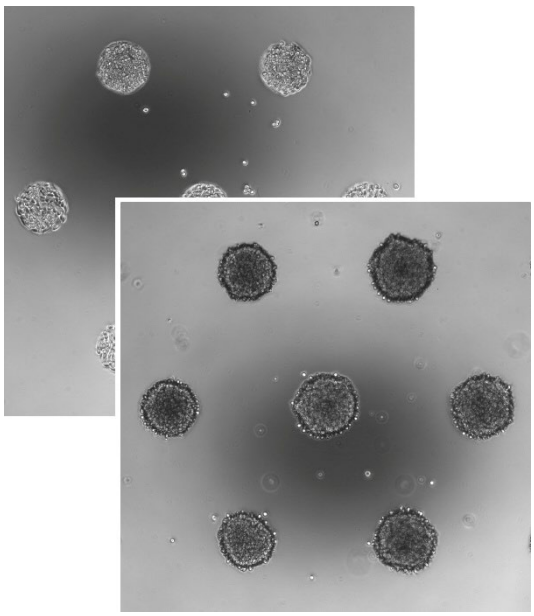
Formation and Long-Term Cultivation of Spheroids in the µ-Slide 8 Well high µ-Pattern ibiTreat

This Application Note outlines a step-by-step protocol on how to create spheroids using the µ-Slide 8 Well high µ-Pattern ibiTreat, cir200, pit600, hex. In this example, the adhesive micropatterns were coated with laminin or Matrigel to support spheroid formation. L929 cells were cultivated on the patterns for multiple weeks, enabling long-term observation and analysis of 3D cell growth.



ibidi Solutions for Micropatterning

- µ-Pattern ibiTreat for Single-Cell Arrays
- µ-Pattern ibiTreat for Multi-Cell Arrays
- µ-Pattern ibiTreat for Line Arrays
- Custom µ-Pattern ibiTreat

Related Documents

- AN 65: Cell Adhesion on ibidi µ-Patterns: Parameters and Optimization (PDF)
- AN 78: Cell Culture and Immunofluorescence Staining in the µ-Slide VI 0.4 µ-Pattern ibiTreat (PDF)
- AN 79: Selective and Localized Cell Adhesion using a CD19-Coated Custom µ-Pattern ibiTreat (PDF)
- Video: The ibidi µ-Patterning Technology—Achieve Spatially Defined Cell Adhesion with Micropatterning
- Rüdiger, D., *et al.*: Selektive und ortsaufgelöste Zellanbindung mit µ-Pattern ibiTreat, 2023, BIOspektrum, 4, 391

1 Materials

1.1 Reagents and Buffers

- L929-Cells (DSMZ, ACC 2)
- Laminin (Biolamina, LN 521N)
- Matrigel (Corning, 354008)
- Sterile PBS (Gibco, 14040-091)
- DMEM (Gibco, 41965047) with 10 % Fetal Calf Serum (Life Technologies, A5256701)

1.2 Equipment

- µ-Slide 8 Well high µ-Pattern ibiTreat cir200, pit600, hex (ibidi, 83812)

2 Coating Procedure

Before seeding the cells, the adhesive spots of the µ-Slide 8 Well high µ-Pattern ibiTreat cir200, pit600, hex can be coated with matrix proteins to improve cell adhesion.

- For coating a µ-Slide 8 Well high, a volume of 150 µl per well is needed of
  - 10 µg/ml laminin in PBS
  - Matrigel diluted 1:40 in PBS at 4 °C
- After adding the coating solution to the wells, the slides are incubated for 2 hours at 37°C.
- Wash the wells and channels three times with 1× PBS.

Important Note: The concentration of the coating proteins can be adjusted to the used cell type. Prolonged coating times or a very high concentration, however, can lead to unspecific cell adhesion. Furthermore, the use of acetic acid to dilute the proteins will lead to unspecific adhesion. Please use hydrochloric acid instead.

3 Cell Seeding and Long-Term Cultivation

Important Note: Before seeding the cells, please read the Instructions of the µ-Slide 8 Well high µ-Pattern ibiTreat cir200, pit600, hex. Perform all steps under sterile conditions. It is recommended that the slides and cell culture medium are placed in the incubator one day before seeding the cells to avoid the formation of air bubbles during handling. It is essential to work swiftly during the whole procedure to prevent the cells from drying.

A mouse fibroblast cell line (L929) was used to generate spheroids on the adhesion spots.

