

## Serial Connection Protocol for Luer-Slides

### 1. General Information

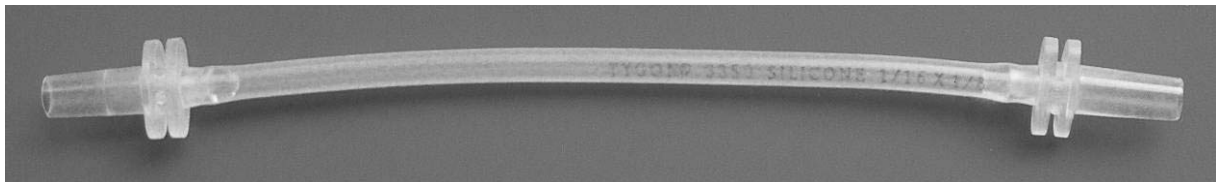
This Application Note describes a step by step protocol how to connect several Luer-Slides in series. The advantage is that observation and incubation of several slides with only one fluidic unit is possible. The flow rate in the slides is identical while utilizing slides of the same geometry. The shear rate can be varied by connecting slides with different heights (e.g.  $\mu$ -Slide I<sup>0.2</sup> Luer and  $\mu$ -Slide I<sup>0.4</sup> Luer).

The protocol only specifies the procedure for two slides. But it can be applied for a larger number of slides.

### 2. Material:

|             |                                                  |                                |
|-------------|--------------------------------------------------|--------------------------------|
| Slides:     | 2 $\mu$ -Slides I <sup>0.4</sup> Luer, ibiTreat  | ibidi                          |
| Cells:      | Endothelial cells ( $2.5 \cdot 10^5$ per slide)  | e.g. Lonza                     |
| Medium:     | Endothelial Cell Growth Medium                   | e.g. Lonza                     |
| Tubing:     | Silicone tubing                                  | e.g. Tygon 3350, ID 1.6 mm     |
| Connectors: | Luer to tubing connector                         | e.g. Value Plastics, MTL5210-6 |
| Syringes:   | Sterile syringe (10 ml) with simple Luer adapter | Various suppliers              |

Plug one plastic connector to each end of the tubing to assemble the connector between the slides. Resulting is a connector tubing with two free male Luer adapters at the ends. Sterilize the connector with ethanol or by autoclaving.



All following steps have to be done under sterile conditions!

### 3. Seeding cells

Important note! To avoid air bubbles emerging over time it is crucial to equilibrate the media, tubing and slides. Place them in the incubator one day before seeding the cells!

- Prepare a cell suspension of  $2.5 \cdot 10^6$  cells/ml.
- Fill 100  $\mu$ l into the channel. Only the channel volume is filled (Fig. 1a).
- Put the caps on the Luer adapters and place the slide for half an hour in the incubator for cell attachment.
- After cell attachment fill the reservoirs with 60  $\mu$ l each.

