Generation and Dynamic Culture of L929 Spheroids in the µ-Slide Spheroid Perfusion

This Application Note is an example protocol for creating multicellular spheroids in the µ-Slide Spheroid Perfusion with subsequent flow application. After formation of spheroids from a suspension of the murine fibroblast cell line L929, perfusion is applied with the ibidi Pump System. This ensures the optimal nutrition of spheroids during long-term cultivation.

Related Documents:

- Instructions µ-Slide Spheroid Perfusion
- Instructions ibidi Pump System

Keywords:

Spheroids, organoids, 3D aggregates, L929 cells, long-term culture, Bioinert, passivation, flow, perfusion, pump, microscopy

Material:

- µ-Slide Spheroid Perfusion Bioinert (80350, ibidi, Germany)
- Murine fibroblast cell line L929 (ACC 2, DSMZ, Germany)
- Cell culture medium (RPMI-1640 (21875034) with FCS (10270106), Gibco)
- Accutase (A1110501, Gibco)
- ibidi Pump System (10902, ibidi, Germany)
- Perfusion Set BLUE, 15 cm, ID 0.8 mm (10961, ibidi, Germany)
- Standard cell culture equipment (sterile working bench, cell culture incubator, culture flasks, PBS, etc.)

Important Note: Equilibrate all required materials, such as µ-Slides, culture medium, and tubing (Perfusion Sets), **overnight** inside the incubator at 37°C and 5% CO_2. This is essential for keeping air bubbles from emerging over time.

1. Cell Preparation & Seeding

- Cultivate L929 cells in culture medium.
- Treat the cells with Accutase for 1–2 minutes for detachment.
- Harvest the cell suspension.
- Centrifuge the cell suspension and dilute it in culture medium; the amount depends on the required cell concentration.
- Count the cells and adjust to a concentration of 5 x 10^5 cells/ml.
- Prepare the µ-Slide according to the instructions.
- Seed the cells into the channel of the µ-Slide. Do not forget to seed cells for a static control.
- Incubate over night at 37°C and 5% CO_2 for spheroid formation.

2. Spheroid Formation

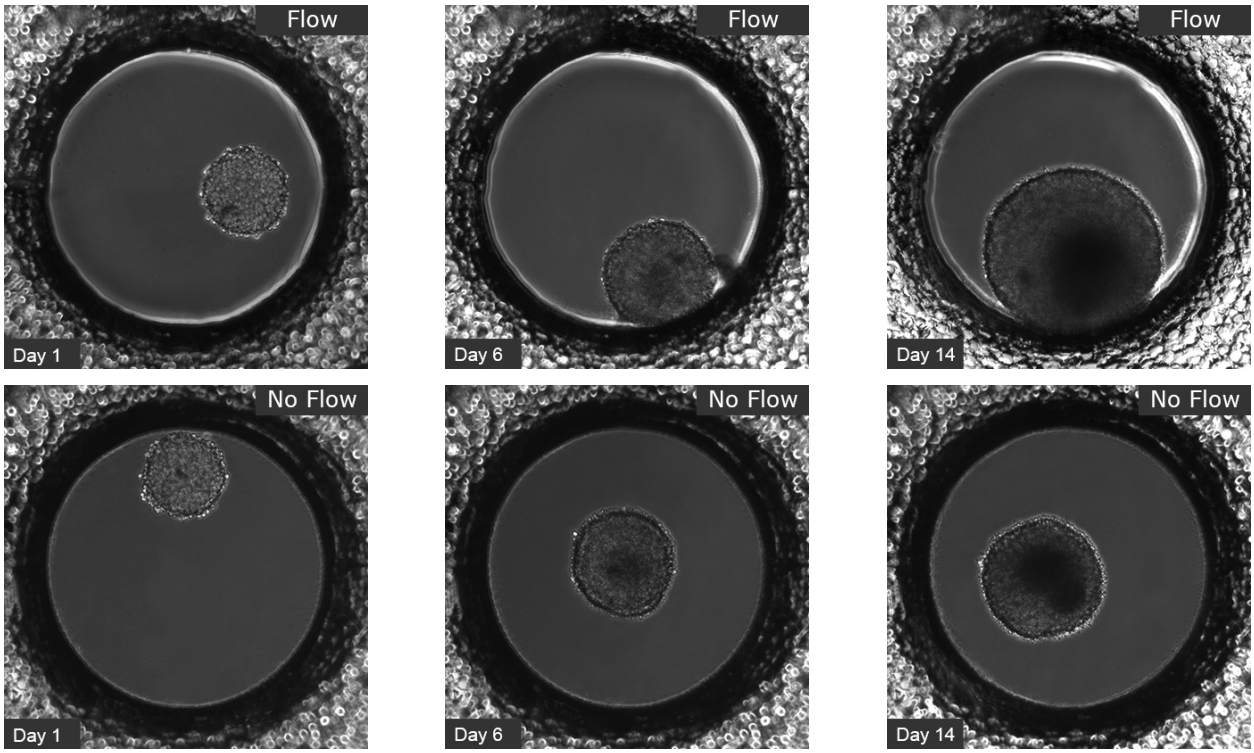
- Control the spheroid formation under the phase contrast microscope.
- Optional: after the spheroids have formed, wash 1x with fresh culture medium.
- Incubate for one hour at 37°C and 5% CO_2 before starting the perfusion.

