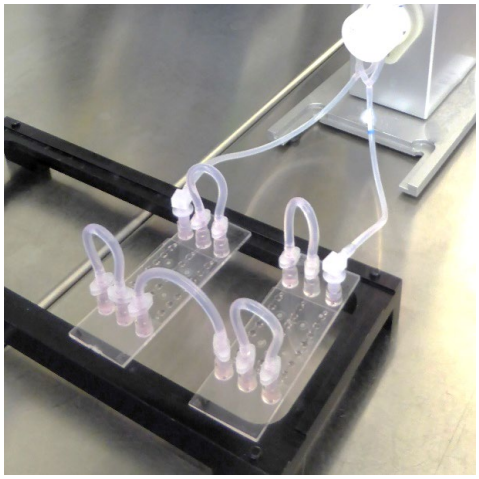
Serial Connection of µ-Slide Spheroid Perfusion Channels for Long-Term Cultivation of 3D Spheroids Under Flow



This application note provides a step-by-step protocol for creating a serial connection between two µ-Slide Spheroid Perfusion and their channels. The protocol only specifies the procedure for two slides but can be applied to a larger number of slides correspondingly.

The ibidi Pump System is specially designed for long-term experiments of cells under perfusion. The standard setup connects one slide to one perfusion set. In the case of the µ-Slide Spheroid Perfusion, the three individual channels and even multiple slides can be connected using the ibidi Serial Connectors for µ-Slides to cultivate several spheroids in separate chambers under perfusion. The spheroids in the chambers of the µ-Slide Spheroid Perfusion are not exposed to shear stress due to the well design. However, the unidirectional, laminar flow of medium through the channels ensures continuous supply of nutrients and stable conditions over long cultivation periods (up to several weeks). Compared to a non-perfused cultivation, where the medium in the channels needs to be regularly exchanged by pipetting, the application of continuous flow promotes the proliferation of spheroids, resulting in an increase of the spheroid diameter due to optimal nutrition over time.

ibidi offers various solutions for spheroid and organoid formation with perfusion:

- µ-Slide Spheroid Perfusion
- µ-Slide I Luer 3D
- µ-Slide III 3D Perfusion
- µ-Slide With Multi-Cell µ-Pattern ibiTreat
- ibidi Pump Systems and Accessories

Related Documents:

- Instructions µ-Slide Spheroid Perfusion (PDF)
- Instructions ibidi Pump System (PDF)
- Application Note 25: Serial Connection of Luer-Slides for Flow Experiments (PDF)
- Application Note 31: Serial Connection of µ-Slide VI 0.4 Channels for Flow Experiments (PDF)
- Application Note 63: Generation and Dynamic Culture of L929 Spheroids in the µ-Slide Spheroid Perfusion (PDF)
- User Protocol 11: Protocol for Spheroid Culture, Staining, and Clearing for 3D Imaging (PDF)

Keywords:

Perfused 3D cell culture, spheroids, 3D-aggregates, organoids, spheroid generation, perfusion, flow, long-term cultivation, ULA labware, Bioinert, passivation, pump, microfluidic chip

1 Material

1.1 Reagents and Buffers

- Harvested cells or spheroids
- Cell culture medium and other standard cell culture reagents (trypsin, PBS, FCS)

1.2 Equipment

- µ-Slide Spheroid Perfusion Bioinert (80350, ibidi, Germany)
- ibidi Pump System (10902, ibidi, Germany)
- Perfusion Set BLUE, 15 cm, ID 0.8 mm (10961, ibidi, Germany)
- Hose Clip (part of the ibidi Pump System)
- Serial Connector for µ-Slides (10830, ibidi; for alternative assembly, see page 2)
- µ-Slide Rack (80003, ibidi Germany)
- Sterile syringe (1 ml) with simple Luer adapter (various suppliers), biocompatible
- Standard cell culture equipment (sterile working bench, cell culture incubator, inverted microscope, culture flasks, pipets, tips, etc.)

