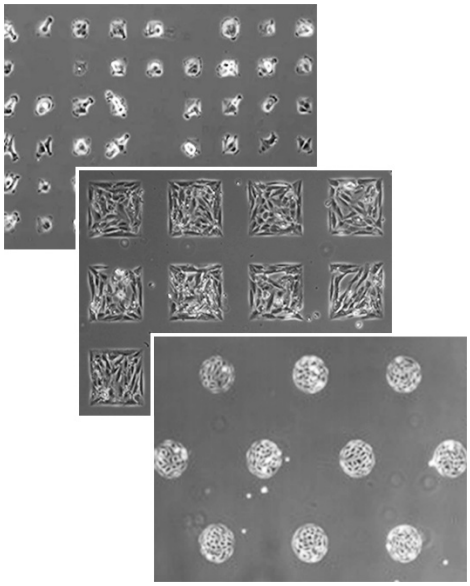
Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization



Micropatterns are powerful tools for optimizing cell assays, by offering precise localization that unlocks the potential to investigate cells within controlled spatial environments.

The ibidi µ-Patterning technology provides spatially defined cell adhesion for various single-cell, multi-cell monolayer or 3D spheroid/organoid applications. The miniaturized, adhesive ibiTreat (tissue culture-treated) patterns on the ibidi Polymer Coverslip are surrounded by a ULA non-adhesive Bioinert surface to prevent non-specific bindings ensuring that cells adhere only to the defined micropatterned areas.

This Application Note outlines the key experimental parameters that influence cell adhesion to ibidi µ-Patterns. It also provides guidance on optimizing both cell adhesion and pattern coverage.

ibidi offers various solutions for cell cultivation on µ-Patterns ibiTreat:

- µ-Slide 8 Well ^{high} µ-Pattern ibiTreat, sqr30, pit75, hex
- µ-Slide VI $^{0.4}$ µ-Pattern ibiTreat, sqr30, pit75, hex
- µ-Slide 8 Well ^{high} µ-Pattern ibiTreat, cir200, pit600, hex
- µ-Slide VI $^{0.4}$ µ-Pattern ibiTreat, cir200, pit600, hex
- µ-Slide 8 Well ^{high} µ-Pattern ibiTreat, cir500, pit1000, hex
- µ-Slide VI $^{0.4}$ µ-Pattern ibiTreat, cir500, pit1000, hex

Related Documents

- Video: The ibidi µ-Patterning Technology—Achieve Spatially Defined Cell Adhesion with Micropatterning

Keywords:

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

1 Parameters That Influence Cell Adhesion on µ-Patterns

1.1 Cell Type

The ibidi µ-Pattern products are designed and optimized for adherent cell types—suspension cells cannot attach to the pattern surface. Furthermore, the adhesion-characteristics to the µ-Pattern surface might depend on the cell type. Therefore, before starting the experiment, it is recommended to test the selected cell type for adequate adhesion to the patterned surface.

1.2 Pattern Geometry

The pattern size and geometry are important parameters, as they control the adhesive capacity and the number of cells grown on one pattern. For example, single cell experiments require single cell occupancy. Therefore, a small pattern size is very important. However, if the pattern size becomes too small, they could hinder proper cell adhesion and spreading, whereas too large patterns may result in increasing numbers of multiple cells on a single cell spot.

Another important parameter is the non-adhesive spacing between the adhesion patches. This distance should be carefully considered, as a small distance enables cell bridging—which is desired in some experiments, such as neuronal growth. However, an extensive distance should be chosen if the cells need to be physically separated.

1.3 Pattern Surface or Surface Coating

The pattern surface with specific or non-specific binding motifs highly influences the adhesion of cells. ibiTreat is the hydrophilic, tissue culture-treated (TC-treated) version of the ibidi Polymer Coverslip. This physical surface modification, which is comparable to the tissue culture treatment of standard cell culture vessels, makes the surface hydrophilic and adhesive to virtually all cell types. Without an additional protein coating, the ibiTreat surface provides excellent non-specific cell adhesion. For specific cell adhesion, the ibiTreat surface can be coated with proteins like non-patterned labware. In general, compared to non-patterned labware, coating on patterned surface requires a significant lower protein concentration. For coating optimization follow the protocol in the instructions.

1.4 Cell Seeding Concentration

The seeding concentration is an additional crucial parameter for optimizing the adhesion process. Low concentrations lead to fewer cells on the pattern or only sparsely occupied spots but less aggregation. High cell concentrations can improve cell coverage but bear the risk of a) aggregation and b) of multiple cells on single-cell spots. Moreover, 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.

In general, compared to non-patterned labware, seeding cells on patterned surface requires a significant lower cell number.