3. Perfusion Experiment

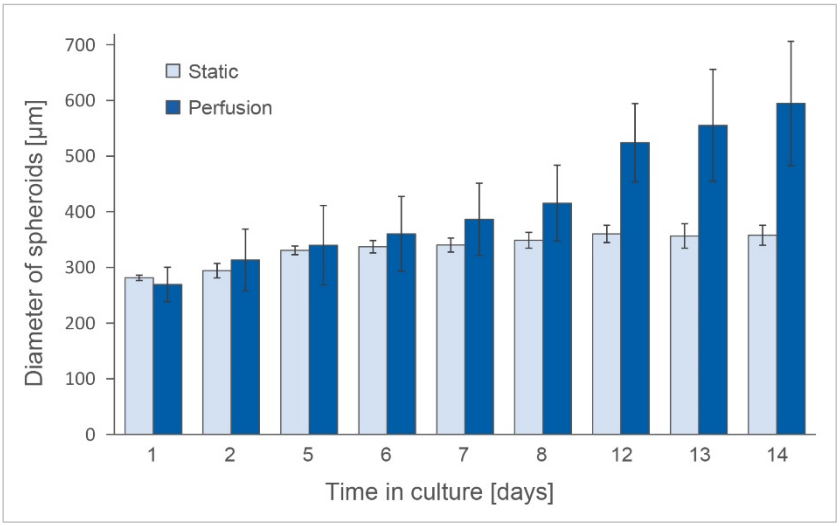
- Prepare the µ-Slide, the ibidi Pump System, and the Perfusion Set for the flow connection according to the instructions.
- To remove air bubbles from the system, let it run 1–2 hours before connecting the µ-Slide.
- Connect the µ-Slide to the tubing and the pump system.
- Start the perfusion experiment with 5 mbar resulting in a flow rate of 0.75 ml/min.
- Determine the flow rate. If necessary, adjust the pressure to create the desired flow rate.
- For the static control, perform a medium exchange every two days according to the instructions of the µ-Slide Spheroid Perfusion.

4. Results

Spheroid formation starts directly after seeding. During long-term culture, the application of flow leads to stronger proliferation of spheroids, resulting in an increase of the spheroid diameter due to optimal nutrition.



L929 fibroblasts show spheroid formation in the µ-Slide Spheroid Perfusion, Bioinert, days 1–14, seeding concentration 5 x 10^5 single cells/ml. Top: perfusion with the ibidi Pump System, 0.75 ml/min. Bottom: no perfusion, medium exchange every second day. Phase contrast microscopy, 10x objective lens, well diameter 800 µm.



Comparison of spheroid formation of L929 fibroblasts in the µ-Slide Spheroid Perfusion, Bioinert, seeding concentration 5 x 10^5 single cells/ml. Static: no perfusion, medium exchange every second day. Perfusion: perfusion with the ibidi Pump System, 0.75 ml/min.