Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization

This Application Note discusses the experimental parameters that are important for cell adhesion to the ibidi µ-Patterns. Further, it explains how to optimize cell adhesion and pattern coverage.

Content

Keywords

- µ-Patterning, adhesion, binding motif, single-cell patterns, multi-cell micropatterning, cell culture, adherent cells

Parameters That Influence Cell Adhesion on µ-Patterns

Cell Type

Successful experiments with the ibidi µ-Patterning are limited to adherent cell types—suspension cells cannot attach to the pattern surface. Further, adhesion to the µ-Pattern surface is highly cell type-dependent since not all cell types bind to all binding motifs. Therefore, before starting the experiment, it is necessary to test the selected cell type for its capability to adhere to the patterned surface.

Binding Motif

The ibidi µ-Patterning technology offers covalently bound binding motifs for cell adhesion. The surface density of these binding motifs may differ from classical protein coatings. As a result, the binding motif may lead to a change of cell morphology or adhesion behavior. The best way to test if your cells are compatible with the binding motif is to seed them on pattern spots with a large adhesive area (e.g., multi-cell patterns). Once cell compatibility is verified, you can switch to smaller patterns, like the single-cell pattern.

Pattern Geometry

The pattern geometry can influence the cells' adhesion behavior due to the amount of binding motif. For example, smaller shapes present less binding motifs, which might reduce the cell adhesion versus larger shapes. Especially for single cell experiments, where it is desired to achieve single cell occupancy on the pattern, the pattern size is very important, because too small patterns hinder proper cell adhesion and spreading, but too large patterns result in the adhesion of multiple cells on a single spot. Furthermore, the spacing in between the adhesion patches influences the ideal cell seeding concentration and the cell bridging from one spot to another, when the pitch is too small for the used cell type.

Cell Seeding Concentration

The seeding concentration is an additional crucial parameter for optimizing the adhesion process. Lower concentrations lead to fewer cells on the pattern or only sparsely occupied spots but less clumping. Higher cell concentrations can improve cell coverage but bear the risk of a) clumping and b) multiples of cells on single-cell adhesion sites. If the cell concentration is too high and cells start aggregating before attaching to the adhesion spots, these floating aggregates might detach adherent cells from the pattern and prevent further cell adhesion.

Cell Suspension Quality

The homogeneity of the cell suspension highly influences the experimental output. A single-cell suspension without any cell aggregates is highly preferable for single-cell assays. Seeding a suspension of cell clusters or spheroids is an option for 3-dimensional multi-cell assays.

Incubation Time

The incubation time after seeding the cells influences the adhesion process. The time necessary for the cells to attach to the patterns might be different compared to other surfaces. Take special care when moving the µ-Patterned slides during the adhesion process. Non-attached or only loosely attached cells might start clumping.

Mechanical Stress and Washing

Partially attached cells may be washed away by mechanical forces. Utilizing this effect helps remove cells or debris. Depending on the harshness of the washing step (shear stress), more or less cells might be washed off the surface.

Cell Fitness

The viability and fitness of the cells is an essential factor for proper cell adhesion. Therefore, make sure to optimize the cell preparation process in terms of minimizing cell stress. Optimize and standardize factors such as chemical detachment, temperature, mechanical stress, time in suspension, culture medium composition and additional cell culture parameters that influence the cell fitness.

Optimizing Cell Adhesion on µ-Patterns

Initial Test: Is the Cell Type Compatible With the µ-Pattern?

If you don't know if your cell type is compatible with the binding motif of the µ-Patterning, we recommend running an initial cell adhesion test on a multi-cell µ-Pattern first. Testing the adhesion on larger patterns reduces the initial compatibility test to the binding motif itself while excluding single cell and pattern geometry effects.

- Use 2-3 different cell seeding concentrations following the protocol in the instructions, and let the cells adhere without disturbance by incubating them for at least 4 h up to overnight.
- Control the cell attachment with the phase contrast microscope before washing away unattached cells. Taking images of the cells always helps to optimize the process afterward.
- Take additional images at least 2 h after washing to give the cells the time to recover from the shear stress experienced while washing.

Please note: At this stage, optimal pattern coverage is not necessary. It is just important that any cells stick to the pattern. If this is the case, you can proceed with optimizing the seeding parameters on your desired pattern. If you do not see any cell attachment on the pattern even after overnight incubation, your cell type is likely not compatible with the used binding motif.

Optimizing Seeding Parameters for Cells That Attach on the µ-Pattern

If you know that the cells bind to the binding motif of the µ-Pattern, you can start optimizing the cell seeding parameters on the desired pattern. Please consider that every pattern needs to be optimized for your specific cell type, as pattern size and distance between the patterns largely affect the seeding parameters. Make sure that the cells are vital and that you have a single-cell suspension.

