IC-CHIP APPLICATION NOTE

DETERMINATION OF INVASION AND MIGRATION OF CELLS INTO 3D MATRIX

Cell migration and invasion into 3D matrix is important in both health and disease states such as embryonic development and cancer. IC-chip provides an easy-to-use and physiologically relevant microenvironment to directly visualize and quantitatively assess migration and invasion of cells 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

DAPI to label nuclei when cells are not labelled and just one time point is analyzed.

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 0

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)

Load the middle channel with the hydrogel. Ex: Matrigel diluted with M0 to 4mg/ml. Pipet 20 µl of hydrogel, load 10 µl per one middle channel.

The hydrogel can also include cells or other agents that is expected to affect invasion and chemotaxis.

Rack the hydrogel loaded IC-chips into a staining jar containing 5 ml of sterile H_20 and place the jar into a cell culture incubator for 30 minutes.

Water provides humidity to prevent drying of the hydrogel.

Passage cells and resuspend at 1 Million cells/ml in **M0**.

** Optimal cell density can be different for different cell types and should be optimized accordingly.*

Load the bottom channel with ~15 µl of MC.

Load the top channel with ~15 µl of cells in M0.

Rack the fully loaded IC-chips into a staining jar with bottom channels at the bottom and place the jar into a cell culture incubator.

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

Day0 images are useful because they serve as reference images for quantifying invasion and migration.

Alternatively, if cells were not labeled, label nuclei using ex: DAPI, at Day 3 for quantification of invasion and chemotaxis.

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

Day 1

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 2

Acquire Day2 images using a fluorescent microscope if desired.

Time point is 24 hours after drug addition.

Day 3

Acquire Day3 images using a fluorescent microscope.

Time point is 48 hours after drug addition.

Alternatively, if cells were not labeled, label nuclei using ex: DAPI, at Day 3 for quantification of invasion and chemotaxis.

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.

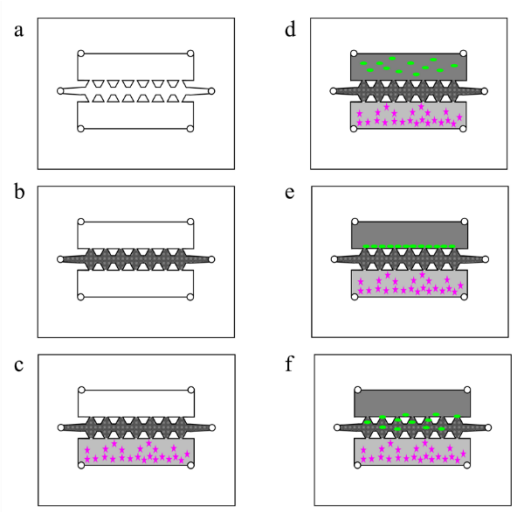
When changing media, place a 20 ul drop at an inlet then aspirate 20 ul** from the other inlet, i.e. instead of pushing new media in, pull out old media which will be replaced by the new (drop) media placed at one of the inlets.

** 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.

SCHEMATIC OF LOADING OF IC-CHIP FOR ASSESSING CHEMOTAXIS AND INVASION



USEFUL SHORT VIDEOS

https://www.youtube.com/watch?v=562Mt2KiNuI
IC-chip 1/3

https://www.youtube.com/watch?v=CtifpIWERw0
IC-chip 2/3

https://www.youtube.com/watch?v=xtuNWjjjZP0
IC-chip 3/3

https://www.youtube.com/watch?v=8Ip_ZJ0z-Tk
DDI-chip

VISUAL PROTOCOL (15 min)

https://www.youtube.com/watch?v=8d2e-KpHZNQ&t=10s

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