DDI-CHIP APPLICATION NOTE

DISTANCE DEPENDENT INTERACTIONS

Cells communicate using different modes such as paracrine, juxtacrine and autocrine signaling depending on the distances between them. These various modes of communication are important in both health and disease states such as embryonic development and cancer. DDI-chip provides an easy-to-use and physiologically relevant microenvironment to directly visualize and quantitatively assess distance dependent interactions with high temporal and spatial resolution.

Materials

Cell culture medium complete with appropriate supplements (MC)

Cell culture medium without any supplements (M0)

Cells expressing a fluorescent protein (GFP, RFP, etc.) or labelled with a fluorescent tracker

ThermoFisher Scientific provides the following dyes among others:

C2925 CellTracker™ Green CMFDA

C34552 CellTracker™ CellTracker™ Red CMTPX

C34565 CellTracker™ Deep Red

Hydrogel such as matrigel (preferably growth factor reduced), collagen, puramatrix

Sterile H_20

Tweezers to handle microscope slides

P20 micropipette, P200 micropipette

Sterile microscope slide staining jar with glass lid (*horizontal*)

Protocol

Day 1

If you are going to use fluorescent trackers, it is better to label the cells according to the manufacturer's instructions 16 – 24 hours before passaging the cells for the experiment.

Day 2

Thaw/prepare the hydrogel according to the manufacturer's instructions. Ex: "matrigel ... by submerging the vial in ice in a 4°C refrigerator, in the back, overnight." (i.e. Day -1)

Mix cells and hydrogel at appropriate ratios for example 1:1 for matrigel and load each side cell channel with one of the cell type laden hydrogel, load the other side cell channel with the other

cell type laden hydrogel. The final concentration of cells will depend on the type of cell used yet 6 Million cells / ml is a good starting point.

Place the hydrogel-cell mix loaded DDI-chips in a petri dish containing pieces of filter paper wetted with sterile H_20 and place the petri into a cell culture incubator for 30 minutes.

Water provides humidity to prevent drying of the hydrogel.

Prepare hydrogel mix with the same final concentration ex: 1:1 using medium without cells and load the middle channel with this mix.

Place the cell-free hydrogel loaded DDI-chips in a petri dish containing pieces of filter paper wetted with sterile H20 and place the petri into a cell culture incubator for 30 minutes.

Load medium to the medium channels.

After 1-2 hours, acquire Day0 images using a fluorescent microscope.

Day0 images are essential because they serve as reference images for quantifying multi-cellular organization.

For drug applications, incubate loaded IC-chips in culture for at least 24 hours before adding drugs to the system.

Day 3

Inspect cells under a fluorescent microscope and capture images if desired.

Change the medium in the bottom and top channels to MC+drug and M0+drug, respectively. If no drug is to be tested, simply change medium.

It is recommended that medium is placed at one inlet of the bottom or top channels and is slowly withdrawn from the other inlet of the bottom or top channels. Repeat once to ensure complete change of medium.

Depending on the cell type, medium in the bottom and top channels can be changed every day or every other day.

Day 4

Acquire Day2 images using a fluorescent microscope if desired.

Time point is 24 hours after drug addition.

Day 5

Acquire Day3 images using a fluorescent microscope.

Time point is 48 hours after drug addition.

