:

S1 – Concentration dependent doxorubicin cytotoxicity on HeLa cells – MTS assay

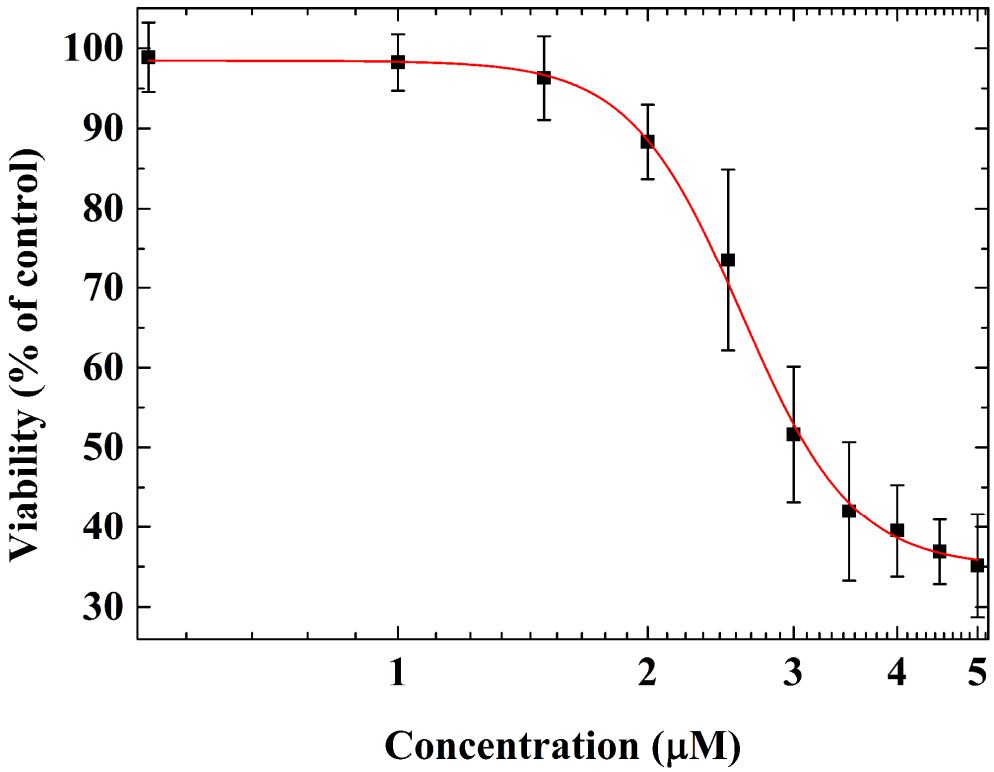

Fig S1. MTS cytotoxicity assay on HeLa cells: 75000 cells/cm^2 were seeded in a 96-well plate and exposed to different Doxorubicin (DOX) concentrations in the range of 0.5 – 5 μM 10 h after cell seeding. The cells were incubated in the presence of DOX for 24 hours before performing the MTS assay. Six cell populations were studied to obtain the average response to each DOX concentration. The error bars represent standard deviation.

