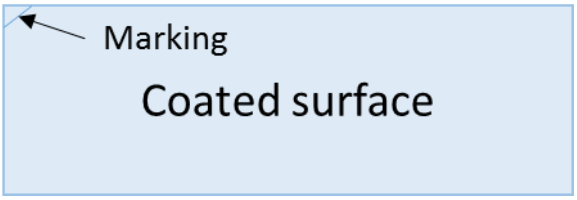
Protocol

Generation of spheroids on Droplet Microarray using *3-D Life* Dextran-CD Hydrogel

Materials and reagents:

DMA with 672 spots (Aquarray: catalog number G-np-102; www.aquarray.com)
Humidifying pad (Aquarray: catalognumber – AQP-0003)
Humidifying Buffer (Aquarray; catalog number – AQP-0003)
L.100 Wells (Dispendix: cataloge number – D16110021819; www.dispendix.com)
3-D Life Dextran-CD Hydrogel FG (Cellendes: catalog number FG91-1; www.cellendes.com)
3-D Life RGD Peptide (Cellendes: catalog number 09-P-001)
I.DOT Mini (Dispendix)
Petri dish

Manipulation with Droplet Microarray (DMA) slides



Droplet-Microarray (DMA) slides are transparent with a coated upper surface and a non-coated lower surface. **The coated surface should not be touched** with gloves or tweezers **to avoid the damage of the coating**. Before taking the slide out of the falcon tube, examine the slide and find the coated surface by a marking at the upper left corner (see the figure on the right). The Droplet Microarray slide can be held with gloves on the sides or with tweezers at the very corner. The Droplet Microarray can be placed in Petri dish, slide adapter or any other container on the non-coated surface.

1) Sterilization of DMA slides

1. Insert one transparent DMA slide into 50 mL falcon tube containing 45 mL of 70 % ethanol for up to one minute (do not incubate in ethanol longer than 10 minutes).
2. Remove the transparent DMA slide from ethanol under the clean bench and place it **on non-coated surface** onto the edge of an open sterile Petri dish to dry at least for 15 minutes until all ethanol evaporated.

2) Preparation of Petri dishes with humidified lids (humidifying chamber)

1. Open the lid of the petri dish and place it upside down under clean bench.
2. Place a sterile humidifying pad into the Petri dish lid using sterile tweezers.

3. Add 8-10 mL of sterile humidifying buffer to the lid and let it evenly spread over the whole surface of the pad. Important: the pad has to be fully wetted; extra liquid might cause detachment of the pad and has to be collected with a sterile pipet from the edge of the lid.
4. If some air is trapped between the pad and the lid squeeze it out with a tip of the pipette.
5. Leave the petri dish at 37°C in the cell incubator and add the DMA after dispensing if the cells in the lower part containing the 2 mL Humidifying Buffer.

3) Creating a Cell Suspension

Creating a cell suspension with $1.5x10^6$ cells in 1 mL to reach 150 cells/ 100 nL droplet:

1. Trypsinize cells following standard procedure, transfer them in 15 mL centrifuge tube and centrifuge for 3 minutes at 1200 rpm.
2. Aspirate the supernatant.
3. Resuspend the cell pellet in 1 mL of seeding medium.
4. Count the cells.
5. Dilute the cells till final concentration of $1.5x10^6$ cells/mL (to obtain 100 cells per droplet) with seeding medium.

For any other cell concentration please adjust accordingly.

4) Dispensing of cells in hydrogel with I.DOT Mini

1. Mix 99 µL pf dd H2O, 16 µL of CB (pH 5.5), 18 µL of FG-Dextran and 5 µL of RGD Peptide in a reaction tube and incubate for 20 min at RT.
2. Add 40 µL of cell suspension and 22 µL CD-Link.
3. Dispense 150 nL the hydrogel/cell suspension immediately on each of the 672 spots using the liquid class “hydrogel” (228 mbar/ms)”.
   Note: Here the I.DOT Mini was used, dispensing can be done with any other compatible nano-dispenser (a list of tested devices can be found on https://www.aquarray.com/product-information - list of compatible devices).
4. Put the DMA immediately into a humidifying chamber and incubate at 37°C, 5% CO_2 for 10 min to allow the hydrogel to solidify.
5. Transfer DMA in a new Petri dish and add 10 mL of the seeding medium containing 1% PenStrep.
6. Incubate at 37°C, 5% CO_2 until analysis.