4Dcell
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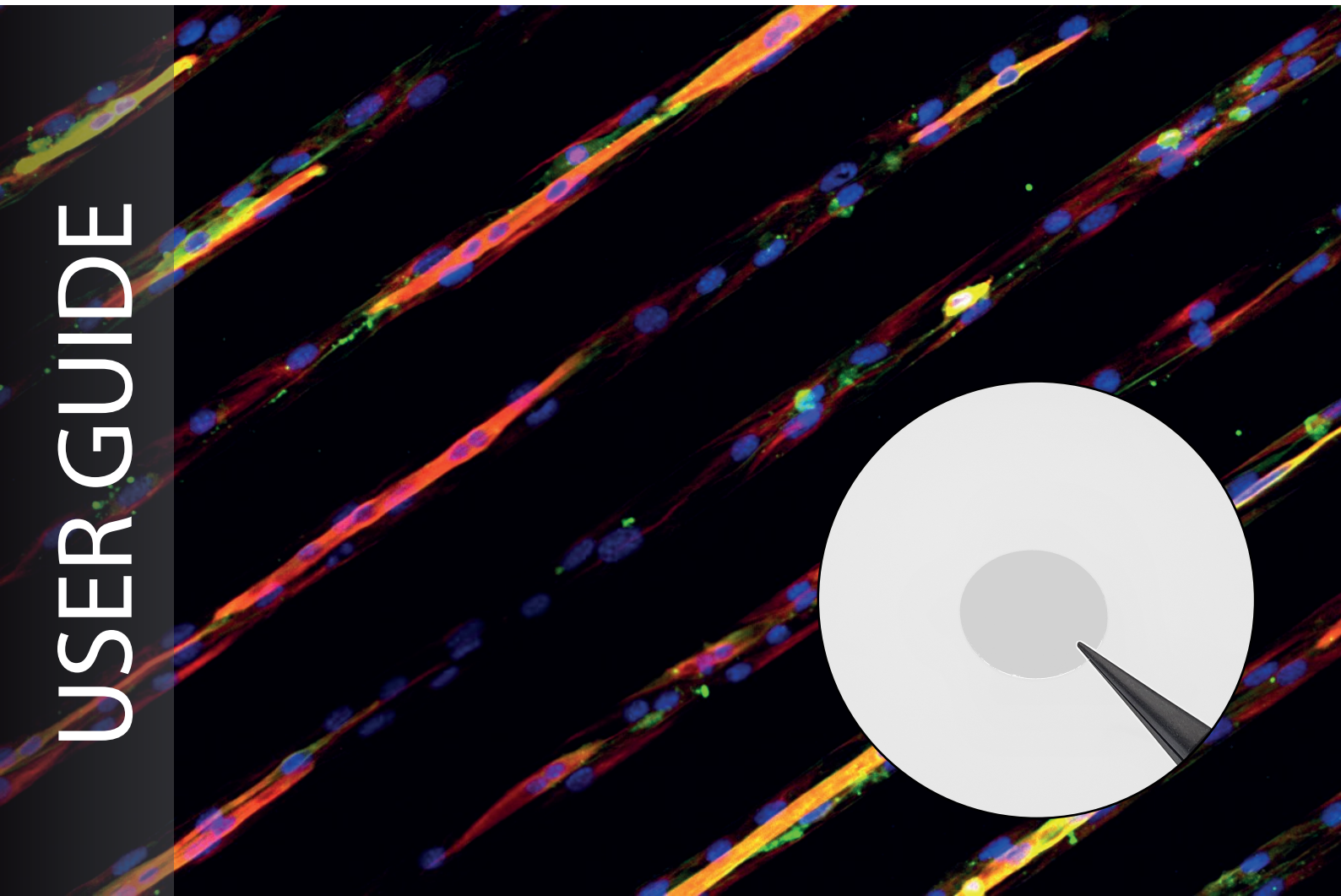
4Dcell dynamic micropatterned coverslips

User protocol

USER GUIDE



4Dcell micropatterned coverslips User Guide

How to use 4Dcell micropatterned coverslips

Materials included

Micropatterned glass coverslips
Triggering Protein (DBCO-RGD, 1616 g/mol) - 50 µg/tube

Materials not included

100-1000 µL micropipette
Distilled water
PBS

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 Petri dish 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 dynamic micropatterned coverslips should be stored at room temperature and used within two months from the date of production. The 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 coverslips 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 onto the coverslips 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 adhesion protein 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. You can also try pre-incubating the dishes in cell medium (media typically contain proteins which can help adhesion).

