

Serial Connection of the six channels in ibidi μ -Slide VI^{0.4}

Also applicable for connecting single channels of other Luer-Slides

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1. Introduction

The ibidi Pump System is specially designed for long term cell conditioning under perfusion conditions. The standard way is to connect one slide to one perfusion set. In some cases it might be useful to increase the number of slides (i.e. cells). For example when various stainings are planned, or if the cell number is not sufficient for the subsequent applications like FACS.

This application note is a step by step protocol for connecting the six channels of μ -Slide VI^{0.4} one after another.

Important Notes!

The serially connected slides are experiencing the very same flow rate and hence the very same shear stress.

We do absolutely recommend connecting the slides in series but not in parallel!

We recommend connecting not more than two μ -Slide VI^{0.4} in the described way.

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2. Material

For the setup you need the following material (sterile):

- Serial Connectors (ibidi #10830), 5 pieces
- Cell culture medium
- ibidi μ -Slide VI ^{0.4} with seeded cells
- Perfusion Set
- 1 ml syringe
- Hose clip, unsterile
- Pipet with tips

Important Note:

Equilibrate the serial connectors, the cell culture medium, the slides, and the perfusion set in the incubator over night!

All steps of this protocol should be done under sterile conditions!

3. Preparations

The whole setup is equal to the standard experiment with only one channel. The only difference is, that you connect the multiple channels serially with each other before you connect the whole assembly to the Perfusion Set.

3.1. Cell seeding

Seed your cells as usual in the channels of μ -Slide VI ^{0.4}. Let the cells attach and fill the reservoirs with 60 μ l cell-free medium after attachment, if further statical incubation is needed. A detailed description is given in [Application Note 13 "HUVECs under perfusion"](#) and in the [instructions of \$\mu\$ -Slide VI ^{0.4}](#).

After cell attachment the slide is ready for the serial connection procedure.

3.2. Pump Setup

Prepare the setup of the pump as described in [Application Note 13 "HUVECs under perfusion"](#). Fill the reservoirs of the perfusion set with 5 ml equilibrated medium each and remove the air bubbles by running the pump with a medium pressure (around 50 mbar).

Important Notes!

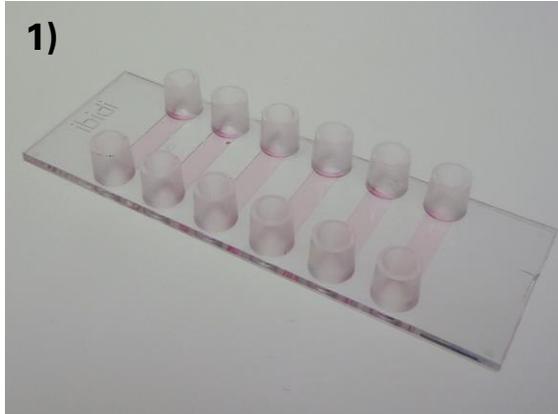
Always take care not to trap air bubbles when adapting a male to a female Luer connector.

Work as fast as possible to avoid cooling out of the slides and cells. The whole procedure has to be done in less than 10 minutes.

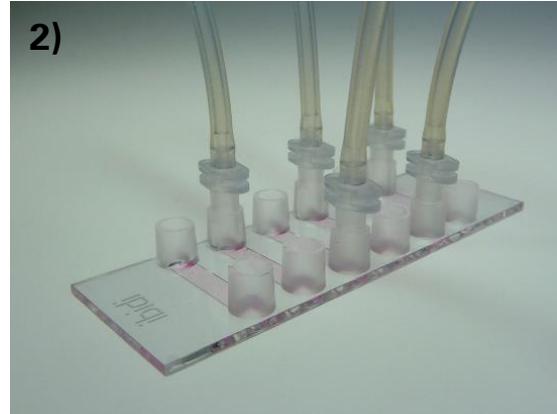
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4. Serial Connection