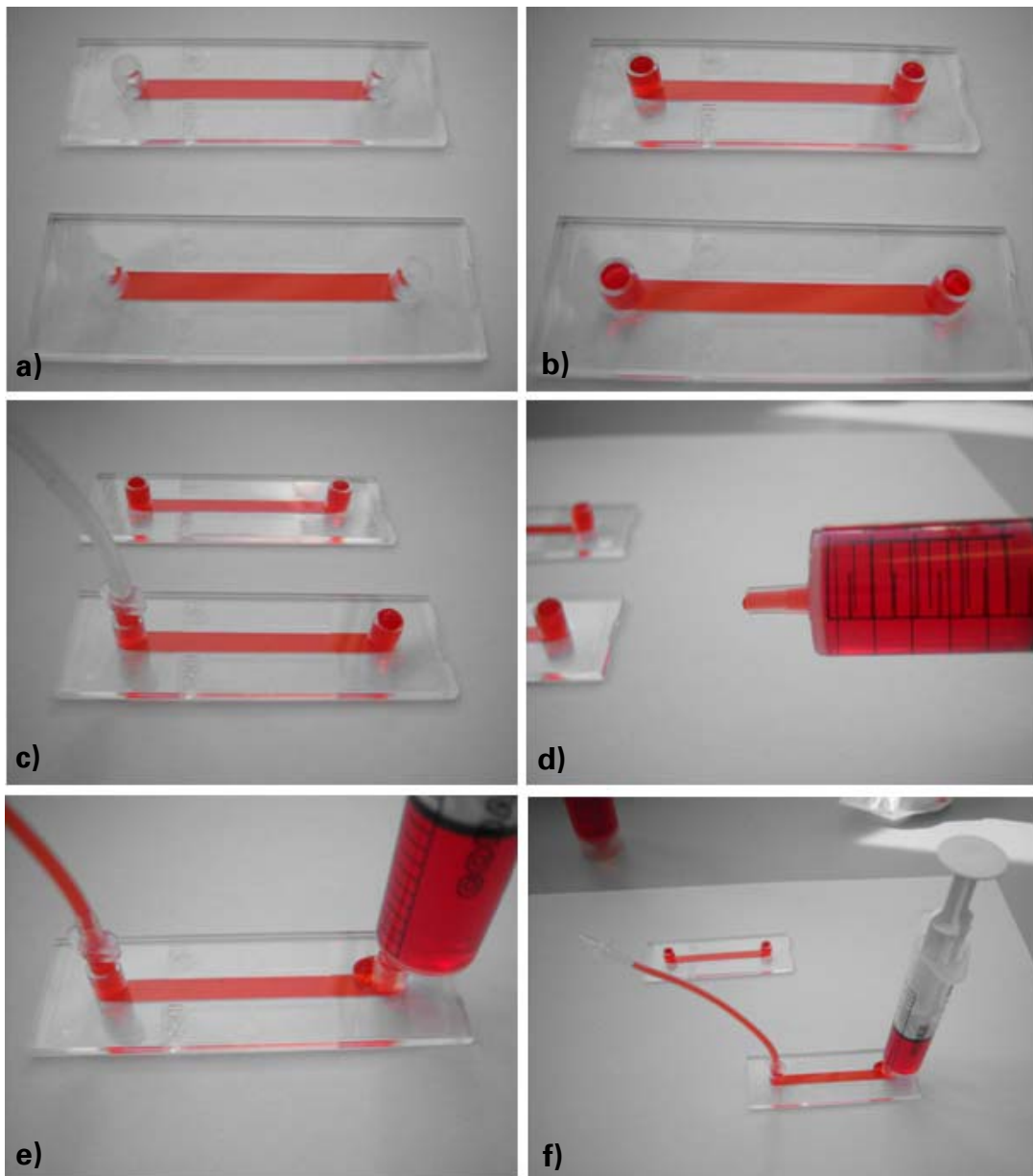
For a detailed description of cell seeding procedure please see Application Note 13 (HUVECs under perfusion).

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### 4. Connecting the Slides

The slides are now prepared with cells growing in the channel and the reservoirs are filled with 60  $\mu$ l cell-free medium (see section 3).