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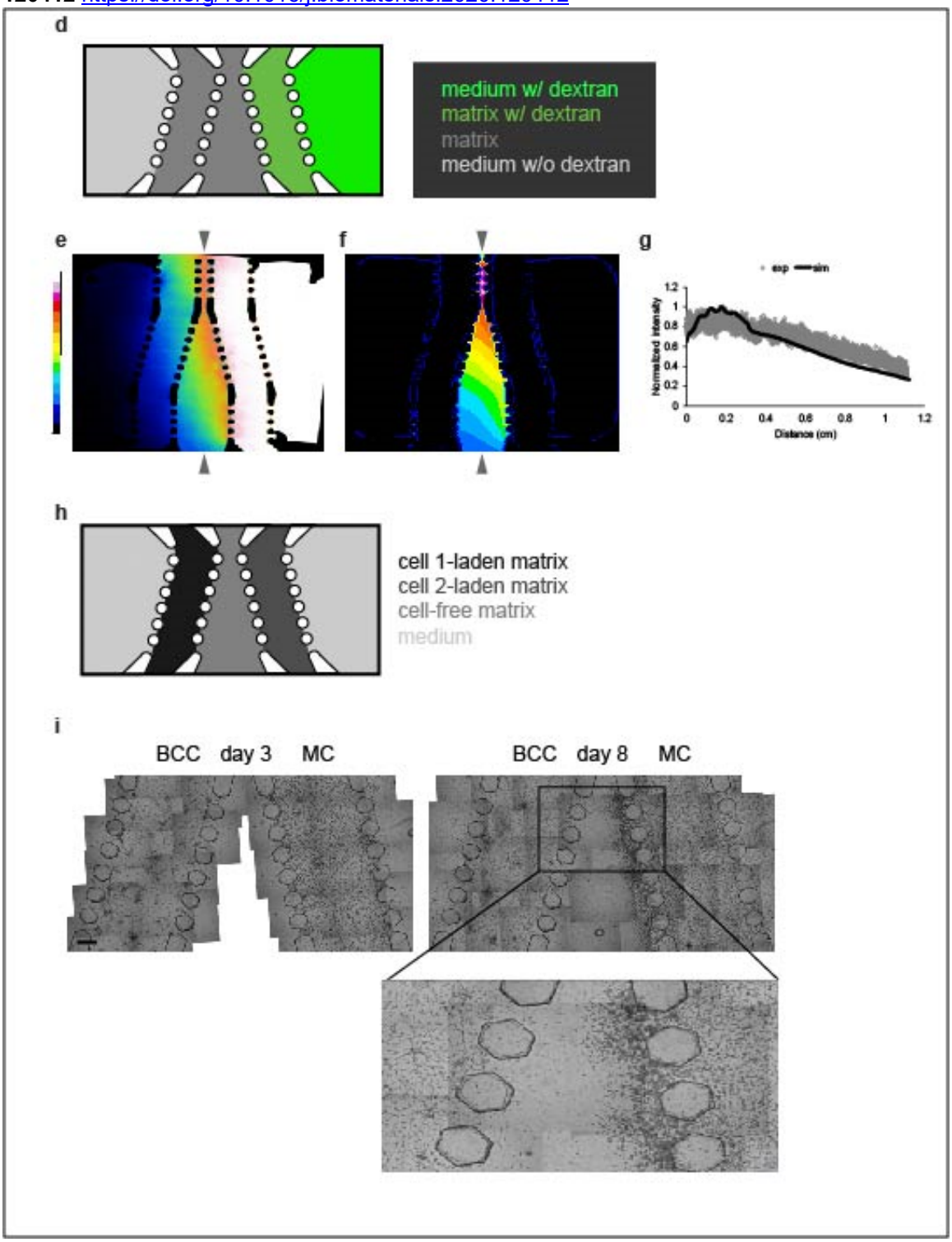

Figure Legend: (d) Cell-on-a-chip design (DDI-chip) to test the diffusion of the dextran molecule (not drawn to scale). Cell-free matrix was loaded into the middle channel. Dextran-laden matrix was loaded into the side channel adjacent to the middle channel. Dextran-free matrix was loaded into the other side channel. The reservoir neighbouring the dextran-laden matrix channel was filled with medium containing dextran. The other reservoir neighbouring the free matrix was filled with dextran-free medium. (e) Fluorescence image of the diffusion of 10 kDa fluorescent dextran in the DDI-chip at day one. (f) Simulation result of the diffusion of 10 kDa dextran molecule in the DDI-chip at day one, generated by VCell. (g) Gradient profiles of the dextran molecule along the distance marked by grey arrowheads in the experimental and simulation results. (h) Cell-on-a-chip design (DDI-chip) to test distant interactions (not drawn to scale). Cell-free matrix was loaded into the middle channel. Cell-laden matrices were loaded into channels on either side of the middle channel. The two reservoirs neighbouring the cell-laden channels were filled with cell culture medium. (i) Representative image for a DDI-chip loaded with BCC and MC (n = 6 cell-on-a-chip devices). (Scale bars, 500 μm)

PLEASE REFER TO THE MAIN IC-CHIP APPLICATION NOTE FOR FURTHER DETAILS