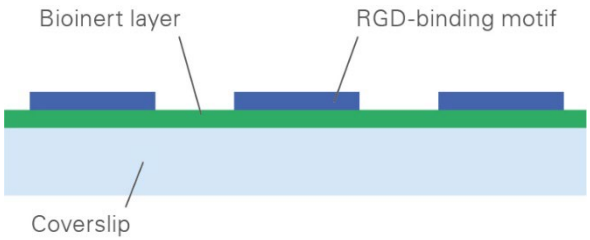
RGD Micropatterning Using the ibidi Micro Illumination System for Spheroid Generation and Cultivation

The ibidi Micro Illumination System can be used for the generation of RGD micropatterns on our Bioinert labware surface, which enables spatially defined cell adhesion. RGD peptides are recognized by integrin receptors on the cell surface, mediating cell—ECM interactions that regulate key processes such as adhesion, spreading, and migration.

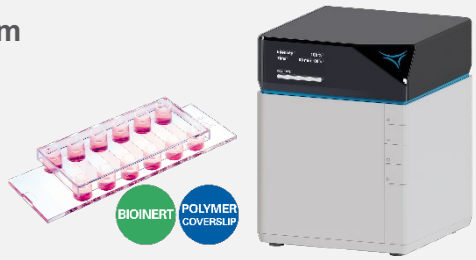
RGD pattern generation involves a two-step functionalization of the Bioinert surface. First, a diazirine linker is attached to the Bioinert surface via UV illumination using the ibidi Micro Illumination System. Next, the RGD ligand is bound to the diazirine linker through click chemistry. The final size and arrangement of the pattern are determined by the photomask. Using this approach, numerous spatially defined functionalized areas can be achieved.

Microstructured surfaces are well suited for a variety of applications, including reproducible large-scale production of spheroids. In this Application Note, we show an example in which a multi-cell RGD pattern is applied to a channel µ-Slide (µ-Slide VI 0.4 Bioinert), enabling spheroid formation of NIH-3T3 fibroblasts under long-term perfusion using the ibidi Pump System.



ibidi Solution for RGD Micropatterning and Long-Term Spheroid Perfusion

- ibidi Micro Illumination System
- µ-Slide VI 0.4 Bioinert
- ibidi Pump System



Related Documents

- Instructions ibidi Micro Illumination System (PDF)
- Instructions µ-Slide VI 0.4 Bioinert (PDF)
- Instructions ibidi Pump System (PDF)
- Application Note 03: Cell Culture in ibidi Channel Slides Using the µ-Slide VI 0.4 as an Example (PDF)
- Application Note 13: Endothelial Cell Culture Under Perfusion With the ibidi Pump System and the µ-Slide I 0.6 Luer (PDF)
- Application Note 31: Serial Connection of µ-Slide VI 0.4 Channels for Flow Experiments (PDF)
- Application Note 73: 2D Whole Protein Pattern Based on a PLL-PEG-Passivated Coverslip Surface Using the ibidi Micro Illumination System (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 Template (ZIP)

1 Material

1.1 Reagents and Buffers

- Mouse embryo fibroblasts (NIH-3T3, ACC 59, DSMZ)
- DMEM with L-glutamine (41965039, Gibco) + 10% FCS (10270106, Gibco)
- Diazirine-alkyne (EN300-7462042, Enamine)
- Cyclo[Arg-Gly-Asp-D-Phe-Lys(Azide)] (cRGD-azide) (17293766, Fisher Scientific)
- BTTAA (CLK067-100, Jena Bioscience)
- $CuSO_4$ (CLK-MI004-50, Jena Bioscience)
- Vit C (CLK-MI005-1G, Jena Bioscience)
- PBS (14190144, Gibco)
- Na_2HPO_4
- NaH_2PO_4
- Sterile, ultrapure water

