Application Note

Generation of spheroids on Droplet Microarray in *3D-Life* Hydrogel

The Droplet Microarray from Aquarray

The Droplet Microarray (DMA) is a revolutionary miniaturized platform for high throughput screenings of all types of live cells in nanoliter droplets with a 10^3-10^4 less reagent and 10-10^2 less cell consumption (Figure 1; https://www.aquarray.com).

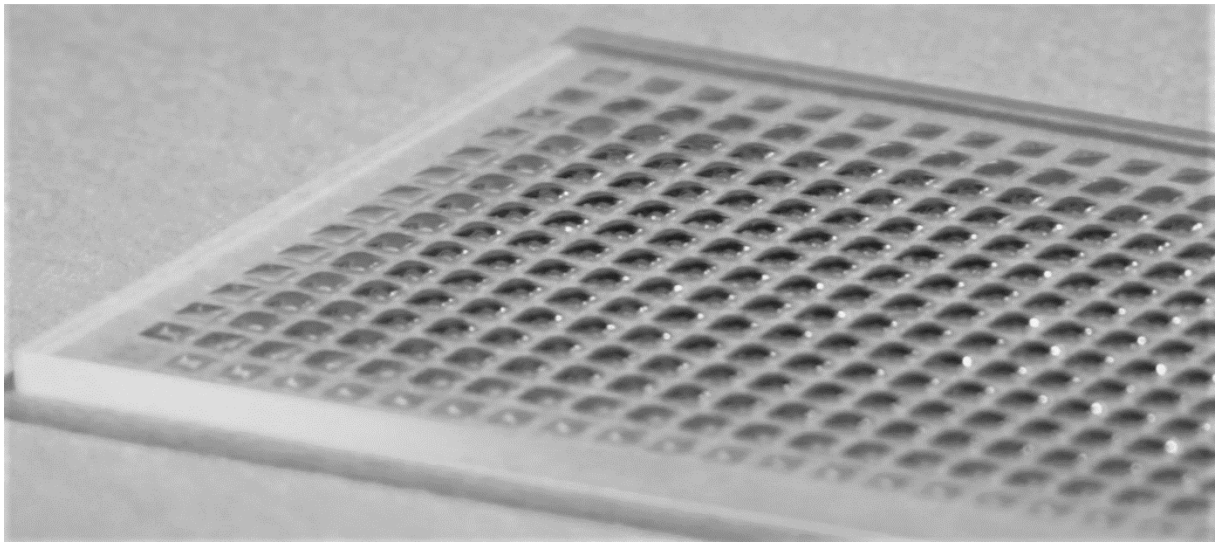

Fig 1: Miniaturized cell culture on DMA in 672 droplets with 150nL medium

Generation of nanodroplet cultures using the *3-D Life* Hydrogel system

Addition of solutions on the DMA is done most precisely using liquid dispensers, however, higher viscosities of solutions can be challenging for dispensing. Since *3-D Life* Hydrogels are quite fluid before crosslinking has advanced, both slow and fast polymerizing hydrogels, were successfully used to generate spheroids in 1mm spots on a 672 spot DMA. The human cervix carcinoma cell line Hela and the human melanoma cell line SK-MEL-28 were dispensed using the I-DOT Mini (A.) with a dosing energy of 228 mbar/ms. 48h after seeding in *3D-Life* hydrogels spheroids with high viability are formed (Figure 2). After one week of culture in *3D-Life* hydrogels Hela cells build round spheroids as well as elongated structures showing the ability to spread in the biomimetic hydrogel (Figure 3 A). SK-MEL-28 form numerous round spheroids in the hydrogel droplets with a typical round phenotype (Figure 3 B).

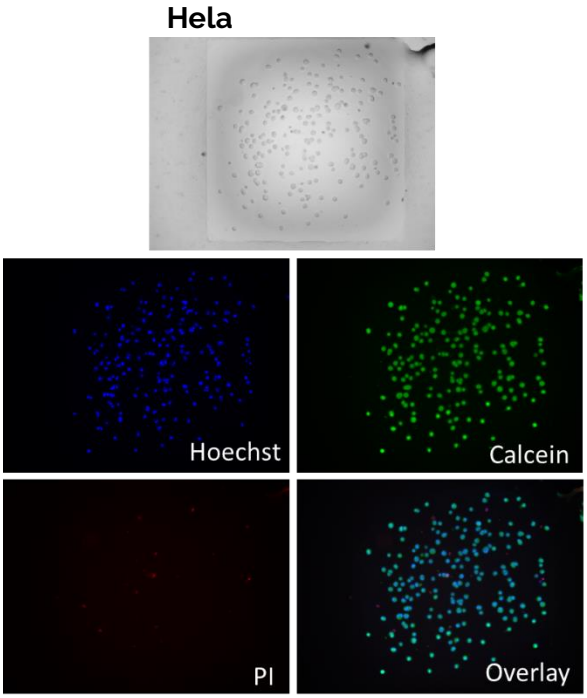

Figure 2: Hela spheroids stained with the fluorescent dyes Hoechst, Calcein and Propidium Iodide 48 hours after dispensing the hydrogel-cell mix on DMA (protocol available at www.aquarray.com)

