Supplementary information

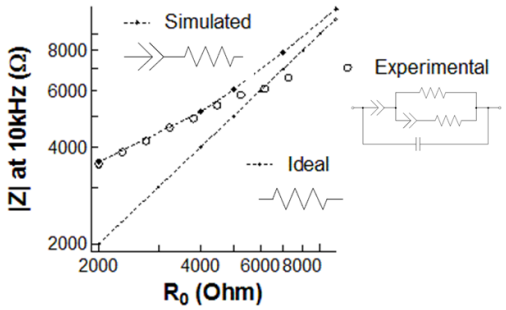

Fig.S1 Impedance magnitude-resistance relationship at LF. Impedance-magnitude measured at 10 kHz is plotted *vs.* LF resistance, R_0. Experimental values (mean ± SD, n=4) are compared with a simulated CPE_{el}-R_0- and a simple R_0-circuit.

In order to study R_{extra} exclusively, we tried to understand the contribution of interface capacitance and tissue capacitance at a low frequency such as 10 kHz. LF-resistance (R_0) dependence on the measured |Z| is demonstrated in Fig. S1. In the case of a simple resistor, impedance only consists of resistor component. Adding a serial CPE_{el}, the relative contribution of CPE_{el} to the absolute |Z| is greater at lower resistances than at higher ones. Therefore, we investigated where our experimental data fits among these models. We found that measured |Z| followed the simulated CPE_{el}-R_0 model up to ~4000 Ω. The experimental signals then converged to the simple resistor profile (ideal case) at 5500 Ω. At higher resistances (> 6000 Ω), |Z| deviates from linearity of the simple resistor model, and lower |Z| was obtained compared to fully resistive behavior. In conclusion, we showed that measurements at 10 kHz are affected by the interfacial capacitance in a resistance-dependent way. This was true especially up to 6000 Ω. To reliably analyze cell dependent extracellular changes and their kinetics, all impedance analysis involves correction of |Z| values by subtracting the electrode interface effect from measured |Z| at 10 kHz. Such correction provides the approximation of experimental values to the ideal linear profile with a slope of unity up to 5500 Ω. Consequently, all toxicity experiments were conducted before 5500 Ω was reached.

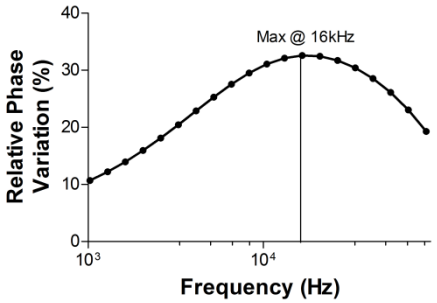

Fig. S2 Relative phase angle variation in the low frequency window (~1 kHz-~80 kHz). $\varphi_{rel}=|((\varphi_{cells}-\varphi_0)/\varphi_0)*100|$. The values of the phase angle with cells (φ_{cells}) and without cells (φ_0) have been extracted from Fig. 3b. 16 kHz is the frequency where the phase angle varies the most with increasing cell density and thus increasing extracellular resistance. We have chosen 10 kHz as our measurement frequency because it is less affected by the membrane capacitance while ensuring a nearly maximal measurement sensitivity.

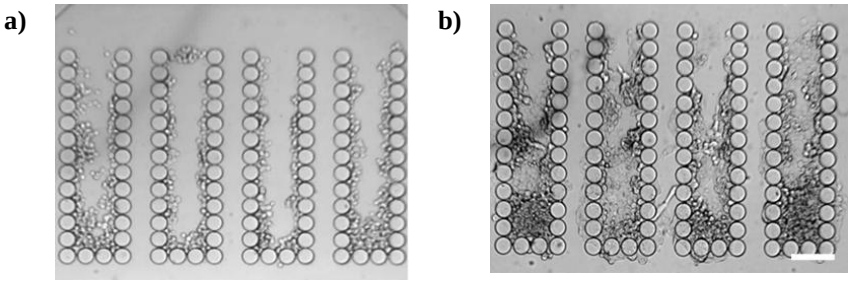

Fig. S3 Cell growth in microfluidic device. a) t= 1 day after cell seeding; b) t=5 days; the presence of more cells shows that cells have grown inside filters; bar=100 µm

