4Dcell
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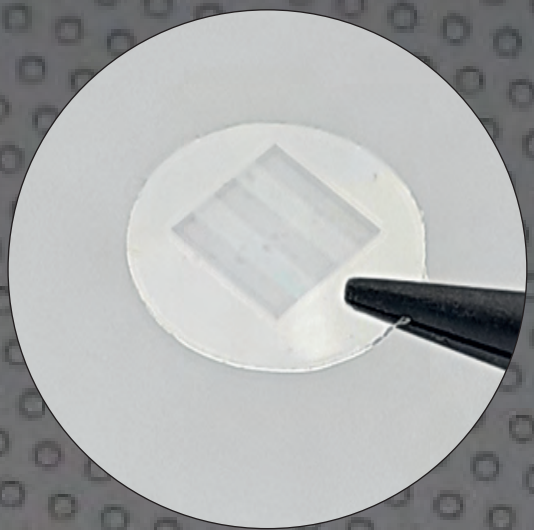
4Dcell micropillars
User protocol

USER GUIDE



4Dcell micropillars User Guide

How to use 4Dcell micropillars

Materials included

Micropillar coverslip: Glass coverlips (16 mm diameter) with one face decorated with a square area 5 mm wide consisting of micropillars.

Stencil: A silicone ring is included that can be used to create a circular wall around the micropillars (similar to a well), reducing the volume necessary for washes and incubation, and concentrating the cells on top of the micropillars.

Materials not included

20-200 µL micropipette
Distilled water
Phosphate Buffered Saline (PBS)
Kimwipes

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 micropillars on the coverslip can be visualized using brightfield optical microscopy. If fluorescent visualisation is necessary, the micropillars can be coated with fluorescent fibrinogen or any other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL.

Shipping & storage

Micropillars can be stored up to 6 months. Longer storage can result in hardening of the PDMS and modifications in the stiffness of the micropillars.

Before starting

The micropillars are sent inside glass-bottom 35 mm Petri dishes, pillars facing upwards. A silicone stencil is assembled onto each coverslip. The central part of the coverslip contains the micropillars. The stencil keeps the coverslip in place, but is easily removed.

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

The micropillars are very sensitive to capillary forces: once the micropillars are wet, do not allow them to dry or they will collapse irreversibly.

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

Incubation with an adhesion protein is optional. Several adhesive coating proteins can be employed if needed (fibronectin, laminin or collagen).

Protocol

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

If coating the Petri dishes with an adhesion protein, proceed to step a.1. Otherwise, proceed directly to step b.

> **a.1.** *Optional: Pipette 100 µL of your adhesive protein on top of the pillars. Pipet slowly to get a clean drop on the top of the pillars and to avoid touching the pillars with the pipette tip*[(1)(2)].
>
> **a.2.** *Optional: Incubate for 5 minutes at room temperature.*
>
> **a.3.** *Optional: Remove the drop carefully with a pipette, avoiding to touch the pillars.*
>
> **a.4.** *Add 100 µL of distilled water or PBS to remove the excess fibronectin and discard*

b. With the stencil in place. wet the micropillars by adding 250 µL of PBS to your Petri dish. Remove and replace with fresh PBS 3 times. The coverslip should be kept wet to avoid collapsing the pillars.

c. Replace the PBS with cell culture medium and repeat the 3 rinses, finishing with 250 µL of cell culture medium in the dish.

d. Prepare a cell solution. For a standard design, we recommend 25 000 cells resuspended in 250 µL of cell culture medium[(3)]. Replace the medium on the micropillars with the cell solution.

e. Transfer the Petri dish from the hood to the incubator.

e. Incubate the micropatterned Petri dish containing the cells at 37°C, 5% CO2.

f. Cells can be observed under the microscope after several hours of incubation. The stencil can be removed at any point, but once the edges of the coverslip are wet the stencil will no longer adhere to the edges of the coverslip and won't be waterproof.

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 pillars 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 into the Petri dish. 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 will promote cell-cell adhesion and cell cluster formation, which is not suitable for single cell seeding.