4Dcell
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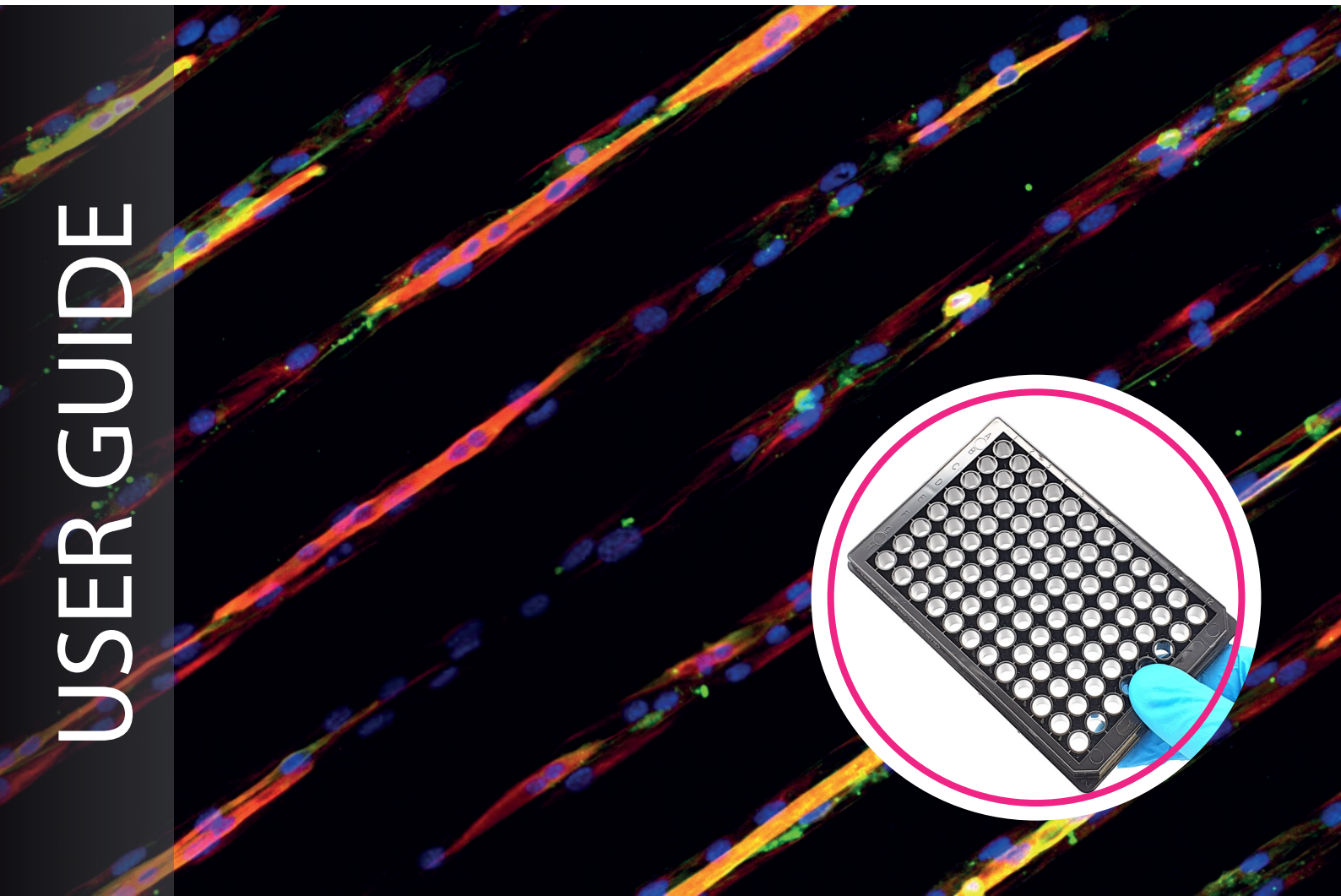
4Dcell Dynamic Micropatterns - Multiwell plates

User protocol

USER GUIDE



4Dcell micropatterned Multiwell plates User Guide

How to use 4Dcell micropatterned Multiwell plates

Materials included

Micropatterned glass-bottom 96 well plate (round wells) coated with anti-adhesive polymer (dynamic)
Migration triggering protein: Click Chemistry Protein (DBCO-RGD, 1616 g/mol) - 40 µg/tube, 3 tubes/plate

Materials not included

20-200 µL/100-1000 µL single or multichannel micropipette
Distilled water
PBS
Cell culture medium
Serum-free cell culture medium

Optional

> Coating solution *(e.g. fibronectin, collagen, laminin).*
The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type.

> Fluorescent-labelled protein *(e.g. fibrinogen, BSA, collagen, laminin).*
The patterns on the wells can be visualized using fluorescent fibrinogen or other fluorescent protein.
The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL.