Alternatively, assemble the Serial Connector using the following parts:

- Silicone Tubing 1.6 mm ID (10842, ibidi)
- Luer Connector Male (10824, ibidi) or Elbow Luer Connector Male (10802, ibidi)

Shorten the Silicone Tubing to a length of 6 cm or shorter. Then add one Luer Connector Male to each end of the tubing. This results in a connector tubing with two free male Luer adapters at the ends to connect channels between slides. Finally, sterilize the connector with ethanol or by autoclaving.

Example of an assembled Serial Connector showing a Silicon Tubing with a Luer Connector Male at each end.

2 Preparation

Important Note:

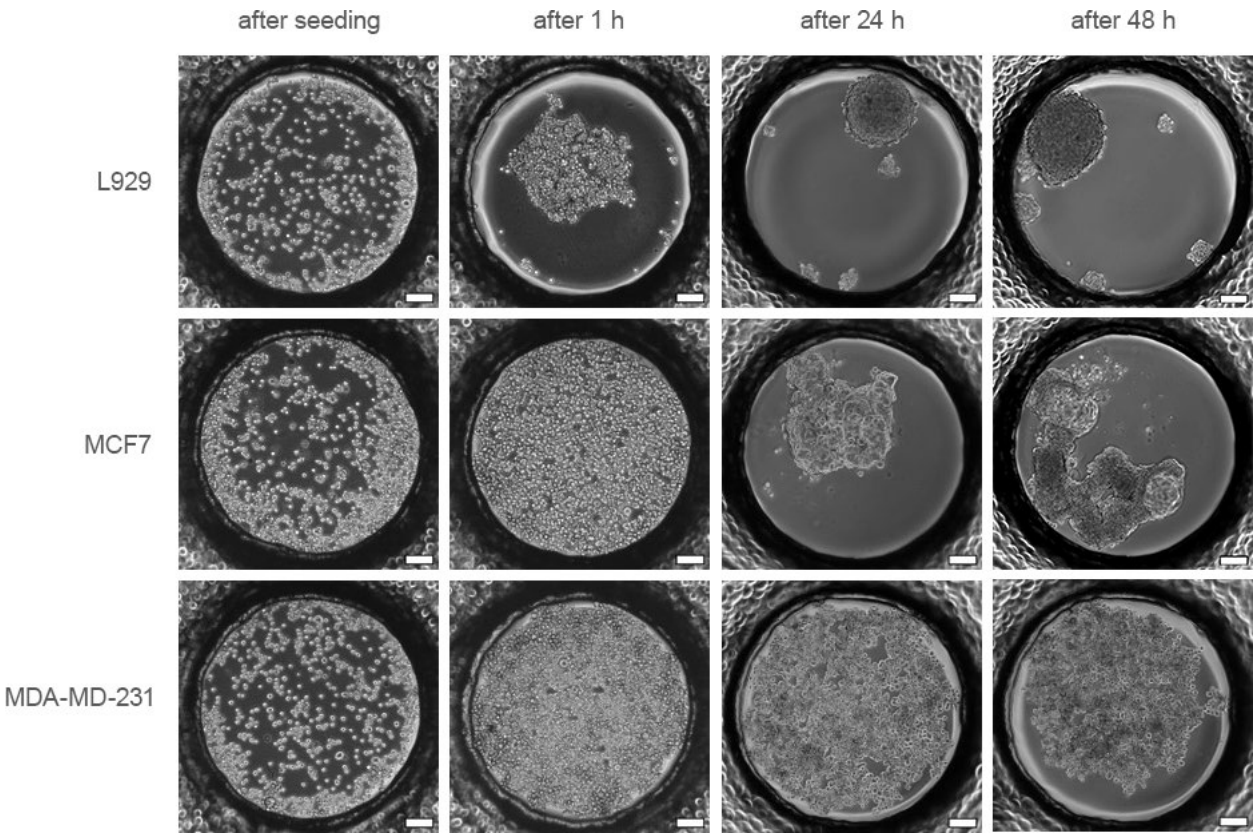
Equilibrate all required materials, such as µ-Slides, culture medium, and tubing (Serial Connectors, Perfusion Sets) overnight inside the incubator at 37°C and 5% CO_2. This is essential for keeping air bubbles from emerging over time.