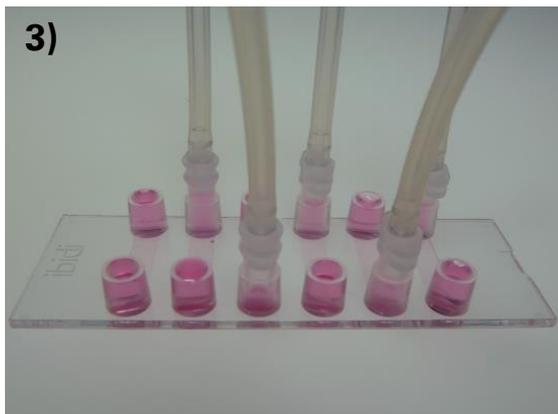
The serial connection of the channels is done after cell attachment and before connecting the slide(s) to the Perfusion Set.



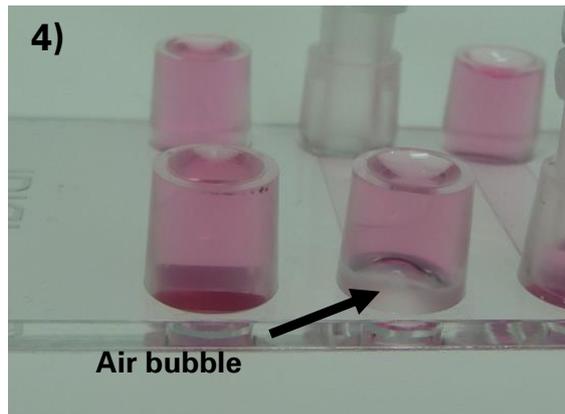
1) Prepare the slides as described in section 3. Cells are seeded and attached.



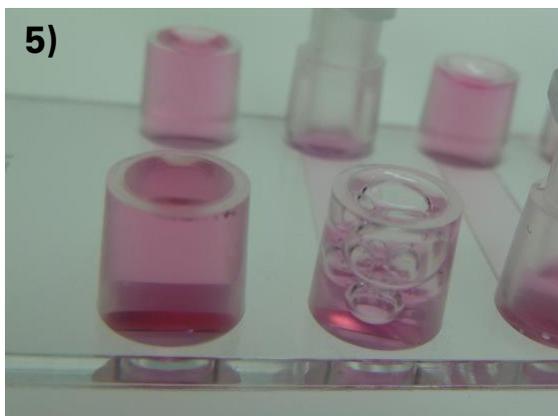
2) Connect five empty Serial Connectors to the Luer ports as shown.



3) Fill the reservoirs with cell free medium until all the reservoirs are filled completely.

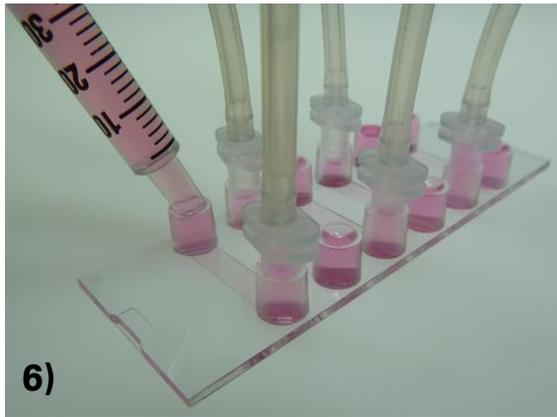


4) In this step take care not to get trapped an air bubble at the bottom of the reservoir.

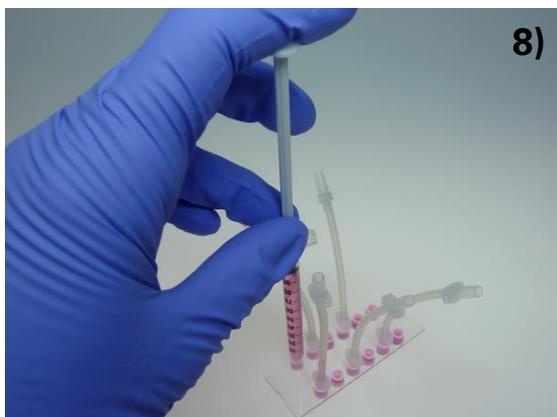


5) Take care not to get trapped air bubbles in the reservoir.