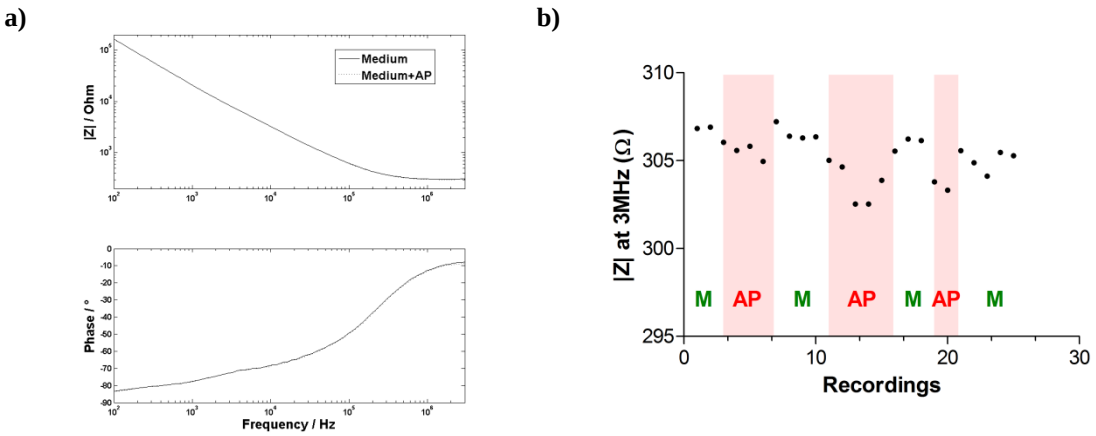

Fig. S4 AP-induced impedance-change in the absence of cells inside the microchip (control experiment). a) Impedance spectrum of cell medium with and without 10 mM AP. Only in the HF region where the phase angle is closest to 0°, a slight change of ~5 Ω and ~2° is observed. b) Impedance magnitude change at 3 MHz after repeated addition of AP and washing with fresh medium. A maximum impedance magnitude change of ~5 Ω was observed when AP was added. This value is negligible compared to the impedance change caused by the cells (>1000 Ω). M-medium; AP-acetaminophen

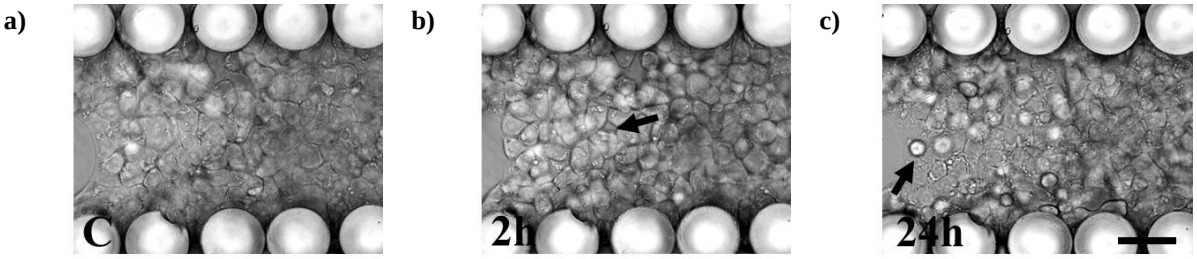

Fig. S5 Time-lapse images of cell culture in microfluidic device before and after AP treatment.a) Confluent cell culture in microfluidic chip before drug treatment. b)2 h treatment with 20 mM AP causes morphological changes such as cell retraction and formation of wider intercellular gapsas indicated by arrow anddarker cell borders. c) After 24 h drug exposure, cell death is observed as indicated by arrow; bar=40 µm.