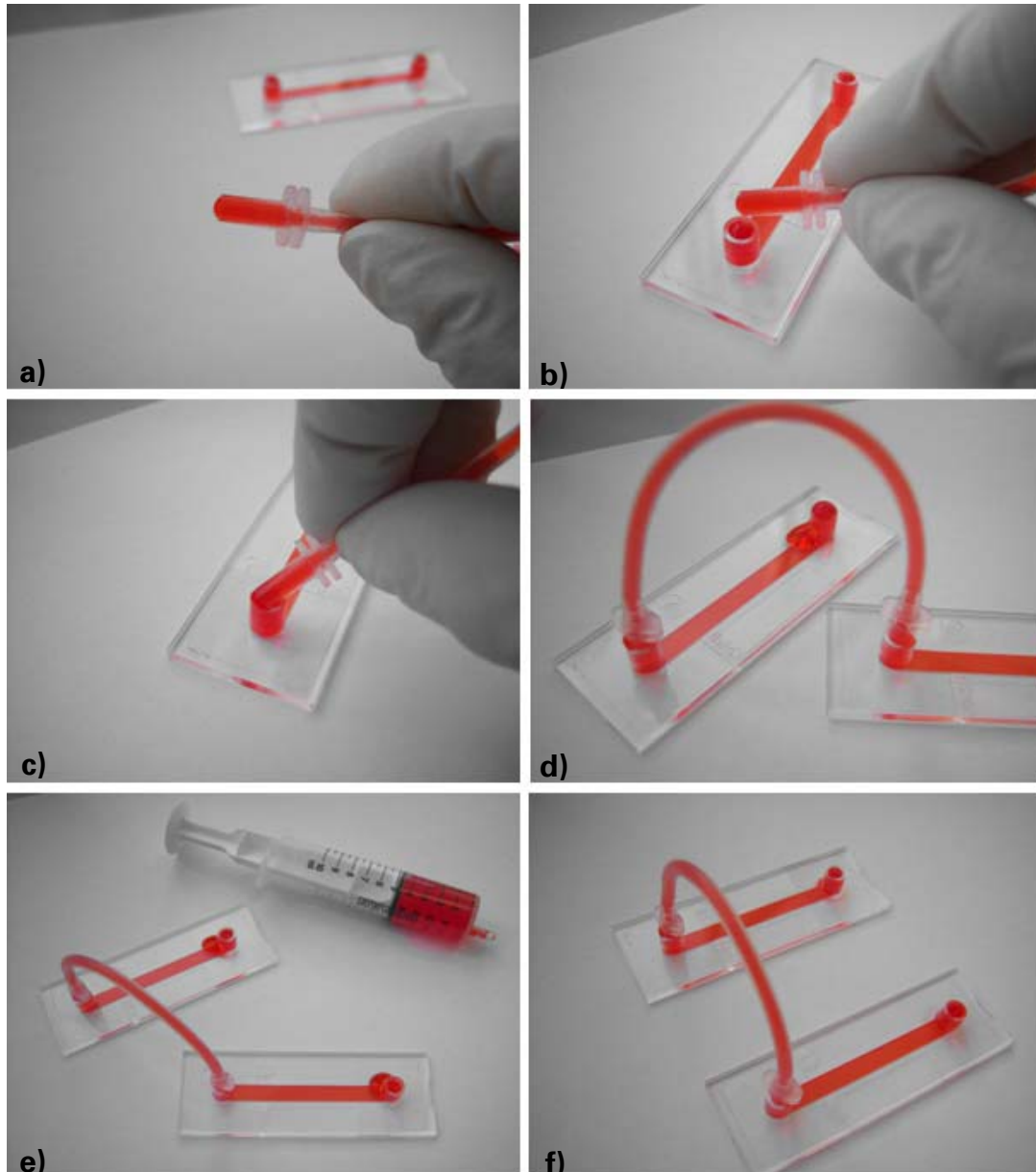
- Right before connecting fill all reservoirs with further 60  $\mu$ l each (Fig. 1b).
- Plug the one Luer adapter of the connector tubing on one of the female Luer adapters of the slide (Fig. 1c).
- Pull some milliliters prewarmed medium into the syringe. Reverse the syringe and push the surplus air out (Fig. 1d).
- Adapt the syringe without air bubbles to the free Luer adapter of the slide. There may be some overspill (Fig. 1e).
- Very carefully push the medium through the slide until the connector tubing is filled with medium (Fig. 1f).



**Fig.1:** (a) Applied volume when seeding cells; (b) Slides filled completely before connection; (c) First slide with connector tubing; (d) Syringe without air bubbles; (e) Syringe plugged into the first slide; (f) Filling the connector tubing by pushing the medium with the syringe.

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- When the connector tubing is completely filled (Fig. 2a) plug it into one of the Luer adapters of the second slide (Fig. 2b-d).
- In case you want to connect more than two slides place a second connector tubing onto the free adapter of the second slide, push through the medium with the syringe, and so on.
- All slides connected slowly remove the syringe from the adapter (Fig. 2e).
- To connect the slides to a perfusion set the two free reservoirs have to be filled with medium onto the top. The excessive medium can be wiped off with a paper towel.