S2 – Microscope images of cell detachment caused by cytotoxicity

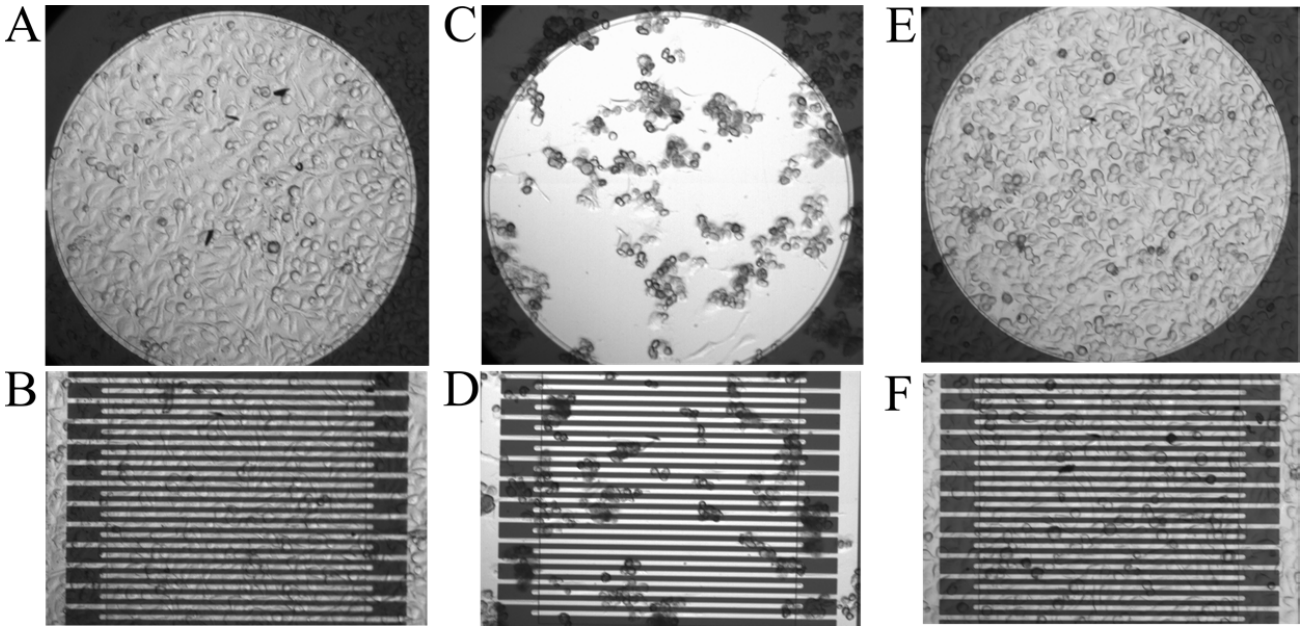

Fig S2. Cytotoxicity induced detachment of HeLa cells: (A,B) Cells on electrodes after 10-h culturing; (C,D) the same cell population as in A/B after 15-h exposure to 5 μM DOX; (E,F) control cell population without drug addition. Impedance measurements were performed every hour.

S3 – Time dependent doxorubicin (5 µM) cytotoxicity on HeLa cells – EIS and MTS assay