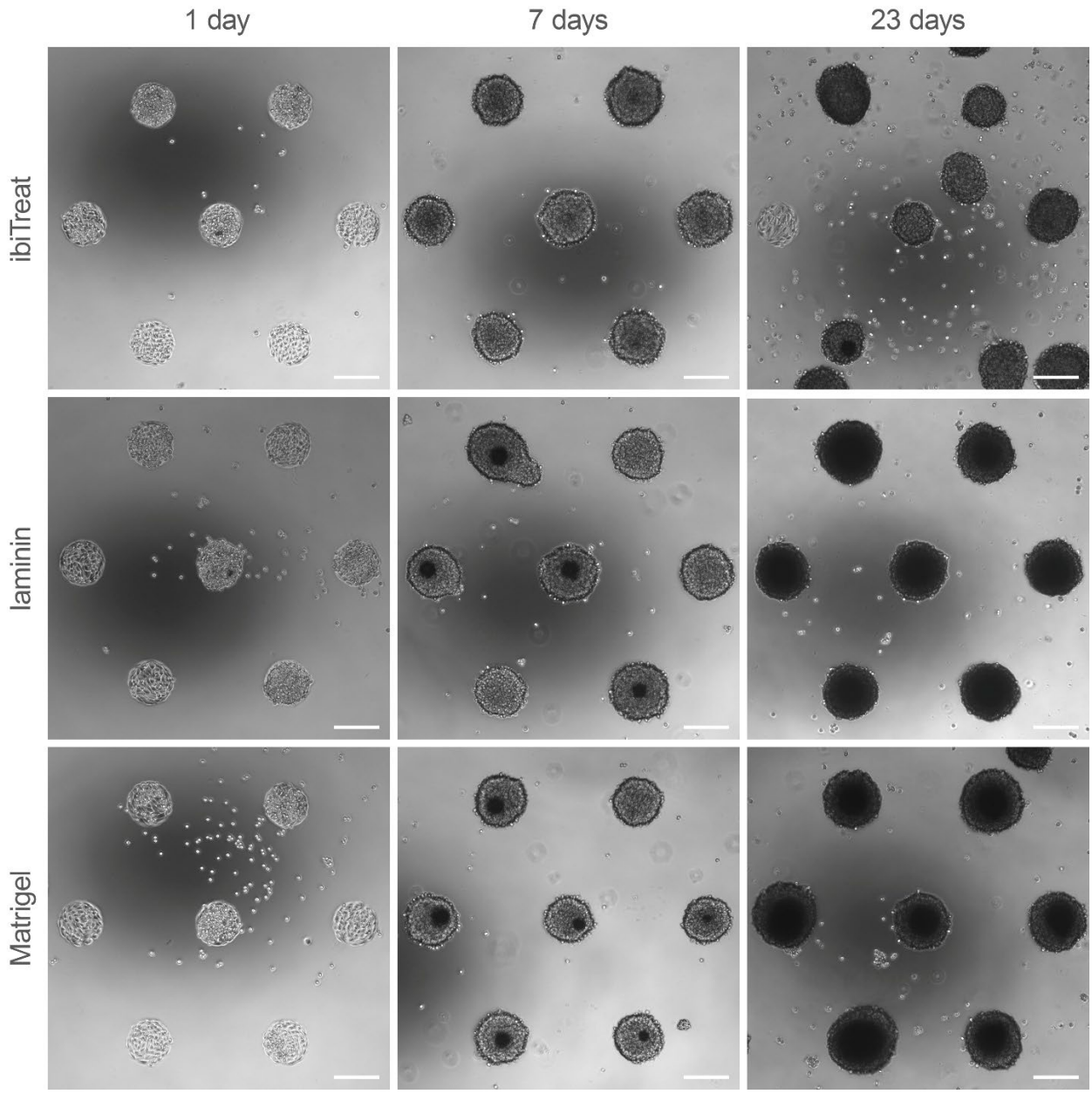
- To seed cells, completely remove the PBS from the wells. Work fast to avoid drying of the surface to prevent damage to the coating proteins.
- Pipet 300 µl of a cell suspension with a concentration of 0.5×10^5 cells/ml into each well.
- Incubate overnight at 37°C.
- Wash 2–3 times with fresh cell media.

- Change the cell media every 2–3 days.

As the spheroids grow larger, the risk of detaching them from their adhesion sites during washing increases. To prevent this, avoid pipetting directly into the center of the wells.

4 Microscopy

The cells were imaged using an EVOS microscope with a 4x objective in the phase contrast mode.



L929 cells growing in the µ-Slide 8 Well ^{high} µ-Pattern $^{ibiTreat, cir200, pit600, hex}$. ibiTreat surface without any additional coating (top) or coated with laminin (middle) or Matrigel (bottom). The cells were cultivated for up to 23 days, and images were taken after 1, 7, and 23 days. Scale bars: 200 µm.