Perform all the following protocol steps under sterile conditions!

- Assemble the µ-Slide Spheroid Perfusion and remove air bubbles, as described in the instructions.
- Harvest your cells as usual and prepare a cell solution in standard cell culture medium as described in Application Note 63. In brief, treat the cells with Trypsin or Accutase, harvest detached cells, and stop enzyme activity by adding the same amount of cell culture medium, centrifuge the cells, and dilute in fresh cell culture medium to a final concentration of e.g., 2–10 × 10^5 cells/ml (according to the cell line and required size of spheroids)

2.1 Spheroid Formation and Transfer in the µ-Slide Spheroid Perfusion

- Seed cells in the channels of the µ-Slide Spheroid Perfusion following the Instructions. **Tip:** Different cell lines can be seeded in the individual channels for co-culture experiments.
- 1 hour after seeding, add medium in reservoirs. Incubate slides under cell culture conditions for 24–48 hours, until the cells self-organize in compact spheroids. Check the spheroid generation process with a suitable microscope.
- Alternatively, generate spheroids using your preferred method (e.g., hanging-drop, agarose-coated plate). Make sure the aggregates are stable enough to transfer them with a pipet into the µ-Slide Spheroid Perfusion as described in the instructions. Close the slide tightly with the lid.
- After compact cell aggregates are formed or placed in the wells, the slides are prepared for serial connection.



Formation of spheroids of human fibroblast (L929) and human breast cancer (MCF7 and MDA-MB-231) cell lines. Within 1 hour after seeding, the cells sink in the wells of the µ-Slide Spheroid Perfusion. Depending on the cell line, compact spheroids form within 24–48 hours after seeding. Scale bar: 100 µm.

2.2 Serial Connection of the µ-Slide Spheroid Perfusion

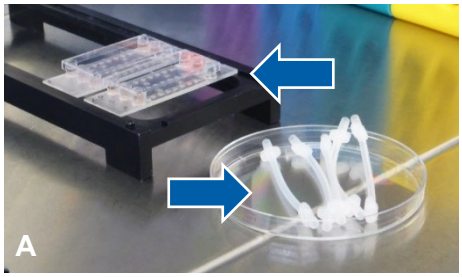
Follow these steps to connect two µ-Slide Spheroid Perfusion serially. Perform these steps as fast as possible to reduce cellular stress caused by temperature fluctuations or agitation to avoid damage to the spheroids.

To help reduce air bubble formation within the channel, use caution when connecting the male Luer adapter to the female Luer port.

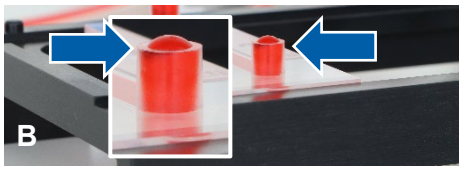
We recommend placing the µ-Slides on a µ-Slide Rack or petri dish to prevent the slide and the cells from cooling down while working on the cold metal surface of the laminar flow hood.

Connecting the Serial Connectors to the µ-Slide

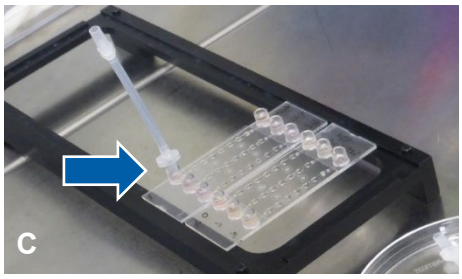
- Put the µ-Slide Rack, the syringe, the equilibrated medium, and the equilibrated Serial Connector into the laminar flow hood.
- **A:** Unpack the serial connectors in a sterile environment. Two connectors are needed to connect the channels on one slide and one additional connector for every two connected slides (e.g., prepare five serial connectors for connecting the channels of two slides). Place the µ-Slides containing the spheroids on the µ-Slide Rack.



