2D Whole Protein Pattern Based on a PLL-PEG-Passivated Coverslip Surface Using the ibidi Micro Illumination System

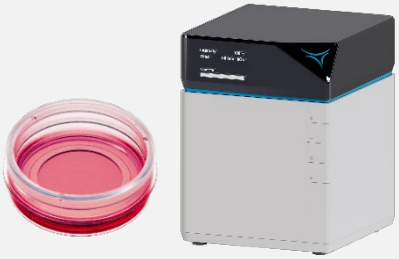
The ibidi Micro Illumination System enables the creation of spatially defined 2D cell-adhesive structures on the ibiTreat (tissue culture-treated) surface of the ibidi Polymer Coverslip bottom. The surface is first entirely passivated and subsequently treated with a photoinitiator. Localized UV illumination through a photomask—which defines the desired pattern—selectively removes the passivation only in the illuminated areas, thereby exposing the underlying ibiTreat surface on the polymer coverslip. These exposed areas can then be coated with extracellular matrix (ECM) proteins, such as fibronectin or laminin, to enhance local cell adhesion.

The applied photomask determines the size, shape, and spatial arrangement of the adhesive patterns, enabling the design of a wide range of custom experimental layouts. These patterned surfaces are suitable for numerous applications, including cell migration studies, single-cell gene expression analysis, and wound healing assays.

In this Application Note, we present a specific example using the µ-Dish $^{35\ mm,\ high}$ ibiTreat, two pattern shapes (circular areas and lines), and MDA-MB-231 cells.

ibidi Solutions for Passivation and Protein Pattern on the ibiTreat Surface

- ibidi Micro Illumination System
- µ-Dish $^{35\ mm,\ high}$ ibiTreat



Related Documents

- Instructions ibidi Micro Illumination System (PDF)
- Instructions µ-Dish $^{35\ mm,\ high}$ (PDF)
- Application Note 72: RGD Micropatterning Using the ibidi Micro Illumination System for Spheroid Generation and Cultivation (PDF)
- Application Note 74: 3D Hydrogel Constriction in the µ-Slide I Luer Using the ibidi Micro Illumination System (PDF)
- Application Note 75: Structuring a Photoresist-Coated Wafer With Photolithography Using the ibidi Micro Illumination System (PDF)
- Application Note 76: Photo-Induced Cell Migration Using the ibidi Micro Illumination System (PDF)
- Photomask Templates (ZIP)

1 Material

1.1 Reagents and Buffers

- Breast carcinoma cells (MDA-MB-231, ACC 732, DSMZ)
- Poly-L-Lysine (PLL) (P8920, Sigma-Aldrich)
- mPEG-succinimidyl valerate, MW 5000 (mPEG-SVA) (MPEG-SVA-5000-1GR, Laysan Bio)
- HEPES, pH 8.3–8.5 (9105.2, Carl Roth)
- [(4-benzoylphenyl)methyl]trimethylazanium bromide (benzophenone) (ENAH97A17DBF, Sigma-Aldrich)
- Ethanol 90% (1.00971, Sigma-Aldrich)
- PBS (14040-091, Gibco)
- Tween 20 (P9416-50ml, Sigma-Aldrich)
- Fibronectin (Far-red HiLyte Fluor 647 labeled, NR04, Cytoskeleton Inc.)
- Sterile, ultrapure water (e.g., Milli-Q water)

1.2 Equipment

- ibidi Micro Illumination System (76000, ibidi)
- 3” photomask (Compugraphics, MacDermid Alpha)
- µ-Dish $^{35\ mm,\ high}$ ibiTreat (81156, ibidi)
- Standard cell culture equipment (pipettes, tubes, sterile working bench, cell culture incubator, culture flasks, hemocytometer, etc.)
- Inverted microscope

2 Micropatterning of µ-Dishes

Please read the Instructions before working with the µ-Dish 35 mm, high ibiTreat. Perform all steps under sterile conditions.

2.1 Passivation of µ-Dishes

Important Notes

Preparation of PEG-SVA solution: Due to the 10-minute half-life of the SVA ester at pH 8.5, the PEG-SVA solution must be prepared immediately prior to use to ensure optimal reactivity.

Storage of passivated dishes: Passivated dishes should be stored protected from light to preserve surface integrity until they are needed for further applications.