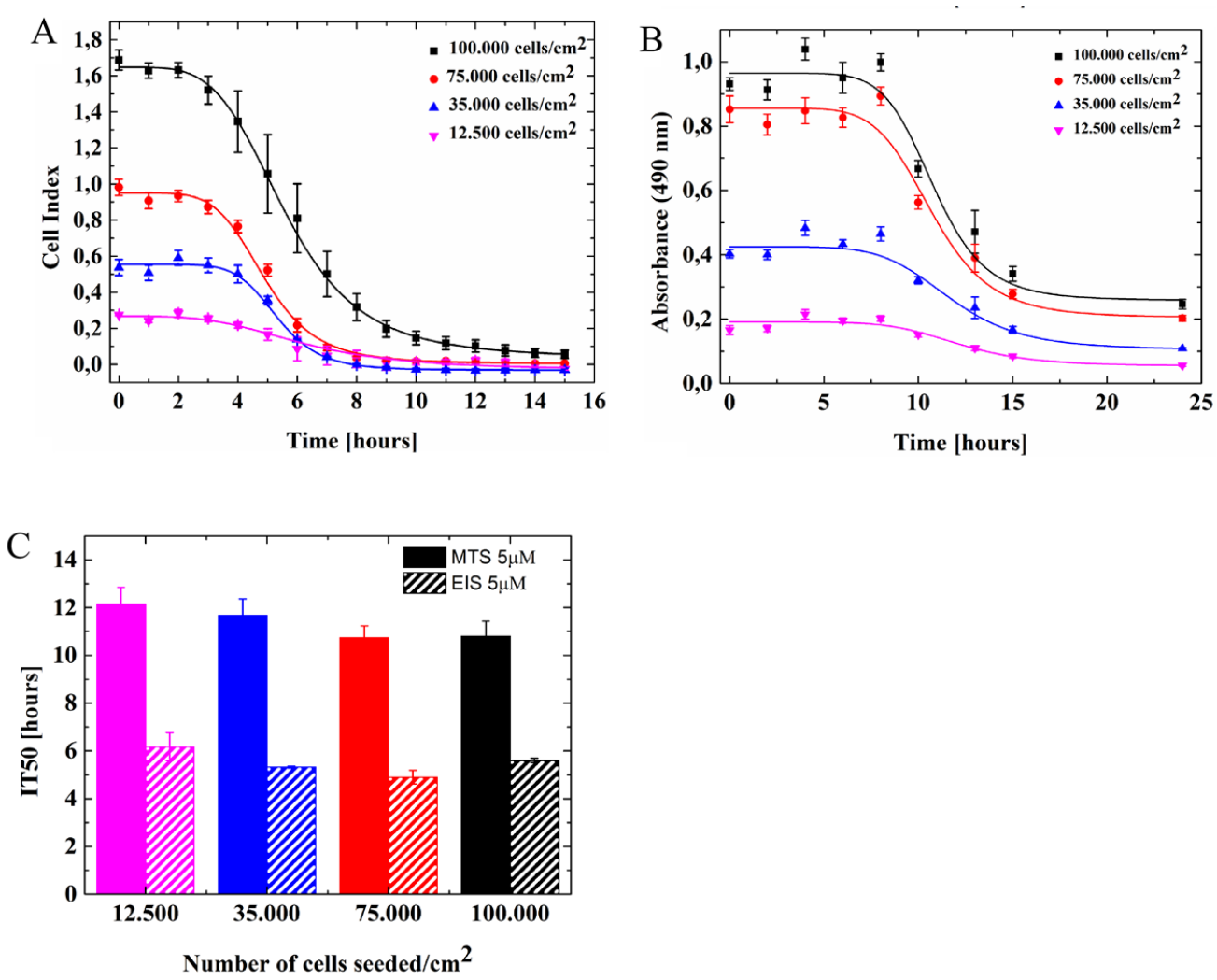

Fig S3. Cytotoxicity determined with the EIS and MTS assay: Comparing the two methods, we can observe that the EIS-based assay gives information on cytotoxicity at an earlier stage than the MTS assay. Time-dependent DOX-induced toxicity studied on four different HeLa cell populations (12500, 35000, 75000, 100000 cells/cm^2) cultured for 10 hours and then exposed to 5 µM DOX. The curves show the cell viability changes after DOX addition. (A) Cell Index vs. time for real-time EIS monitoring of cytotoxicity over 15 h (EIS measured using the coplanar configuration every hour). (B) Absorbance at 490 nm vs. time for MTS assays over 24 h (end-point assays performed 2, 4, 6, 8, 10, 13, 15 and 24 h after DOX addition). (C) Comparison between EIS and MTS assay: IT50 values for the four different cell populations exposed to 5 µM DOX. Error bars represent s.e.m., n ≥ 20.

S4 – Time dependent doxorubicin (2.5 µM) cytotoxicity on four different HeLa cell populations – MTS assay