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

Protocol

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

If coating the coverslips with an adhesion protein is necessary, proceed to step a.1. Otherwise, proceed directly to step b.

> **a.1.** *Optional: The coverslip is attached to the Petri dish, and the patterned side of the coverslip faces up. To remove a coverslip, fill the Petri dish with distilled water, and wait until the coverslip floats.*
>
> **a.2.** *Optional: Remove the coverslip from the Petri dish and let it air dry. Remove the water from the Petri dish as well and let it air dry.*
>
> **a.3.** *Optional: Pipette 100 µL of your adhesive protein on parafilm*[(1)(2)]*.*
>
> **a.4.** *Optional: Carefully place the micropatterned side of the coverslip on the drop of fibronectin.*
>
> **a.5.** *Optional: Incubate for 30 min to 1 hour at room temperature.*
>
> **a.6.** *Optional: Rinse the coverslip with distilled water and allow it to air dry before proceeding to the cell seeding step. Once dried, place the coverslips back in the Petri dishes or in a 6 well-plate. The patterned side of the slide facing up.*

b. Prepare a cell solution. For a standard design, we recommend 30 000 – 60 000 cells/mL[(3)]. For some patterns that have larger cell seeding areas (for instance wound healing), we recommend a higher density of cells.

c. Pipet 400 µL of cell suspension onto each coverslip.

d. Carefully transfer the coverslips from the hood to the incubator[(4)].

e. Incubate the micropatterned coverslips with the cells in typical cell culture conditions (for example 37°C, 5% CO2).

f. ***Optional***: after initial adhesion (30 minutes - 2 hours, depending on the cell type), carefully remove the medium to remove unadhered or poorly adhered cells. Tilt the petri dish and allow the medium to flow into the edge of the petri dish. Remove the medium and replace with fresh warm medium in the corner of the petri dish to avoid perturbing the newly adhered cells. The cells can also be rinsed with PBS solution before adding warm medium[(5)]

g. Patterned cells can be observed under the microscope after several hours of incubation.

h. Dynamic micropattern activation

> **h.1.** Resuspend Triggering Protein in the appropriate amount of warm medium (without serum). The molecular weight of the protein is 1616 g/mol. For the recommended concentration of 25 µM, resuspend the aliquot (50 µg) in 1.237 ml[(6)]
>
> **h.2.** Carefully remove cell supernatant without scratching the cell layer or the polymer coating. Replace with an appropriate amount of Triggering Protein solution. (We recommend a volume of 200 µl.)
>
> **h.3.** Incubate the petri dish with the Triggering Protein solution for 5 - 30 minutes.[(7)]
>
> **h.4.** Replace Triggering Protein solution with fresh warm medium.[(8)]

Notes

(1) The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type. The incubation with fibronectin can be performed directly on the coverslips attached to the Petri dish (higher volume of protein solution is need to cover the whole glass coverslip).

(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.

(3) The seeding density is optimized for HeLa cells. The number of cells added and the adhesion or culture times could vary for other cell types. Make sure the cell suspension is thoroughly re-suspended into individual cells before pipetting onto the coverslips. It is better to re-suspend cells that are at a confluency around 50% or less. High confluency of cells before re-suspension and seeding onto the micropatterns will promote cell-cell adhesion and cell cluster formation, which is not suitable for single cell patterning.

(4) Avoid allowing the solution to swirl, which will cause the cells to accumulate in the center of the dish and result in uneven cell adhesion. To encourage even cell seeding, if your cell type allows, the dish can be kept on a warm surface (37°C) for the first 20 minutes to allow initial adhesion without disturbing the cell solution.

(5) Removing the unattached cells will reduce cell clustering and bridging between micropatterns.

(6) Lower concentrations of Triggering Protein solution will result in a lower coverage of RGD peptides on the surface and can reduce adhesion and migration speed of cells on the surface. This property can however be used to tune the adhesion ability of cells to the surface. We have observed adhesion of some cell types onto activated surfaces using concentrations as low as 1 µM.

(7) In our experiments, we observed adhesion onto surfaces with incubation times as low as 1 minute, with no discernible difference in migration speed or cell phenotype.

(8) To avoid perturbing the cells you can also skip this step and dilute the triggering protein solution with medium rather than changing the solution.