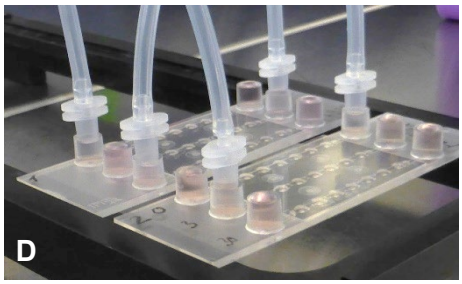
- **B:** Fill all reservoirs with cell-free medium until the top. The reservoirs should be slightly overfilled (a small convex meniscus of liquid should be on top).



- **C:** Plug the male Luer adapter of one of the Serial Connectors into the first female Luer ports of the first channel of the µ-Slide. Twist the adapter while tightly pressing it into the Luer port. Wipe off any overspilled medium using a paper towel. The first serial connector is now connected to the slide.



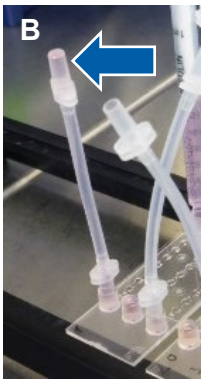
- **D:** Repeat this procedure with the other connectors at the two following channels of the same µ-Slide, alternating between front and back Luer ports. In the same manner, connect the remaining two connectors to two channels of the second µ-Slide



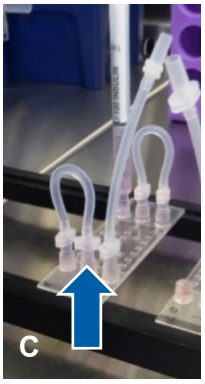
Connecting the Channels of the µ-Slide Spheroid Perfusion

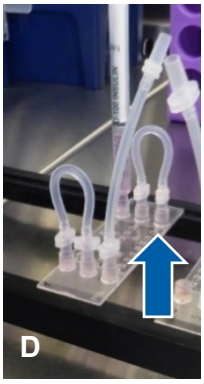
- Fill the syringe with at least 1 ml of prewarmed medium. Reverse the syringe and push out the surplus air. There should be a small hump of medium.
- **A:** Connect the syringe to the free Luer adapter of the first channel of the µ-Slide. Make sure no air bubbles are trapped inside. Wipe away any spillover with a paper towel.
- **B:** Very carefully, push the medium through the first channel of the µ-Slide until the Serial Connector tubing is filled entirely with medium and you see a small hump of liquid.

  Important: Do not push the medium too fast; to not displace spheroids from their wells.

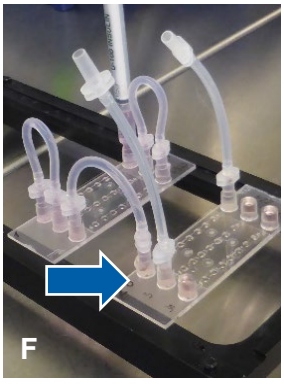


- **C:** Plug the male Luer adapter of the Serial Connector into the free female Luer port of the second channel of the µ-Slide.
- **D:** Keep the syringe connected to the µ-Slide. Continue to push the medium through the channels using the syringe until the second Serial Connector is filled entirely and you see a hump of liquid at the Luer adapter. Plug it into the free female Luer port of the third channel.