1.2 Equipment

- ibidi Micro Illumination System (76000, ibidi)
- 3” Photomask containing 200 µm circles with 600 µm pitch, hexagonal layout. (compugraphics, MacDermid Alpha)
- µ-Slide VI $^{0.4}$ Bioinert (80600, ibidi)
- ibidi Pump System (10902, ibidi)
- Perfusion Set YELLOW-GREEN, 50 cm, ID 1.6 mm, 10 ml Reservoir (10964, ibidi)
- Serial Connector for µ-Slides (10830, ibidi)
- Hose Clip (10821**,** part of the ibidi Pump System)
- Sterile 1 ml syringe with simple Luer adapter, biocompatible (various suppliers)
- Standard cell culture equipment (pipettes, tubes, sterile working bench, cell culture incubator, culture flasks, hemocytometer, etc.)
- Inverted microscope

2 RGD Micropattern Generation

2.1 Preparation of Stock Solutions

1. Right before the experiment, freshly dilute all reagents under sterile conditions in the respective solvent to a final stock solution concentration as shown in Table 1. Vortex until the reagents are completely dissolved.

   Table 1: Preparation of the stock solutions for the different reagents.

Reagent	Solvent	Stock solution concentration [mM]
Diazirine-alkyne	Ultrapure water	5
$CuSO_4$	Ultrapure water	100
BTTAA	Ultrapure water	50
Cyclo RGD-azide	PBS	2
Vit C	Ultrapure water	1000

2. Prepare a 0.1 M Sodium phosphate buffer at pH 7.

   For example, mix 28.9 ml of 1 M Na_2HPO_4 with 21.2 ml of 1 M NaH_2PO_4 and fill up to a final volume of 500 ml with ultrapure water.

2.2 Illumination

For the pattern generation, use either the demo photomask provided with the ibidi Micro Illumination System, or your customized photomask with your desired pattern. For this Application Note, please download the photomask template here.

1. Dilute diazirine-alkyne to **3 mM** with ultrapure water.
2. Fill each channel of the µ-Slide VI $^{0.4}$ Bioinert with 30 µl of the 3 mM diazirine-alkyne solution.
3. Put the µ-Slide VI $^{0.4}$ Bioinert into the ibidi Micro Illumination System and follow the ibidi Micro Illumination System Instructions.
4. Illuminate the slide for **1 minute** with **100% intensity.**
5. After illumination, immediately wash the channels 4 times with 120 µl ultrapure water per channel.
6. Dry the channels with compressed air.

2.3 Click Functionalization

Important Note

The click functionalization does not have to be performed on the day of illumination. However, for optimal results, we recommend carrying out the click functionalization within **24 hours** after illumination.

1. Mix the stock solutions listed in Table 1 according to the order and final concentrations given in Table 2.

   Please Note: Fill the channels with the solutions immediately after adding the Vitamin C.

Table 2: Pipetting scheme for the solution for the click functionalization. All reagents are listed in the order of pipetting.

Reagent	Resulting final concentration [mM]	Volume [µl]
100 mM $CuSO_4$	2	4
50 mM BTTAA	10	40
0.1 M Sodium phosphate buffer, pH 7	-	116
2 mM Cyclo RGD-azide	0.2	20
1000 mM Vit C	100	20
		Total volume: 200

2. Fill each channel with 30 µl of the prepared solution for the click functionalization.
3. Incubate for 1 hour at room temperature in the dark.
4. Wash each channel 4 times with 120 µl PBS.
5. Incubate the slide filled with PBS overnight at room temperature in the dark.
6. The next day, wash each channel again 4 times with 120 µl PBS and proceed with cell seeding.

3 Cell Seeding and Cultivation

3.1 Cell Seeding and Static Cultivation

1. Prepare the NIH-3T3 cell suspension with a concentration of 2 × 10^6 cells/ml in cell culture medium.
2. Before seeding the cells into the functionalized µ-Slide VI $^{0.4}$ Bioinert, aspirate the PBS completely from the channels.
3. Fill each channel with 30 µl of the NIH-3T3 cell suspension.
4. To avoid drying of the channels, carefully fill the reservoirs with cell culture medium. Make sure that the cells are not washed out of the channel.
5. Change the cell culture medium in the channels every 2–3 days.