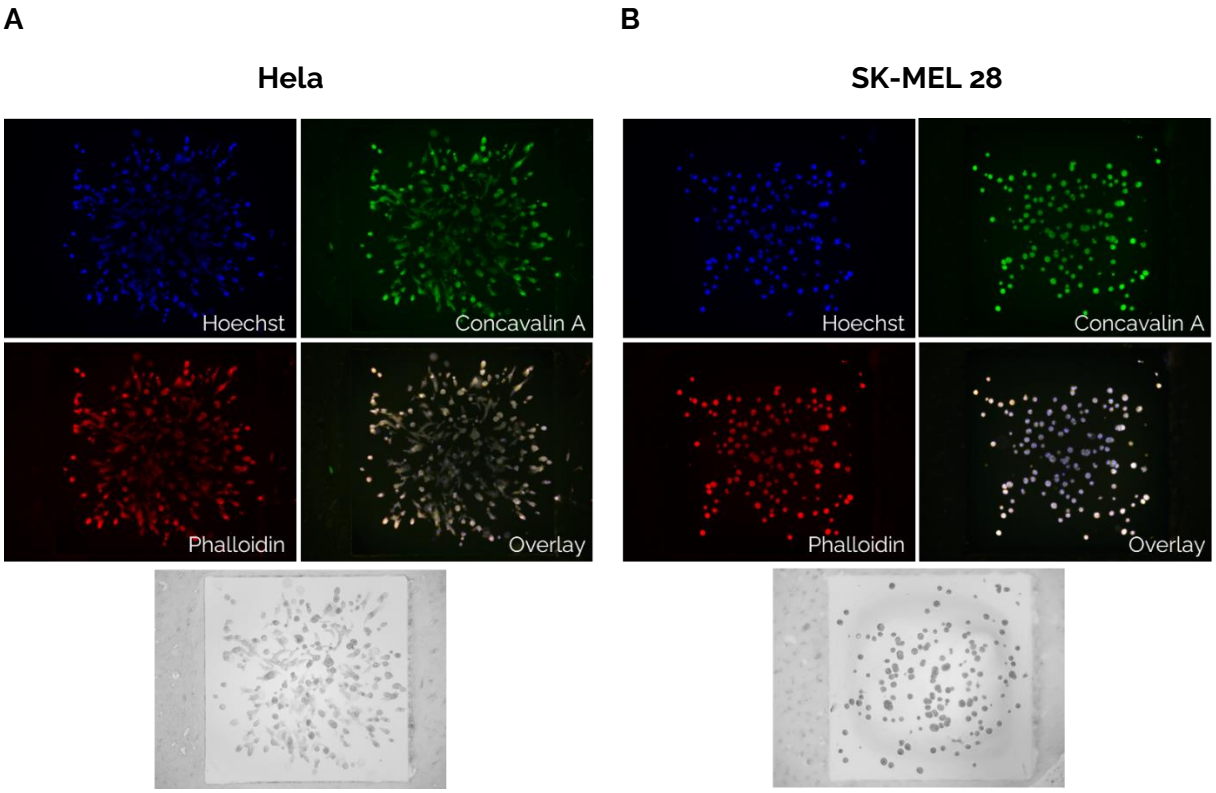

Figure 2: Hela (A) and SK-MEL-28 (B) spheroids stained with the fluorescent dyes Hoechst, Concavalin A and Phalloidin one week after dispensing the hydrogel-cell mix on DMA (protocol available at www.aquarray.com)

More information under www.aquarray.com and www.cellendes.com

Methods

200 cells (Hela and SK-MEL 28) were dispensed using the liquid Dispenser I-DOT Mini AQ-edition in 100 nL droplets containing either *3-D Life* SG-Dextran polymer or *3-D Life* FG-Dextran respectively crosslinked with *3-D Life* CD-Link at a crosslinking strength of 2,2 mmol/l. Before crosslinking, SG-Dextran or FG-Dextran was covalently modified with 0,5 mmol/L *3-D Life* RGD Peptide. After 7 days of culture the spheroids were stained with Hoechst (nucleus), Concavalin A (Endoplasmic Reticulum) and Phalloidin (actin).

Products used

Aquarray: Droplet Microarray 672 spots (Cat # G-np-102), I-DOT Mini AQ edition (https://www.aquarray.com/i-dot-mini-aq-edition)

Cellendes: *3-D Life* Dextran-PEG Hydrogel FG (Cat # FG 90-1), *3-D Life* Dextran-CD Hydrogel SG (Cat # SG 91-1**,** *3-D Life* RGD Peptide (Cat # 09-P-001) https://www.cellendes.com/index.php/product-line/products

Reagents and materials not included in the Aquarray or *3-D Life* products: DMEM, 10% fetal calf serum, 100 µg/ml streptomycin, Sterile PBS w/o Ca/Mg (PBS$^-$), HeLa and SK-MEL-28: cell suspension in DMEM + 10% FCS$^-$ at a cell density of 1,33x 10^6 cells/mL

Contact: Dr. Alexandra Matzke-Ogi

alexandra.matzke-ogi@aquarray.com

Contact: Dr. Brigitte Angres

angres@cellendes.com