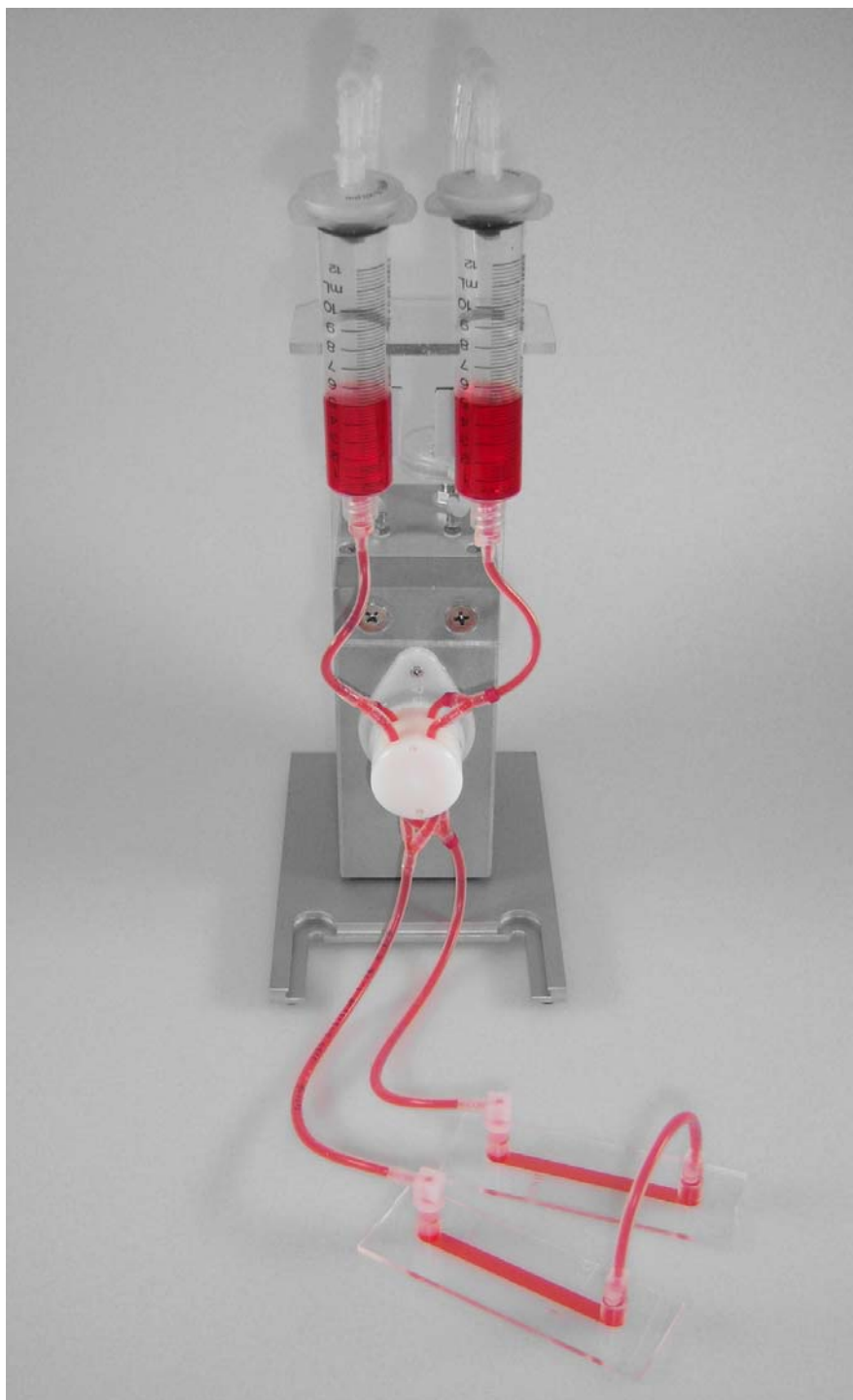


**Fig.2:** (a) Connector tubing completely filled; (b-c) Insertion of the connector tubing on the Luer adapter of the second slide; (d) Slides with adapted connector tubing; (e) Slides after removal of the syringe; (f) Overflow medium is wiped off and the reservoirs filled for connection to the perfusion set.

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### 5. Connection to the perfusion set

For a detailed instruction for connecting the slides to the perfusion set please refer to Application Note 13, section 6 "Connecting the slide to the perfusion set".



**Setup of one fluidic unit with two  $\mu$ -Slides I Luer in serial connection**