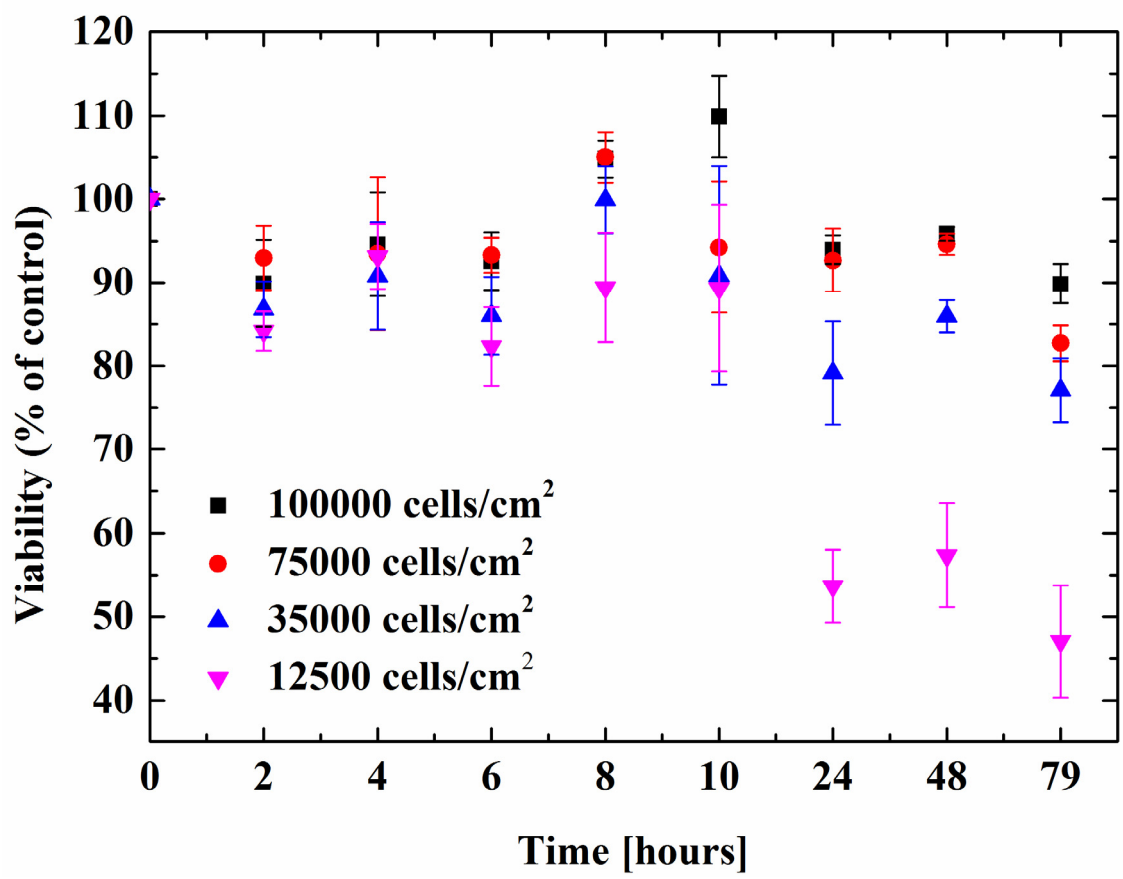

Fig S4. MTS cytotoxicity assay on HeLa cells: Time dependent cytotoxic effect of doxorubicin on four different HeLa cell populations (12500, 35000, 75000, 100000 cells/cm^2) cultured for 10 h and then exposed to 2.5 µM DOX. The cytotoxicity was evaluated by MTS assay at different time points (2, 4, 6, 8, 10, 24, 48, 79 h) after introduction of DOX.

