

Application Note 5: Microdroplet Cell Cultures in 3-D Life Hydrogels on the Aquarray Droplet Microarray

The Droplet Microarray

The Droplet Microarray (DMA, Fig. 1) of Aquarray is a new miniaturized platform for cell-based high throughput screenings (HTS) of all types of live cells in nanoliter droplets requiring 10^3 - 10^4 -fold lower amounts of reagent and 10 - 10^2 -fold lower amounts of cells compared to conventional screening platforms. For more information on this technology visit the website of Aquarray GmbH at <https://www.aquarray.com>.

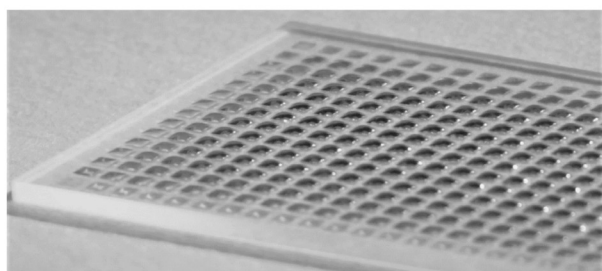
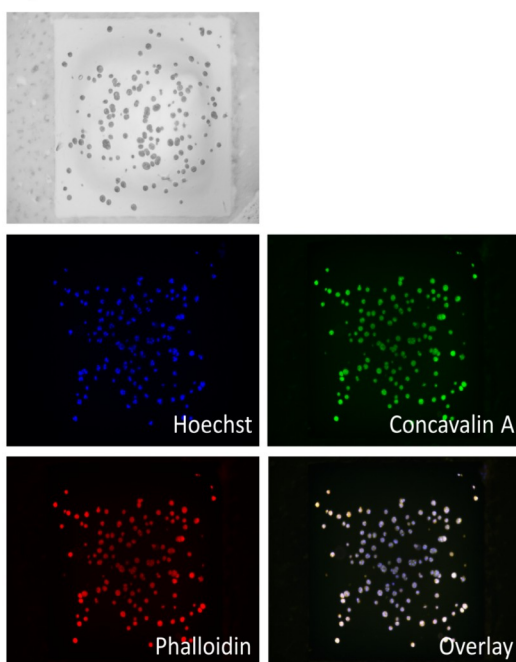


Figure 1: Miniaturized cell culture in microdroplets of 150 nL medium on DMA (672 hydrophilic spots).

Generation of nanodroplet cultures using 3-D Life Hydrogels

The DMA technology is well suited to generate arrays of 3-D cell cultures in a miniaturized way. At Aquarray both, slow and fast polymerizing 3-D Life Hydrogels, were successfully used to generate spheroids in an array of 672 spots, each with a diameter of 1 mm. The human melanoma cell line SK-MEL-28 and the human cervix carcinoma cell line HeLa had been dispensed using the I-DOT Mini. After one week of culture in 3D-Life fast gelling Dextran-CD Hydrogel modified with 3-D Life RGD Peptide SK-MEL-28 form numerous round spheroids in the hydrogel droplets with a typical round phenotype (Figure 2 A). Similarly, beyond forming spheroids, HeLa cells spread throughout the gel demonstrating the ability to migrate within the biomimetic hydrogel (Fig. 2 B).

A) SK-MEL-28



B) HeLa

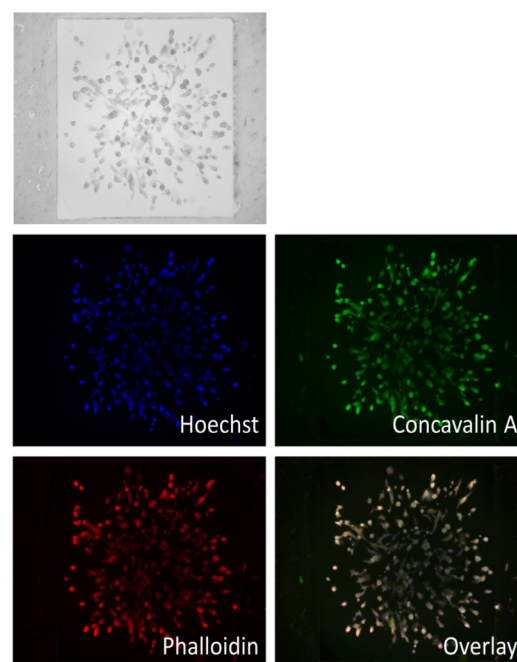


Figure 2: Cultures of SK-MEL-28 (A) and HeLa cells (B) on the Droplet Microarray with 672 spots. Examples of cultures shown in phase contrast (upper panel) and cultures stained with the fluorescent dyes Hoechst (nucleus), Concavalin A (endoplasmic reticulum) and Phalloidin (actin) one week after dispensing the hydrogel-cell mix on the DMA (lower panels).

Methods

The experiments were carried out by Aquarray in their facilities. Detailed protocols can be found on Aquarray's website <https://www.aquarray.com/protocols>. In brief, 200 cells (Hela or SK-MEL-28) were dispensed in 100 nL droplets of 3-D Life Mal-Dextran polymer mixed with 3-D Life CD-Link at a crosslinking strength of 2.2 mmol/l reactive groups using the liquid Dispenser I-DOT Mini AQ-edition with a dosing energy of 228 mbar/ms. Before crosslinking, Mal-Dextran had been covalently modified with 0.5 mmol/L 3-D Life RGD Peptide. After solidification of the hydrogels the DMA was transferred into a cell culture dish and covered with 10 mL medium. The DMA with the cell-laden hydrogels was incubated at 37°C and 5% CO₂ and stained according to Aquarray's protocol "Cell painting protocol using PhenoVue Kit on Droplet Microarray".

Products used

Cellendes:

3-D Life Dextran-CD Hydrogel FG, Cat. No. FG91-1

3-D Life RGD Peptide, Cat. No. 09-P-001

Aquarray:

Droplet Microarray 672 spots, Cat. No. G-np-102

Dispendix:

I-DOT Mini AQ edition (<https://dispendix.com/idot-non-contact-liquid-handler/i-dot-mini/>)