1. Add 200 µl of PLL with a final concentration at 100 µg/ml (diluted in Milli-Q water (1:10)), to each dish.
2. Incubate for 30 minutes at room temperature.
3. Rinse the dish three times with 5 ml 0.1 M HEPES (1.0 M stock solution diluted with Milli-Q water), ensuring the pH is between 8.3 and 8.5.
4. Prepare a PEG-SVA solution in 0.1 M HEPES, pH 8.3–8.5 at 100 mg/ml.
5. Remove the HEPES solution and immediately fill the dish with 200–250 µl of the freshly prepared PEG-SVA solution, ensuring the surface does not dry during the process.
6. Incubate for 1 hour at room temperature.
7. Wash the dish thoroughly at least 10 times with 5 ml of Milli-Q water. Adequate washing is indicated by rapid dewetting of the surface.
8. Dry the surface using compressed air or allow the dish to stand for up to 30 minutes. Passivated dishes can be stored for hours or even several days.

Note: If the passivation layer appears inconsistent or non-uniform by eye, the dishes may be subjected to oxygen plasma treatment (20 % concentration, 1.5-minute duration) prior to further use.

2.2 Photoinitiation and Photopatterning

Additional Information for the Mixture of Benzophenone, Ethanol and Tween 20

The final solution should be applied at approximately 20 µl/cm². Ethanol and Tween 20 are included to enhance the spreading of the solution. A typical starting ratio of 1 µl benzophenone solution, 1 µl of Tween 20 (0.5 %) and 5 µl of ethanol provides effective coverage. However, both the ratio and total volume may require optimization depending on the specific application or surface area.

1. Prepare a solution of benzophenone in Milli-Q water at a concentration of 400 mg/ml.
2. Mix 10 µl of the benzophenone solution with 50 µl of 90 % ethanol and 10 µl of 0.5 % Tween 20 (diluted in Milli-Q water).
3. Add 70 µl of the benzophenone/ethanol/Tween 20 mixture to the dish, ensuring complete coverage of the surface.
4. Allow the mixture to dry for approximately 20 minutes under the hood.
5. Position the dish on the photomask containing the desired structures and place it into the ibidi Micro Illumination System. Follow the Instructions of the ibidi Micro Illumination System.
6. Illuminate for 1 minute at 100 % intensity. Make sure to avoid long incubation periods and use the patterned surface immediately, without any storage.