First Optimization Run

- Seed at least three different concentrations of the cells on the slide following the instructions.
- Let the cells adhere for at least 4 h without disturbance before washing.
- Take images of the cells before washing and at least 2 h after washing.

Possible Results and Troubleshooting

- After washing, the cells sit nicely on the pattern, and pattern coverage is good.

  → You already found suitable seeding parameters for your experiment. You can start with your experiment using the same conditions.

- Before washing, there are only a few cells floating and aggregating. After washing, there are very few cells on the pattern, and pattern coverage is poor.

  → The cell density might be too low. Try higher seeding concentrations and consider longer incubation times before washing away unattached cells.

- Before washing, there are a lot of floating, aggregating cells. After washing, there are very few cells on the pattern, and pattern coverage is poor.

  → The cell density might be too high. Try reducing the initial seeding concentration, as a too high cell density often leads to cell aggregation, hindering cell binding to the pattern. Also, check the cells 2 h and 3 h after seeding to see if a shorter incubation time leads to less aggregation and, therefore, better pattern coverage.

- After washing, there is undesired cell aggregation on the pattern.

  → This indicates a too high initial seeding concentration and/or a too long incubation time before washing. Try a lower seeding concentration and check the cells already after 2 h and 3 h to see if a shorter incubation time is enough for good cell adhesion.

- There is no cell adhesion on the pattern or the cells are rounded and do not spread when using small single cell spots or thin lines.

  → If you know from previous experiments that the cells can bind to the used binding motif of the pattern, the pattern dimensions might be too small for your cell type, and the cells do not have enough space to spread and adhere properly. Therefore, use bigger pattern dimensions for your experiments.

Second Optimization Run

- Use optimized cell culture parameters according to your results from the first optimization run, as described above.
- Compare your results with the first optimization run and, if necessary, go into another round of optimization.

Example Images

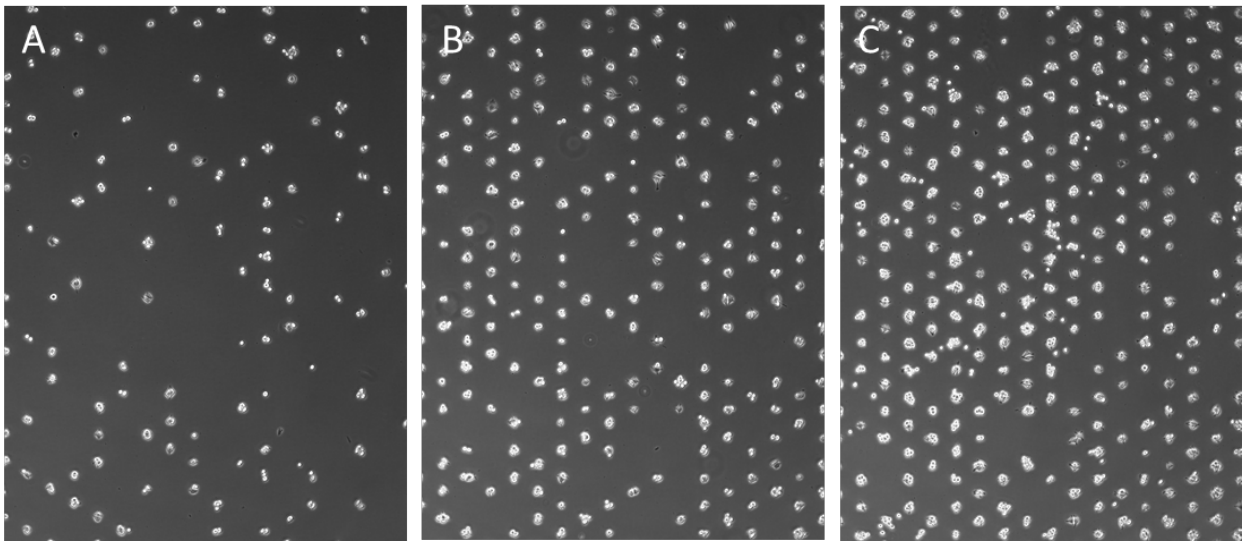

Figure 1: Influence of different seeding concentrations of L929 cells on a single cell pattern. Cells were seeded into a µ-Slide VI $^{0.4}$ with Single-Cell µ-Pattern at concentrations of (A) $1*10^5$ cells/ml, (B) $3*10^5$ cells/ml, (C) $5.25*10^5$ cells/ml. After 24 h, unattached cells were washed away and images were taken 2 h after washing. At low seeding densities, many spots are not covered with any cell. However, at too high seeding concentrations cells start to aggregate on the pattern.

