INITIO CELL

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 the middle channel with the mix. The final concentration of cells will depend on the type of cell used yet 6 Million cells / ml is a good starting point. Use 20 µl of the mix per one middle channel.

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 side channels 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.

TIPS & TRICKS

Use a P20 micropipet to minimize pressure while loading channels.

If you realize a leak while loading the gel (with or without cells) into the middle channel, stop and load the rest of the gel from the other inlet.

Holding the chip upright such that the longest axis is perpendicular to the ground can help, too. Otherwise holding the chip flat is also fine, depends on the viscosity of the gel you loading.

Inspect the IC-chip under the microscope to check for complete filling of the middle channel with the gel. If the gel does not fill the inter-post gaps fully*, make sure the gel reaches the top of the outlet before stopping loading.

*The gel should fill in the middle channel such that the gel reaches the long bottom of the posts facing the media channels.

When changing media in the top and bottom channels, beware of air bubbles trapped in the inlets. If there is air, gently remove the air first then load new media. If there is no air, you should see the media move as you touch the media from one inlet/outlet.

When changing media, place a 20 ul drop at an inlet then aspirate 20 ul** from the other inlet, i.e. instead of pushing new media in, pull out old media which will be replaced by the new (drop) media placed at one of the inlets.

** Aspirate less if there has been evaporation to avoid generation of air bubbles.

When changing media, repeat change once (total 2 aspirations).

Change media every other day unless cells are metabolically very active, if so change media every day.

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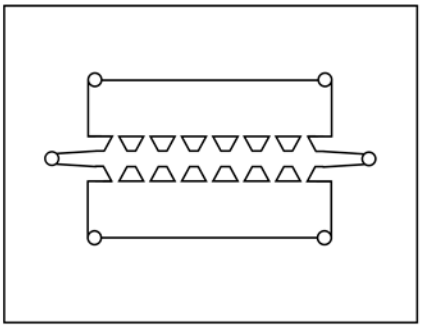

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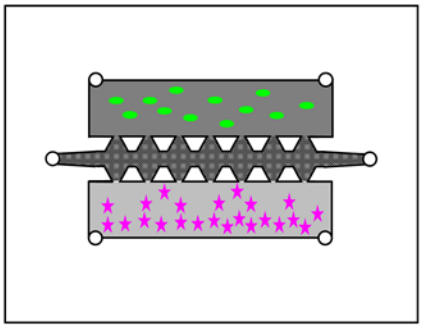

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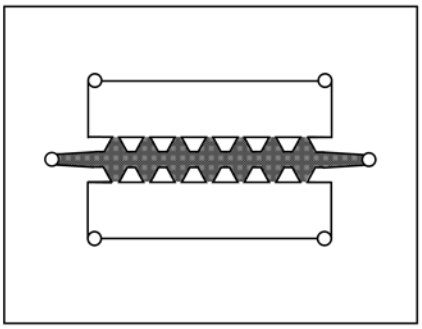

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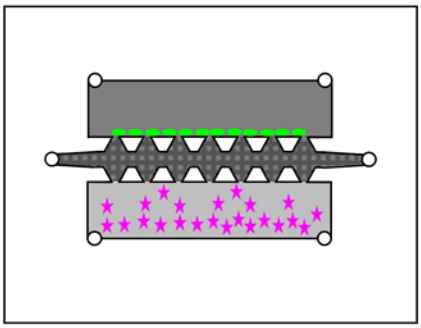

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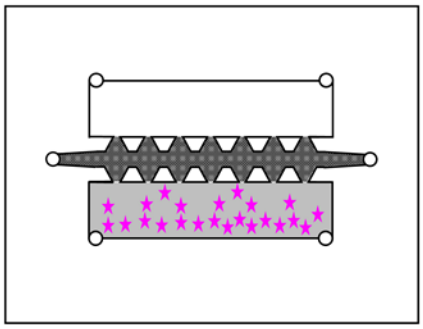