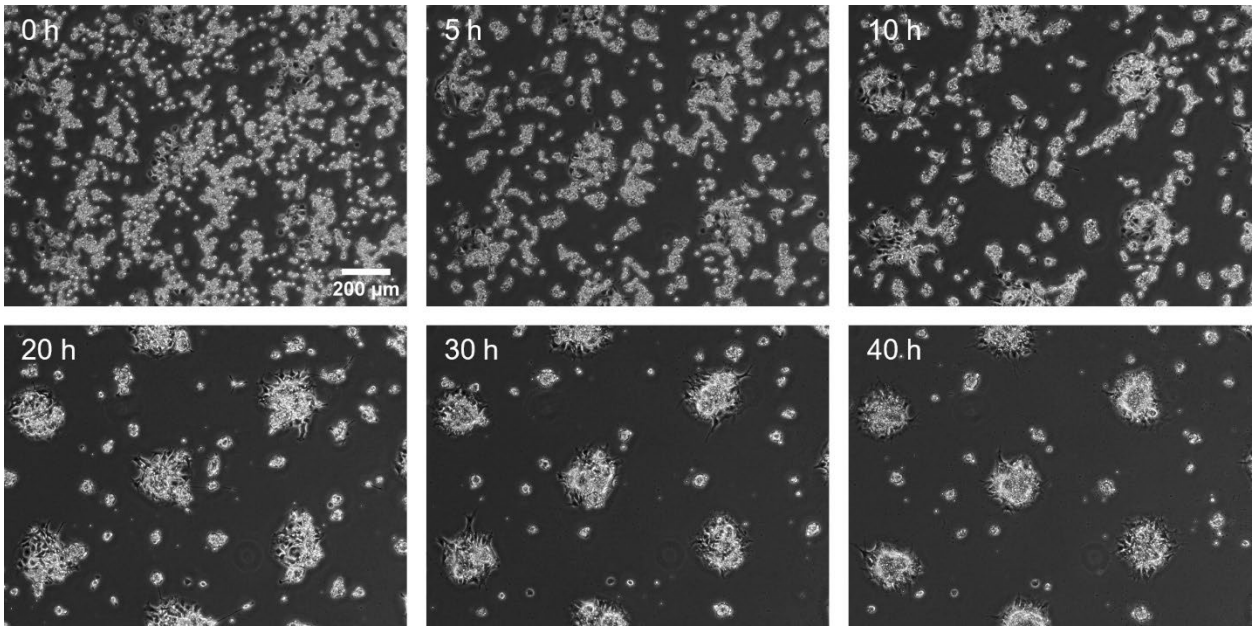


Figure 1: NIH-3T3 cells were seeded on a 200 µm RGD pattern in the channels of the µ-Slide VI $^{0.4}$ Bioinert. Over a period of 40 hours, the cells accumulate on the pattern and form spheroids.

3.2 Perfusion of the Spheroids for Automated Long-Term Cultivation

After 10 days of static cultivation and manual medium change, the µ-Slide VI $^{0.4}$ Bioinert is connected to the ibidi Pump System for automated long-term perfusion.

1. Prepare the ibidi Pump System as described in the ibidi Pump System Instructions.
2. Fill the cell culture medium into the reservoirs.
3. Connect the µ-Slide VI $^{0.4}$ Bioinert to the Perfusion Set. Detailed step-by-step instructions to connect the slide to the perfusion system are described in Application Note 13: Endothelial Cell Culture Under Perfusion With the ibidi Pump System and the µ-Slide I $^{0.6}$ Luer. A serial connection of µ-Slide VI $^{0.4}$ channels is described in Application Note 31: Serial Connection of µ-Slide VI $^{0.4}$ Channels for Flow Experiments.
4. Use a similar stepwise increase of the shear stress, as described in Application Note 13. The perfusion is started at 2 dyn/cm^2 and increased to 3 dyn/cm^2 after 4 days.