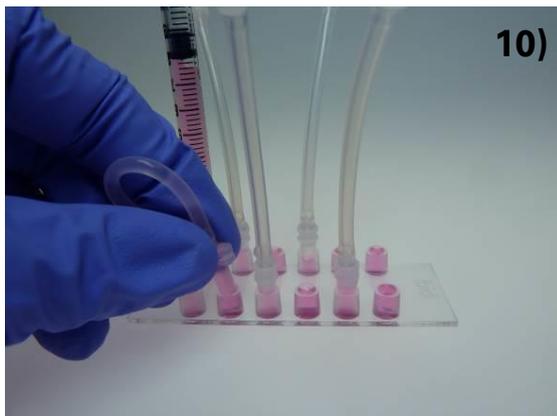
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Take up 1 ml fresh, prewarmed medium in a syringe and adapt the syringe without trapping air.



Slowly push the medium through the channel and the first serial connector until the medium fills the Luer connector completely

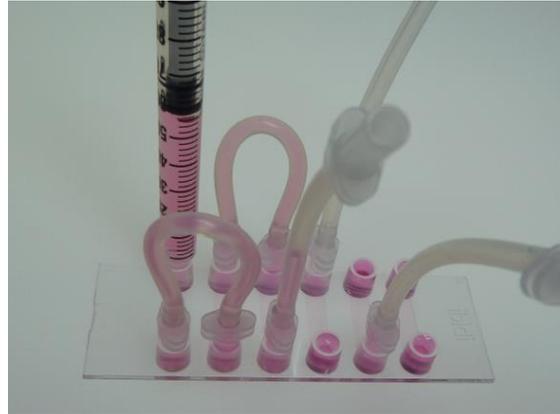


Bend the Serial Connector and adapt the free male Luer adapter to the neighboring Luer port on the slide. Take care not to trap an air bubble in the Luer port!

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Now repeat the last four steps for every new connection. First fill the next Serial Connector with medium...



...then connect the male Luer adapter to the neighboring Luer port on the slide.



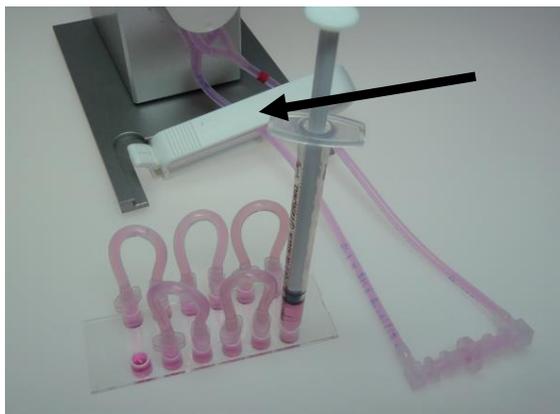
Three Serial Connectors are connected.



Four Serial Connectors are connected.



Now all five connections are closed. For the adaption of the slide to the Perfusion Set, leave the syringe on the Luer port...



... and begin with connecting the free Luer port. Before starting clamp off the tubing of the Perfusion Set with the hose clip!