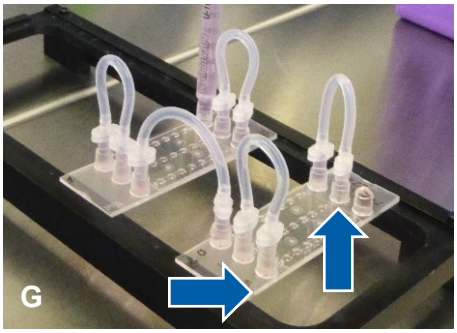


- **E:** Continue to push the medium through the last channel of the first slide until the third Serial Connector is filled entirely.
- **F:** Plug this Serial Connector into the free female Luer port of the first channel of the second µ-Slide.



- **G:** Repeat until all Serial Connectors are secured into the respective Luer Ports and all channels of the second µ-Slides are connected. Wipe away any spilled excessive medium with a paper towel.

  The two µ-Slide Spheroid Perfusion are now serially connected.



Tip: If you want to connect more µ-Slides, repeat the steps until all channels of all µ-Slides are connected, as shown below.

Four serially connected µ-Slide Spheroid Perfusion, connected to the ibidi Pump System.

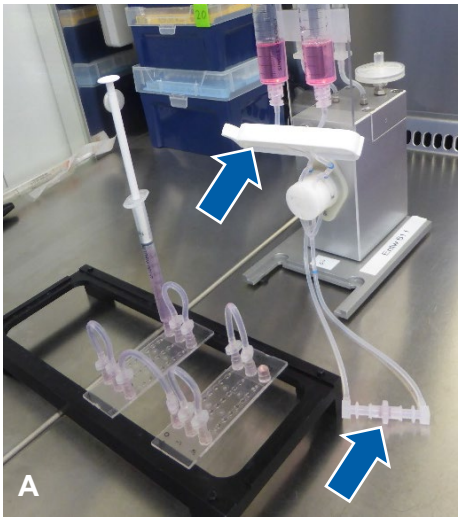
2.3 Connection to the Perfusion Set

Please find detailed instructions about connecting the slides to the Perfusion Set in Application Note 13: Endothelial Cell Culture Under Perfusion (Chapter 6, “Connecting the µ-Slide I $^{0.6}$ Luer to the Perfusion Set”.)

Perfusion Set BLUE (length 15 cm, ID 0.8 mm, 10 ml; Cat. No. 10961) is recommended to perfuse multiple, serially connected µ-Slides Spheroid Perfusion. Equilibrate the Perfusion Set and the other material overnight inside the incubator at 37°C and 5% CO_2. This is essential for keeping air bubbles from emerging over time.

- Mount the Perfusion Set onto the Fluidic Unit as described in the ibidi Pump System Instructions.
- **A:** Pinch off the Perfusion Set tubing using the white plastic hose clip that comes with the ibidi Pump System.

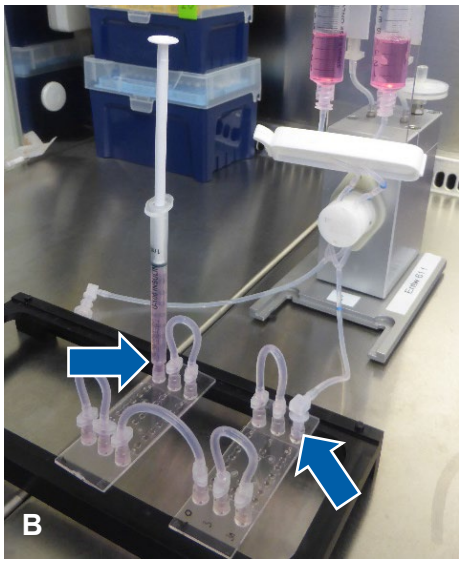
  Pull out one male Luer adapter of the Perfusion Set from the middle connector by holding it upwards. Avoid trapping air bubbles inside.