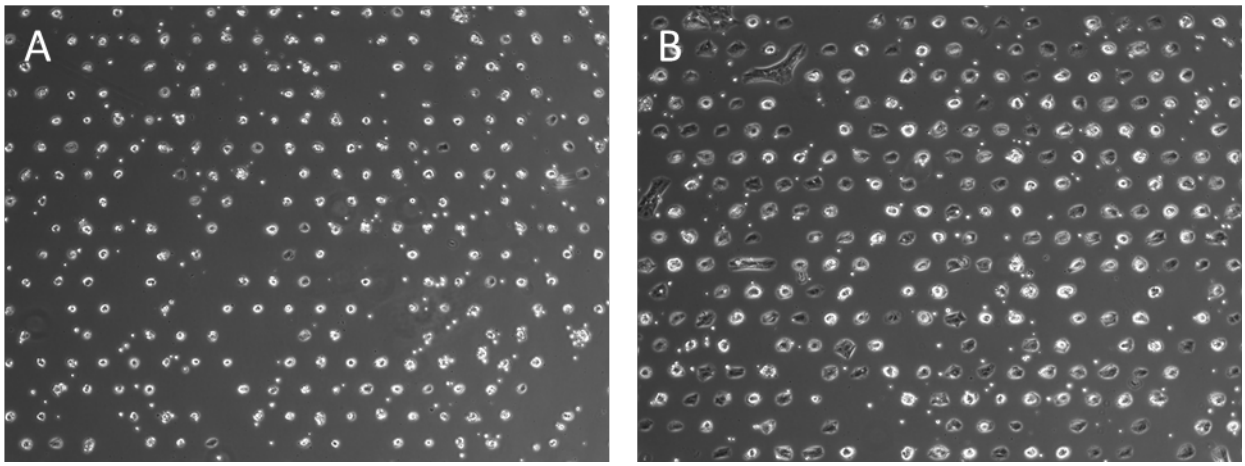

Figure 2: Influence of different pattern sizes on the spreading behavior of Rat1 cells on a single cell pattern. Cells were seeded into a µ-Slide VI $^{0.4}$ with a µ-Pattern of (A) 20 µm squares with a distance of 110 µm or (B) 30 µm squares with a distance of 110 µm at concentrations of $9*10^5$ cells/ml. After 24 h, unattached cells were washed away and images were taken 2 h after washing. For Rat1 cells, 20 µm squares are too small for a good cell spreading, whereas 30 µm squares provide enough area for the cells to flatten on the pattern.

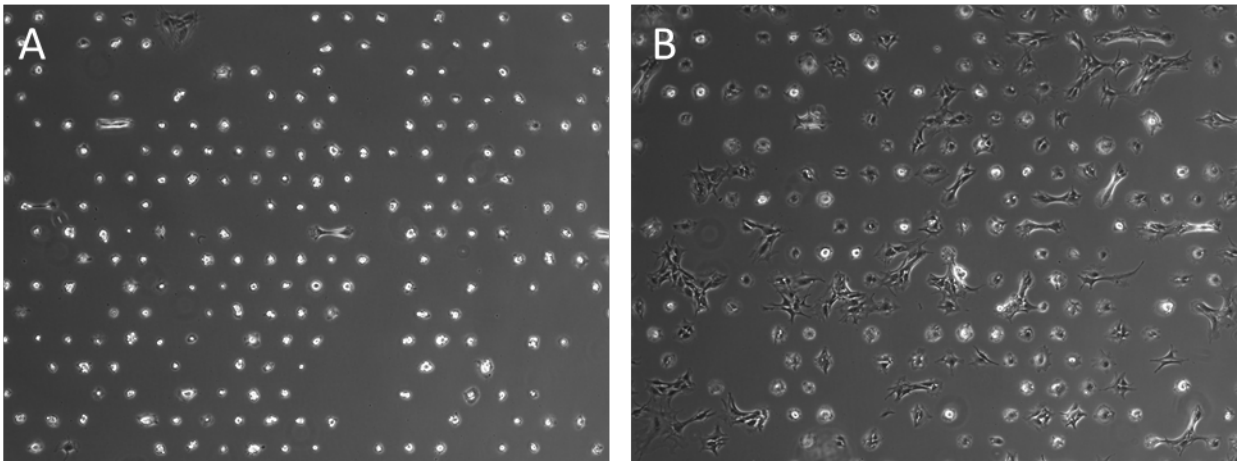

Figure 3: Influence of different pattern geometries on the spreading behavior of NIH3T3 cells on a single cell pattern. Cells were seeded into a µ-Slide VI $^{0.4}$ with a µ-Pattern of (A) 20 µm squares with a distance of 110 µm, or (B) 30 µm squares with a distance of 110 µm at a concentration of $3*10^5$ cells/ml. After 24 h, unattached cells were washed away and images were taken 2 h after washing. For NIH3T3 cells, 20 µm squares are too small for a good cell spreading, whereas 30 µm squares provide enough area for the cells to flatten on the pattern. However, due to the 10 µm smaller distance between the pattern edges, cells are able to bridge from one spot to another. For this cell line, a higher distance between the patterns is needed.