1.5 Cell Suspension Quality

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

1.6 Incubation Time

The incubation time after seeding the cells influences the adhesion process. The time required for cells to adhere to µ-Patterned surfaces may differ from that on conventional substrates. Handle µ-Patterned slides with extra care during this phase, as cells that are not fully attached—or only loosely attached—can easily detach or start to aggregate.

1.7 Mechanical Stress and Washing

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

1.8 Cell Viability

Cell viability 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 cell viability.

2 Optimizing Cell Adhesion on µ-Patterns

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

If you don't know if your cell type is compatible with the µ-Pattern, we recommend running an initial cell adhesion test on a multi-cell µ-Pattern first—even though your final experiment will be on the single-cell level. By this, only the adhesion compatibility of your cell type to the patterns will be tested, while excluding single cell and pattern geometry effects.

- Decide if your application needs unspecific adhesion (=ibiTreat surface without coating) or specific adhesion (=ibiTreat surface with coating).
- Use 2–3 different cell seeding concentrations following the protocol in the instructions. Incubate the cells at least 4 hours up to overnight and avoid disturbance by e.g., moving.
- Control the cell attachment with using a phase contrast microscope before the washing steps to remove unattached cells.

  Note: Saving cell images during the test experiment can help to optimize the process.

- Capture additional images at least 2 hours after washing to give the cells sufficient time to recover from the shear stress induced during the washing process.

Note: At this stage, optimal pattern coverage is not necessary. It is just important that a sufficient number of cells adhere 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, a different coating protocol might be necessary.

2.2 Optimization of Seeding Parameters

Please consider that every pattern needs an optimized protocol 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 suspended homogeneously.

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 hours without disturbance before washing.
- Take images of the cells before washing and again at least 2 hours after washing.

Possible Results and Troubleshooting

- After washing, the cells are positioned 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 hours and 3 hours after seeding to see if a shorter incubation time leads to less aggregation and, therefore, better pattern coverage.
- After washing, there is an undesired cell aggregation on the pattern.
  → This indicates too high initial seeding concentration and/or too long incubation time before washing. Try a lower seeding concentration and check the cells already after 2 hours and 3 hours 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 round and do not spread when using small single cell spots or thin lines.
  → If you know from previous experiments that the used cell type adhere to the patterns, the pattern dimensions might be too small, 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.

3 Example Images

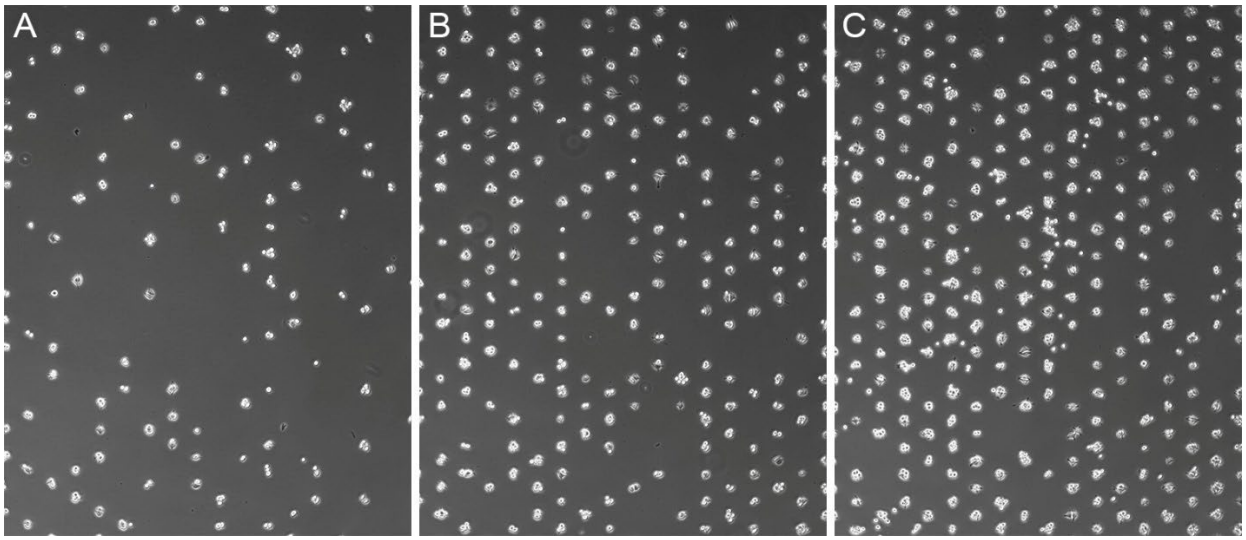


Figure 1 Influence and optimization of different seeding concentrations of L929 cells on a single cell pattern. Cells were seeded into a µ-Slide VI $^{0.4}$ at concentrations of (A) 1 × 10^5 cells/ml, (B) 3 × 10^5 cells/ml, (C) 5.25 × 10^5 cells/ml. After 24 hours, unattached cells were washed away,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