

An *in-vitro* method to study anti-apoptotic signaling from the extracellular environment

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INTRODUCTION: The emergence of acquired multidrug resistance (MDR) remains a major hurdle in the successful treatment of cancer. Signaling from the extracellular environment has shown to induce *de novo* drug resistance, a pre-state to MDR.¹ Hence, identifying such signaling pathways may pave the way for novel targeted therapies that can prevent the occurrence of MDR. This creates a demand for a predictive *in vitro* method for the study of the dependence of anti-apoptosis signaling on the environment.

So far, *in vitro* studies of the effect of environmental parameters on drug response have typically focused on one parameter at a time, such as the interaction with matrix proteins² or the effect of enhanced cell-cell contacts in 3D organized cells³. Therefore we are working on an *in vitro* platform, with which it is possible to simultaneously study the effect of several extrinsic parameters. This platform consists of a microwell array molded into a polyethylene glycol (PEG) hydrogel.⁴ The material properties of this hydrogel make it possible to mimic tissue-like stiffness. This allows us to explore many parameters of the environment, such as dimensionality, composition of the interfacing protein matrix and rigidity.

METHODS: Hydrogel microwell arrays were prepared as described previously.⁴ Arrays were used for cell experiments one day after preparation. After UV-sterilization, MCF-7 cells were seeded into the wells at a density of 750 000 cells / ml. After 1 hr of adhesion the samples were rinsed to remove cells from the microwell plateau. The cells were pre-cultured in the microwells for 24 hrs. Thereafter 10 nM taxol or vector only were added to the samples for another 24 hrs. To visualize the apoptotic ratio the samples were fixed in paraformaldehyde and stained with Hoechst 33342. Samples were imaged with a Leica confocal microscope equipped with a water objective, 20x magnification and 0.7 NA. For each microwell 3 images separated by 15 μ m in z-direction were obtained and analyzed for the percentage of fragmented nuclei. The experiment was performed in duplicates and repeated three times.

RESULTS: MCF-7 breast cancer cells formed dense clusters similar in size when cultured within 100 μ m wide microwells coated with collagen I. It was found that cells organized in clusters were more resistant to

treatment with taxol compared to cells cultured on flat substrates. The apoptosis detection on single cell level allows us to measure non-symmetric distributions of cell phenotypes. In this experiment there were no significant differences in cell death ratios over the clusters.

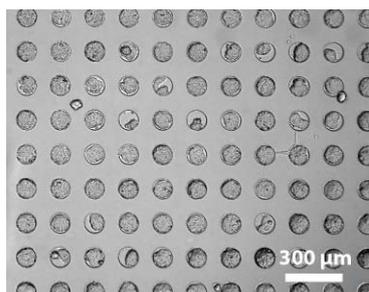


Fig. 1: MCF-7 cells form cluster in 100 μ m wide microwells. This image is from 48 hrs post seeding.

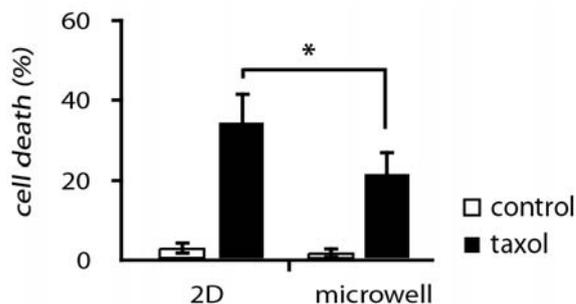


Fig. 2: The 3D organization of the cells in the microwells provides an anti-apoptotic effect.

DISCUSSION & CONCLUSIONS: This work aims at the development of a tool to study the dependence of drug response on environmental parameters. The preliminary results demonstrate that the 3D organization of cancer cells induce an anti-apoptotic signaling. Understanding and targeting such signaling might help to overcome novel MDR.

REFERENCES: ¹ Shain *et al.*, *Mol Cancer Ther*, **1**, 69, 2001. ² Aoudjit *et al.*, *Oncogene*, **20**, 4995, 2001. ³ St. Croix *et al.*, *Nat Med*, **2**, 1204, 1996. ⁴ Lutolf *et al.*, *Integr Bio*, **1**, 59, 2009.