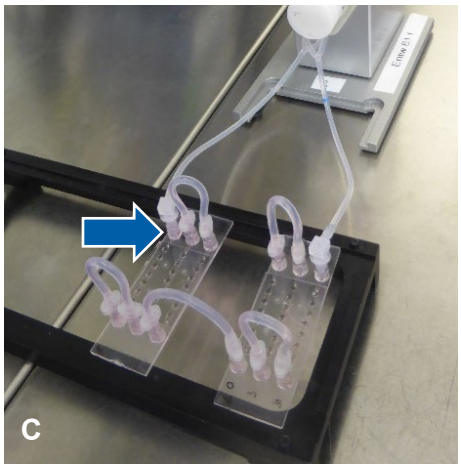
- **B:** Tightly connect this male Luer adapter to the free female Luer port of the second µ-Slide. The Fluidic unit is now connected to the µ-Slide.

  Slowly remove the syringe from the other µ-Slide and check the Luer port for air bubbles (if necessary, aspirate them with a syringe or pipet).

  Now, re-fill the Luer port with medium until a meniscus is clearly visible.



- **C:** Disconnect the second male Luer adapter of the Perfusion Set from the middle connector by holding it upwards and connect it to the remaining female Luer port on the first µ-Slide.

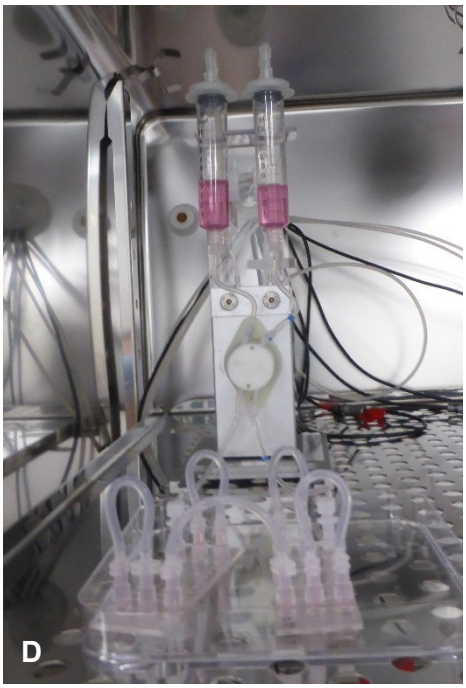


- The serially connected µ-Slides Spheroid Perfusion are now connected to the Fluidic Unit. Wipe off any excessive medium with a paper towel and remove the white plastic clamp (A).

  Note: Before starting your experiment in the next step, it is strongly recommended to check the cells under the microscope first.

- Put the whole assembly into the incubator and connect the Fluidic Unit to the ibidi Pump System.

  Now, start the flow experiment. A flow rate in the range of 0.3–1 ml/min is recommended for the perfusion of the serially connected µ-Slides Spheroid Perfusion.



2.4 Adapting the Flow Rate

The PumpControl Software does not provide a standardized setup of more than one µ-Slide. Therefore, you will have to manually adapt the parameters of the ibidi Pump for the new demands.

In the software, you can still select the µ-Slide Spheroid Perfusion and your Perfusion Set color (BLUE). However, the resulting flow rate (and hence also the shear stress) will be lower than in the single-channel application.

Due to the sophisticated well design of the µ-Slide Spheroid Perfusion, it allows continuous perfusion of the cells without applying any shear stress. Thus, adjusting the precise flow rate in the software by calibration is not needed. For good results, it is sufficient to apply a low flow rate of e.g., 0.75 ml/min.

2.5 Long-Term Cultivation of 3D Cell Aggregates

Perfused cultivation of 3D cell spheroids allows for extended cultivation periods of several weeks without the need for frequent manual medium exchange, providing a more efficient and time-saving approach. Furthermore, this technique facilitates the co-culturing of aggregates of diverse cell types, enabling the study of their metabolic interactions.

Exchange medium in the reservoirs of the Perfusion Set (total exchange: 10 ml), as described in section 6.10.1. of the ibidi Pump System Instructions. Exchange medium 1–2× weekly, or as required, depending on the number of connected µ-Slides and the metabolic rate of your cells. Control the spheroids regularly under the microscope.