S5 – Schematic view of the measurement setup

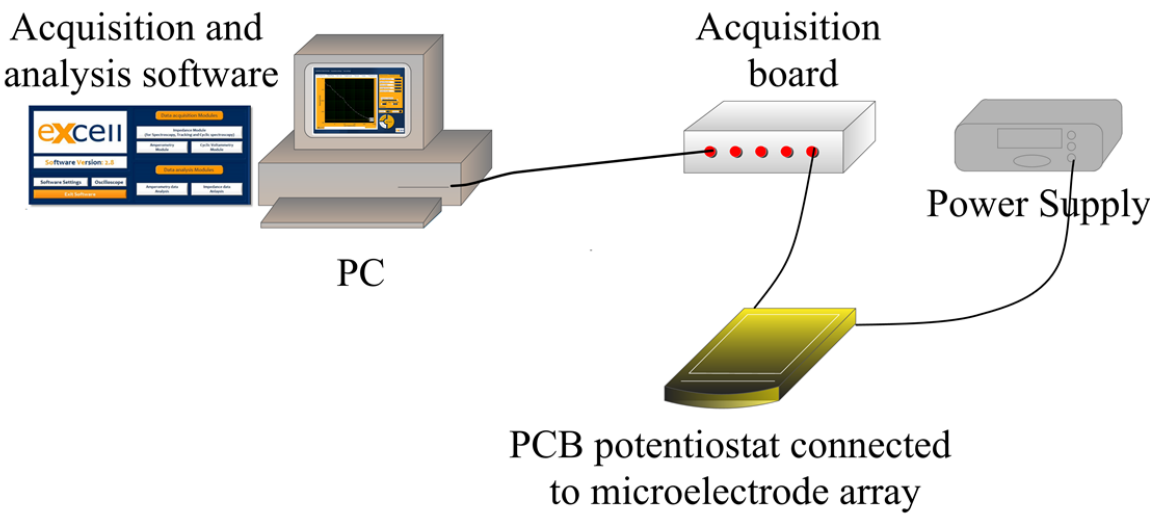

Fig S5. Schematic of the measurement setup: The cell culture and detection unit (containing the microelectrode array) integrated with the PCB potentiostat is connected to a computer and controlled by a tailor-made data acquisition and analysis software. Data acquisition is performed through a portable USB acquisition board. Power supply is externally provided.

S6 – Normalized impedance spectra recorded for cultured cells (seeded at the density of 75000 cells/cm^2)

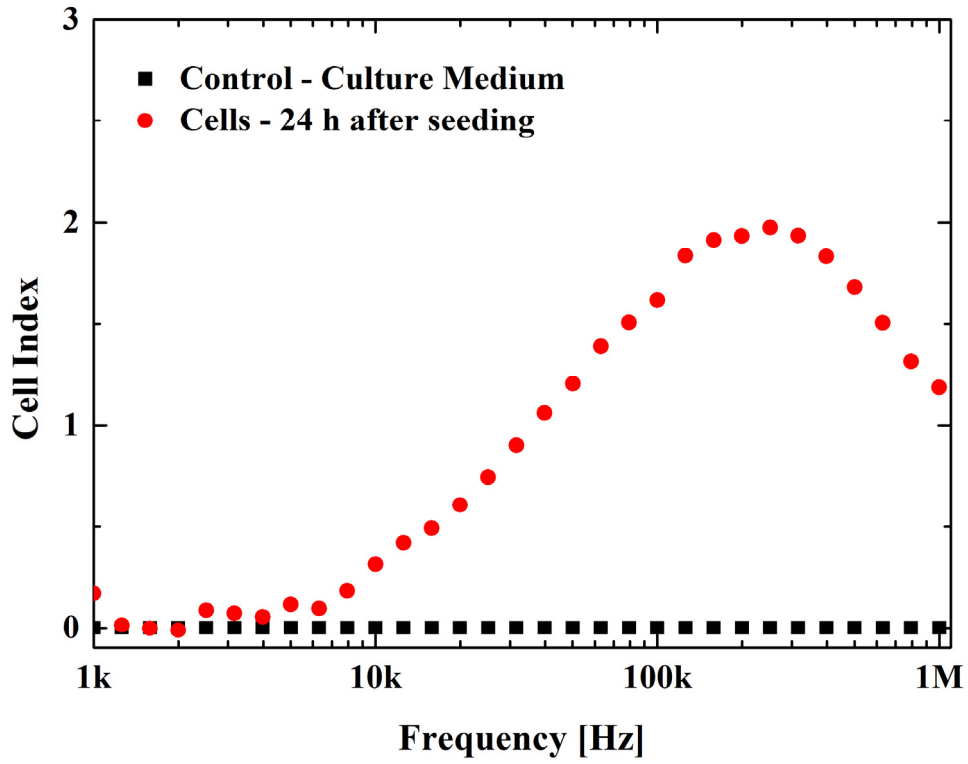

Fig. S6. Normalized impedance spectrum recorded for cultured cells (seeded at the density of 75000 cells/cm^2): A normalized impedance spectrum acquired in the frequency range between 1 kHz and 1 MHz (10 data points per decade) using Solartron 1260A impedance analyzer. The cells were cultured for 24 h. The highest value of normalized impedance is obtained at 250 kHz, where the measured impedance is still influenced by extracellular resistance and membrane capacitance. Hence, for fast multiplexed monitoring of cellular responses, impedance spectra recorded in a frequency range up to 100 kHz provide a general overview of cytotoxic responses.