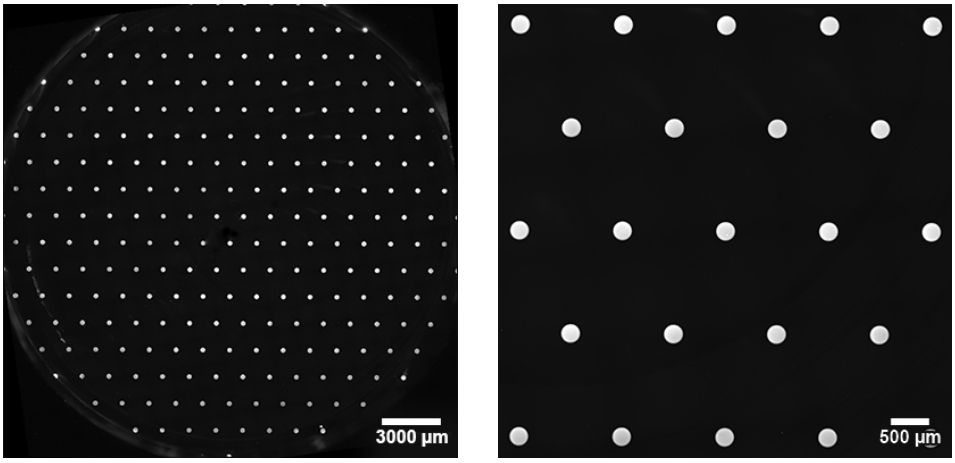
2.3 Protein Coating

Additional Information for the ECM Protein Incubation

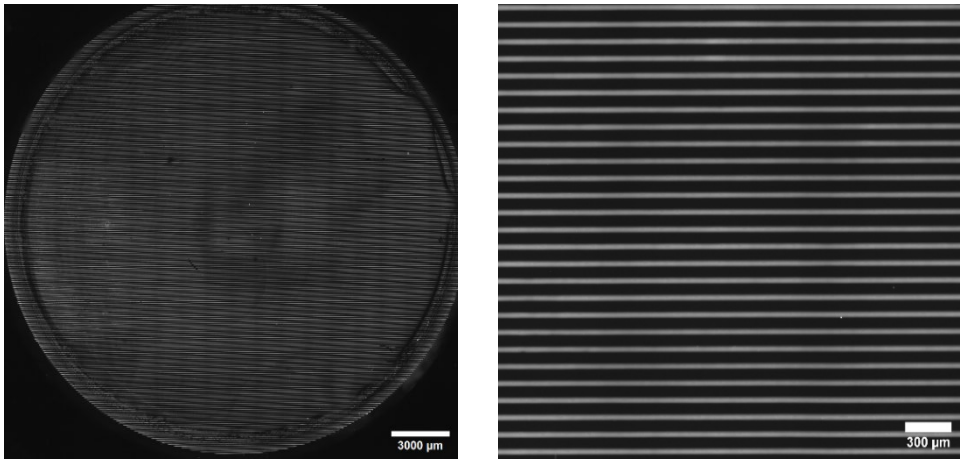
Incubation times longer than 15 minutes result in increased protein concentrations on the patterns but also in higher background levels.

The use of fluorescently labeled fibronectin is optional but highly recommended, as it enables verification of successful patterning prior to cell seeding and adhesion.

1. Rinse the dish three times with 5 ml Milli-Q water.
2. Rehydrate the substrate with PBS for 5 minutes at room temperature.
3. Prepare a 20 µg/ml solution of fluorescently labeled fibronectin (HiLyte 647).
4. Remove the PBS from the dish and immediately add 300 µl of the prepared fibronectin solution.
5. Incubate for 15 minutes at room temperature, protected from light.
6. Rinse the dish three times with 5 ml PBS, ensuring some PBS remains to prevent drying.
7. Examine the resulting pattern using a fluorescence microscope.



Patterning of an entire µ-Dish 35 mm, high ibiTreat was performed using a photomask containing circles with a diameter of 200 µm and a pitch of 1500 µm. The patterning was visualized using fluorescently labeled fibronectin (HiLyte 647).



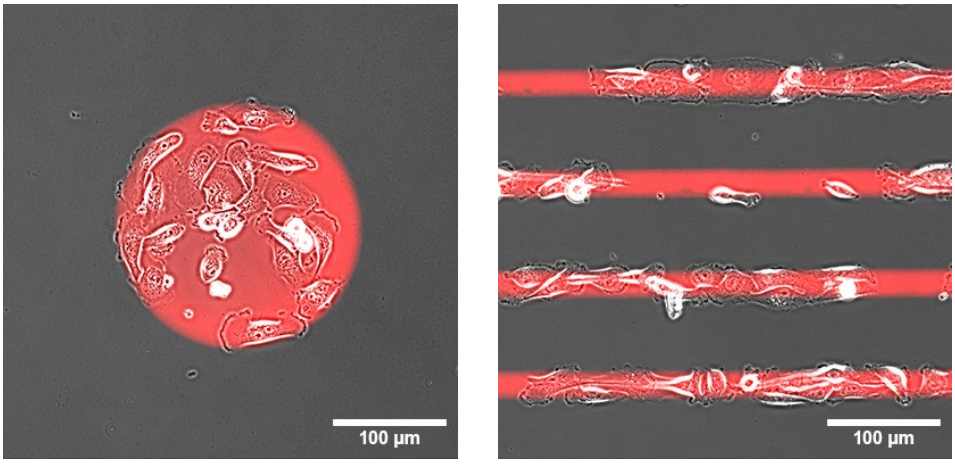
Patterning of an entire µ-Dish 35 mm, high ibiTreat was performed using a photomask containing lines with a width of 8 µm and a pitch of 100 µm. The patterning was visualized using fluorescently labeled fibronectin (HiLyte 647).

3 Cell Seeding and Cultivation

Perform all steps under sterile conditions. Before starting the experiment, prepare the MDA-MB-231 cells in a standard cell culture flask with the cells adherent at the bottom. The cells should be healthy and optimally subconfluent on the day of the experiment. If not stated otherwise, all incubation steps are at room temperature.

Please note that the required cell suspension concentration may vary depending on the experiment and the cell line used.

1. Prepare the MDA-MB-231 cell suspension at a concentration of 3×10^5 cells/ml in cell culture medium.
2. Add 100 µl of the cell suspension to the micropatterned dish.
3. Fill up with cell growth medium.
4. Allow the cells to adhere for a minimum of 1 hour before starting the imaging.



MDA-MB-231 cells seeded onto the patterned substrates demonstrated cell adhesion to the defined geometries within 2 hours post-seeding. Overlay of phase contrast microscopy of the label-free cells and fluorescence microscopy of the patterning with fluorescently labeled fibronectin (HiLyte 647).