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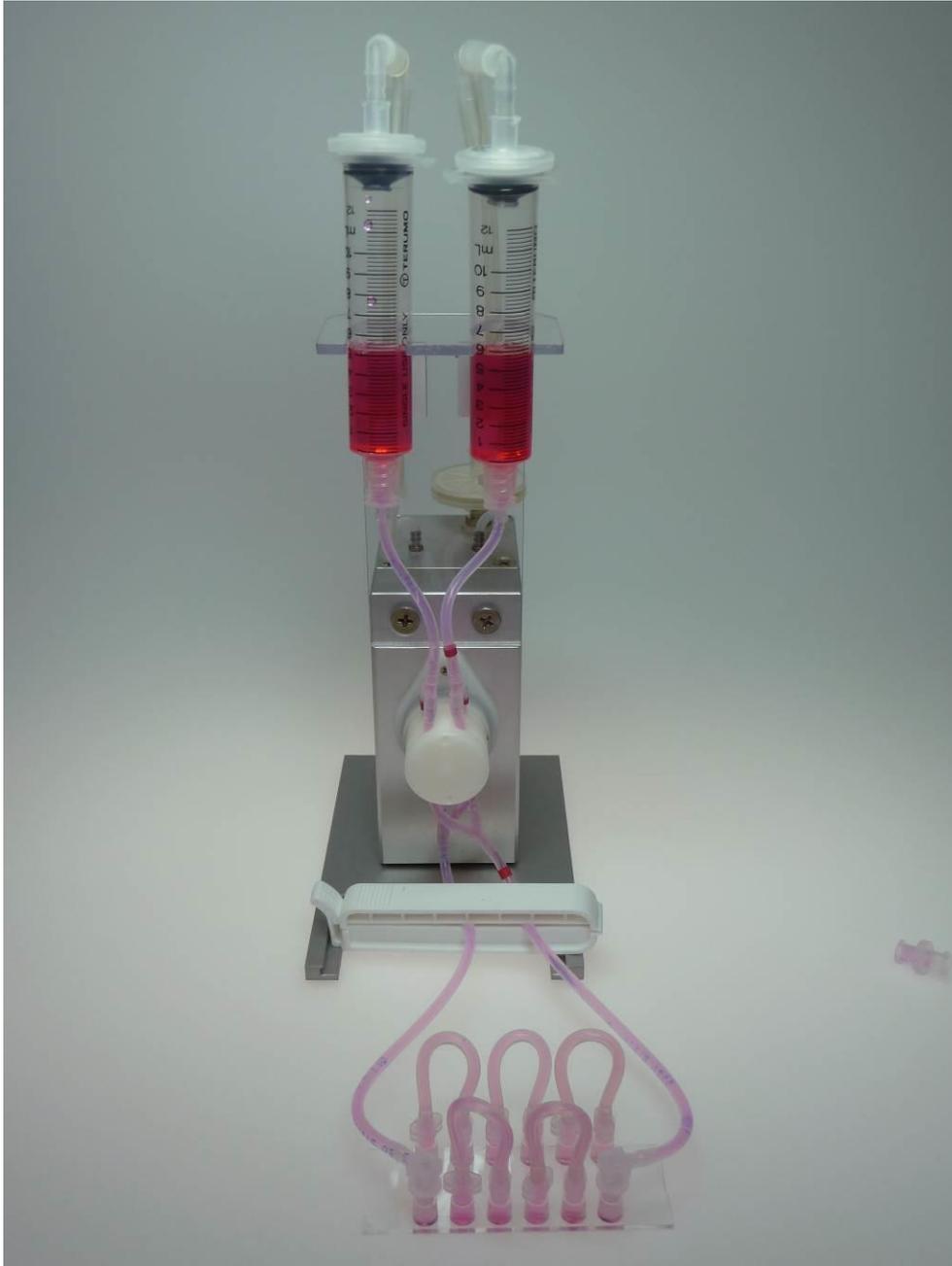
Pull the male Luer connector out of the Female Luer Lock Coupler of the Perfusion Set and place it into the remaining Luer port on the slide. Now slowly pull out the syringe and fill the Luer port to the top with fresh medium and remove the air bubbles.



After removing the syringe, pull the second male Luer connector out of the Female Luer Lock Coupler of the Perfusion Set and place it into the remaining Luer port on the slide.



The setup is finished and ready for starting the flow. Don't forget to remove the hose clip!



5. Adapting the flow rate

The setup of more than one slide is not foreseen in the software. This means you will have to adapt the parameters of the pump for the new demands.

As a guideline you can indicate in the software, that you are using the μ -Slide VI^{0.4}. The resulting flow rate (and hence also the shear stress) will be lower than in the single channel application.

Start a cycle with the desired shear stress and measure the flow rate by hand (see the [ibidi Pump Instructions](#), page 40 et sqq). Then go to the recalibration dialogue and insert the calculated (given flow rate of the software) and the measured flow rate.