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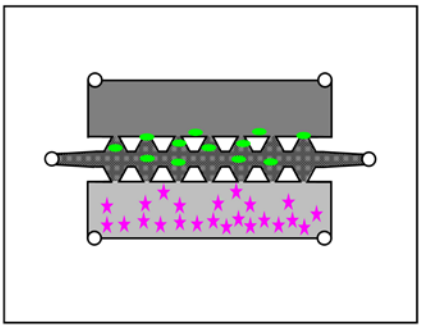
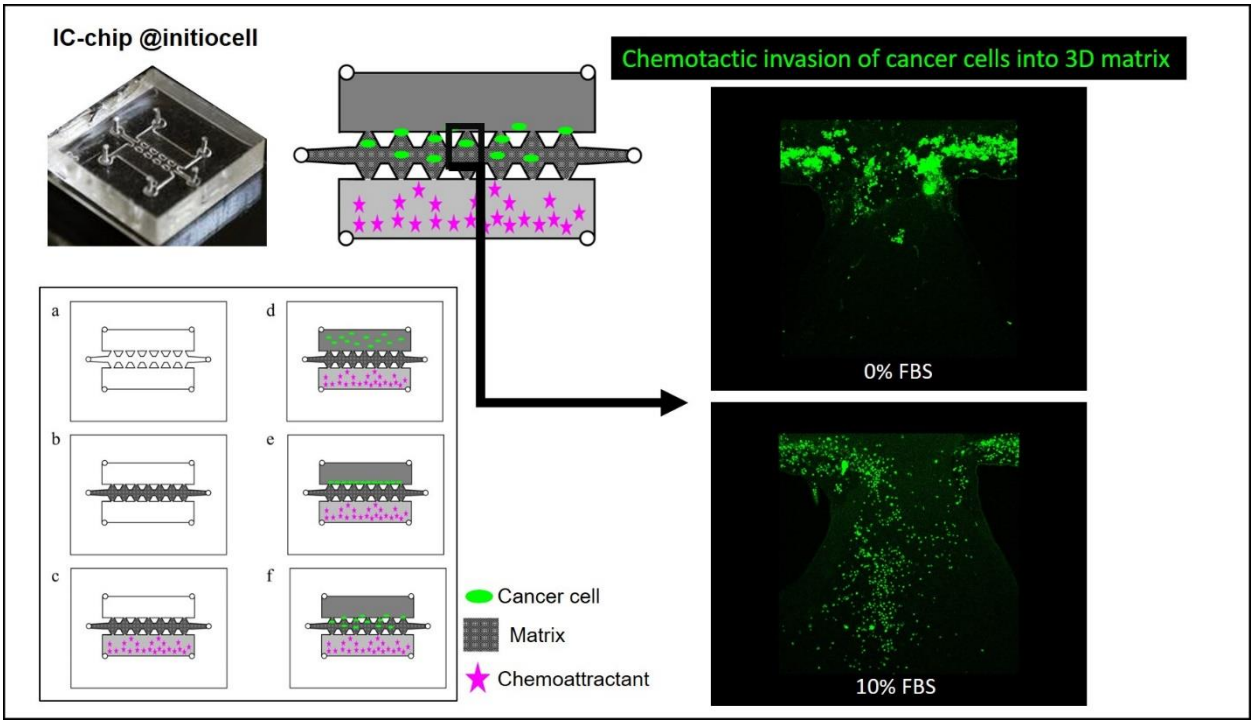
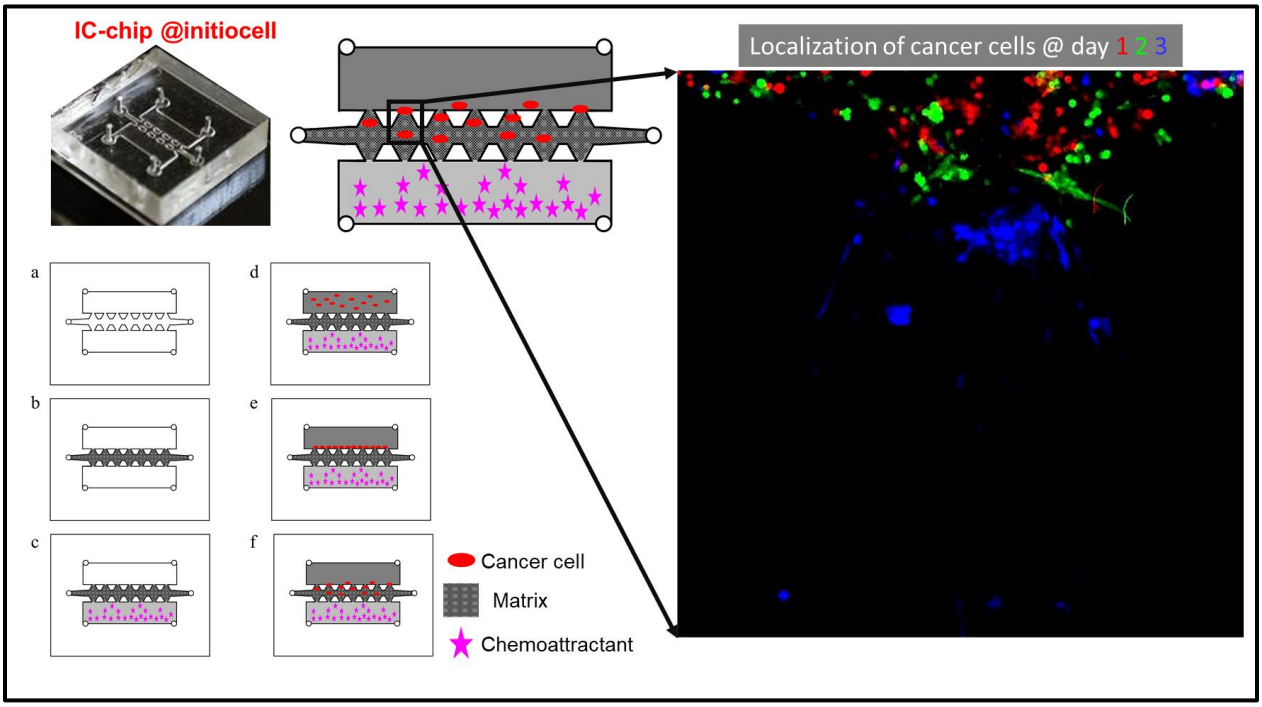




#chemotactic invasion of #cancer cells in #ICcellchip @initiocell. Image courtesy of M. Karabicici and Prof. Esra Erdal @ibgizmir



#chemotactic invasion of #cancer cells in #ICcellchip @initiocell. Image courtesy of Dr. A. Kısım and Prof. Ö. Yalçın Özuysal @iyteedutr