DDI-CHIP *AUTOCRINE* 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

Cell culture medium without any supplements

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, etc.

Sterile H_20

Tweezers to handle microscope slides

P20 micropipette

Petri dish

Protocol

Day 0

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 1

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 0)

Mix cells and hydrogel at appropriate ratios for example 1:1 for matrigel. The final concentration of cells will depend on the type of cell used yet 6 Million cells / ml as final cell concentration is a good starting point. Use 20 µl of the mix per one middle channel. For precious e.g. patient

samples, 10 µl of the mix can suffice. Otherwise, working with a P20 adjusted to a 20 µl volume for all channels facilitates loading of the DDI-chip.

Load the middle channel from the "wide" end so that the pressure applied is enough to fill the wide part and the rest of the middle channel. Holding the chip upright while loading also helps.

A P20 micropipette is ideal for loading. Using larger volume micropipettes will increase pressure and will not provide proper control of loading.

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.

If cells tend to settle at the glass bottom, 15 minutes' incubation on PDMS side and 15 minutes incubation on the glass side is recommended. Please note PDMS will stick to the petri surface so place spacers when incubating on the PDMS side.

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. Use 10 µl of the mix per one side channel.

Holding the chip upright while loading helps.

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. Use 10 µl medium per one medium channel.

When loading medium, hold the DDI-chip horizontal such that the inlet and outlet of the medium channel to be loaded are facing upwards to allow air to be pushed out.

If cells can survive in serum free medium, it is better to use it than complete medium to avoid influence of supplements on cell-cell interactions. Alternatively, medium with reduced serum concentration can be used.

After 1-2 hours, acquire reference images using a phase contrast and/or a fluorescent microscope.

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

For drug applications, incubate loaded chips in culture for at least 24 hours before adding drugs to the system to allow cells to accommodate to the microenvironment.

<u>Day 2</u>

Inspect cells under a phase contrast and/or fluorescent microscope and capture images if desired.

Change the medium in the medium channels to contain medium+drug. 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.

Day 3 and afterwards

Acquire images using a phase contrast and/or fluorescent microscope at desired time points.

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 a 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 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 neighboring channels.

When changing media, 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 10 ul drop at an inlet then aspirate 10 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 or use 20 µl of medium 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.

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

SCHEMATIC OF LOADING OF DDI-CHIP FOR ASSESSING AUTOCRINE SIGNALING