Shipping & storage

The micropatterned 96-well plates should be used within 3 months from the date of delivery.
The migration triggering protein is shipped lyophilized. Upon reception, it should be immediately stored at -20°C in the dark.

Before starting

To avoid contamination, only manipulate the the plates using aseptic techniques in a laminar flow hood.

The micropatterns consist of a thin layer of polymer bound to the surface of the glass. To avoid scratching this layer, pipette solutions into the plates carefully and avoid touching the surface of the glass.

The timing of protein and/or cell incubation can vary depending on your needs. The protocol suggested by 4Dcell was optimized for mouse embryonic fibroblasts (MEFs) and HeLa cells. You may need to adapt this protocol for your cell type.

Incubation with an extracellular matrix protein to improve cell adhesion to the micropattern is optional. We suggest only using an adhesive protein if your cell type does not readily adhere to glass substrates. Several adhesive coating proteins can be employed if needed (fibronectin, laminin or collagen).
Be careful not to confuse the extracellular matrix protein with the trigger protein.

Many steps of the following protocol require preparation of solutions and reagents in advance: read the full protocol before starting experiments.

Protocol

Step 1 - Cell seeding

a. Open the individual plastic bag containing the plate using aseptic technique in a laminar flow hood.

If coating the wells of the plate with an extracullular matrix protein is necessary, proceed with step a.1. Otherwise, proceed directly to step b.

a.1. *Optional: Pipette 50 µL of the fibronectin solution[1][2] or any other adhesion solution directly in each well.*

a.2. *Optional: Incubate for 30 min to 1 hour at room temperature.*

a.3. *Optional: Discard the adhesive protein solution from the wells.*

a.4. *Optional: Rinse the wells 3x with 200 µL sterile PBS before proceeding to the cell seeding step.*

b. Prepare a cell solution. We recommend 5 000 – 20 000 cells/well. Prepare at least 20 mL to ensure you have enough solution for your entire plate[3].

c. Pipet 200 µL of cell suspension into each well. To prevent cells gathering in the center of the wells, do not move the plate at this stage. Cell adhesion can be encouraged by placing the plate on a warm surface (30°C) before pipetting and not touching the plate during or after pipetting.

d. Incubate the micropatterned plate containing the cells for 1 hour (or more, depending on cell type) at typical cell culture conditions (e.g. 37°C, 5% CO_2).

e. Remove medium and unattached cells and wash the plate with pre-warmed culture medium or sterile PBS[4].

f. Discard medium or PBS, add 200 µL of fresh pre-warmed culture medium per well and incubate cells over-night. Patterned cells can be observed under the microscope after several hours of incubation (24-36 hours).

NOTES

[1] The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type.

[2] The patterns can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL. Mix fluorescent fibrinogen and fibronectin at a 1:1 ratio (50 µL per well).

[3] The seeding density is optimized for the MEFs and HeLa cell lines therefore, the number of cells added, and the adhesion or culture times could vary. Carefully estimate the number of cells needed to seed per well so that confluence is reached within 24 to 48 h (at this point, after 24-48h, the wells should be ready to proceed to Step 2: Activation of cell migration).

[4] Removing the unattached cells will reduce cell clustering and bridging between micropatterns.

Step 2 - Activation of cell migration

For best results, the following steps should only be performed once the cells fill the micropatterns. Before starting, have pre-warmed, ***serum-free*** *cell culture medium ready.*

a. Collect the provided vial containing the lyophilized triggering protein.

b. Allow the vial to equilibrate at room temperature for 10 minutes before opening it.

c. Tap down the vial to ensure that all the lyophilized powder is collected at the bottom. The powder may not be visible to the naked eye.

d. Collect 1 mL of appropriate media[5] and add it to the tube to make a 25 µM (40 µg/mL) stock solution of triggering protein.

e. Homogenize the triggering solution by pipetting up and down 2-3 times into the tube. Make sure all the solution is in the bottom of the tube and not in the lid. Dilute to the appropriate concentration (we suggest 10-25 µM).

f. Transfer the 96 well plate with the patterned cells from the incubator to the microscope and take pictures of all the wells before proceeding with the protocol (t=0h).

g. Transfer the plate to the cell culture hood and discard the medium from the wells to be activated.

h. Activate cell migration by pipetting 25 µL of triggering solution in each well and incubate for 5 to 30 minutes at 37°C / 5% CO_2 [6].

i. Discard the triggering protein solution from the wells[7].

j. Add 200 µL of pre-warmed complete cell culture medium in each well and incubate at 37°C / 5% CO_2 [8].

NOTES

[5] We recommend dissolving the triggering protein in serum-free culture medium, but PBS can also be used.

[6] In our experiments 5 minutes were sufficient to trigger migration, but you may want to leave the protein in contact with the surface longer to maximise surface coating.

[7] To avoid perturbing the cells you can also skip this step and dilute the triggering protein solution with medium rather than changing the solution.

[8] Images may be acquired at this point to monitor the medium exchange.