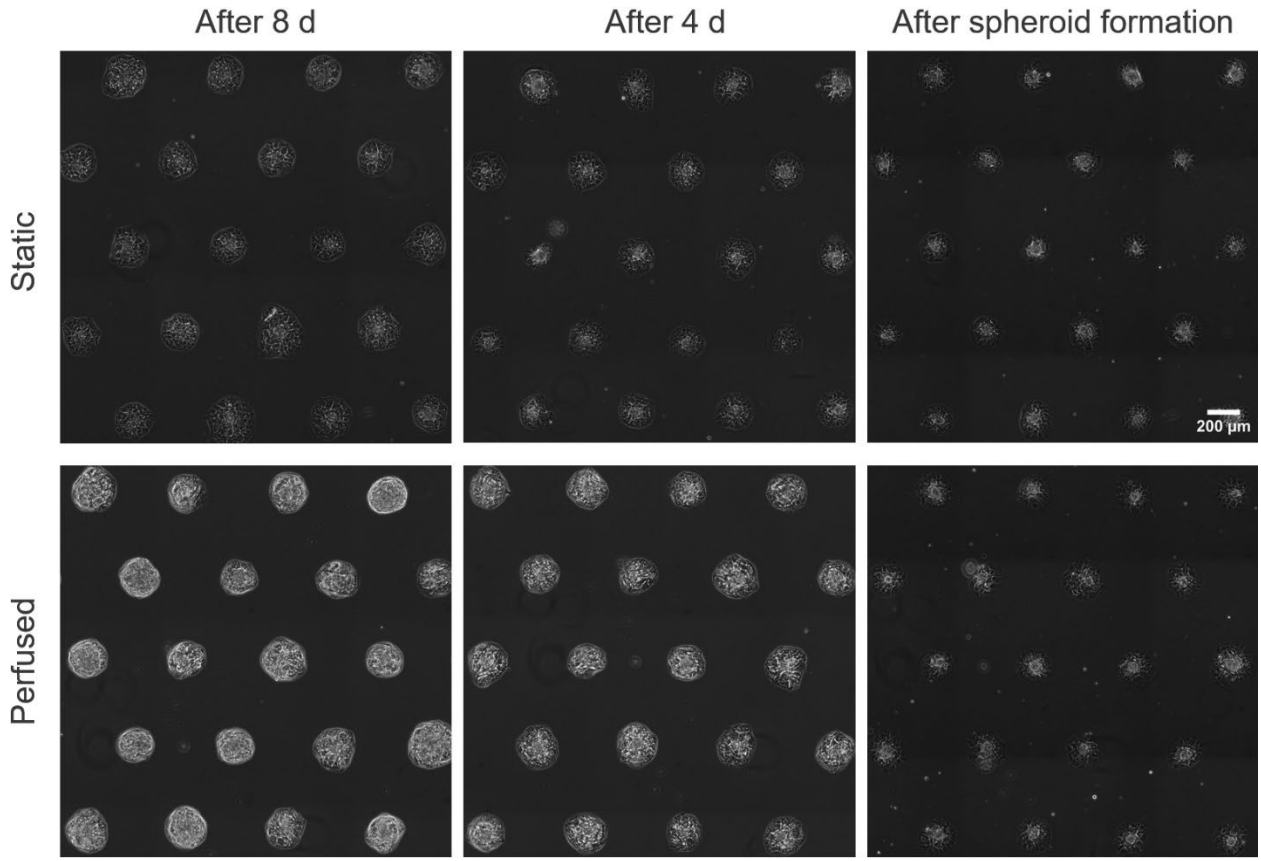


Figure 2: Comparison of static and perfused cultivation of NIH-3T3 spheroids. Spheroids cultured under perfusion show faster growth over time and a higher packing density than those cultured under static conditions.