General Handling Guide for Cross-flow Membrane Chips

Example: Cross-flow Membrane Chip Fluidic 480 – Basic Chip Module for Cell Culture Assays

Application cases:

Suspension cell culture

Adherent cell culture

And co-culture of bacteria, yeast and mammalian cells

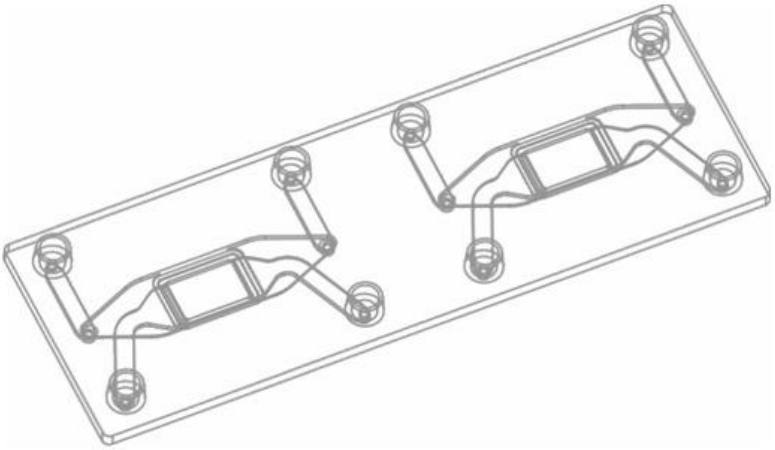

Table of content

1 Introduction

The cross-flow membrane chip represents a microfluidic chip system for adherent cell culture and thus the generation of diverse cellular tissues or organoids. The cell cultivation is conducted in two units per chip (Fig. 1), each representing a membrane-divided two-chamber system itself. Cell culture takes place solely on the integrated membrane, e.g. PET, with a hydrophilic property in contrast to the hydrophobic chip body.

Furthermore, the cross-flow membrane chip can be used as a membrane filtration element for the defined selection of cells and particles depending on particles size and the membrane pore size.

The cavities are enclosed to the environment except of an inlet and outlet for each sub-chamber. This allows the contamination free application of different cultivation media on each cavity. Cavities are connectable *via* the ports and allow chamber comprehensive applications and examinations.

2 Chip description

Various cross-flow membrane-chip designs are available at *microfluidic ChipShop* as summarized in ***7 Available cross-flow membrane chips***. All chips have a similar operation procedure. As example the following descriptions highlight our fluidic 480 cross-flow membrane chip.

Fluidic 480, see **Figure *1***, consists of two independent cross-flow membrane units both separated by a thin membrane with defined pore size, forming two compartments connected via the pores of the membranes.

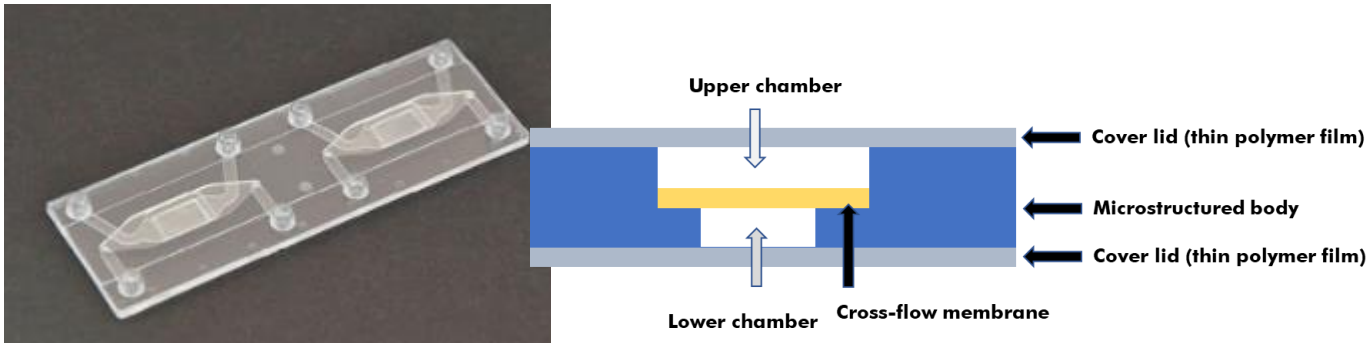

Figure 1: Fluidic 480 assembled cross-flow membrane chip (left) and cross section of the membrane area (right) highlighting the sandwich of cover lid – lower chamber – cross-flow membrane – upper chamber – cover lid.

Cell cultivation is possible in up to four cavities per chip, differing in cultivation area and volume between upper and lower chamber (**Figure 2**). The size and volume of the upper chamber is bigger than that of the lower one, as shown in **Figure 3**. The different size is also illustrated by differently colored fillings in **Figure 2** (green: upper chamber, red: lower chamber).

The chip is available with solvent resistant PET membranes of different thickness, pore-size and pore-density.

Liquid supply and removal are possible via two Mini Luer in- and outlets per chamber as shown in **Figure 2**: A1, A2, B1 and B2.

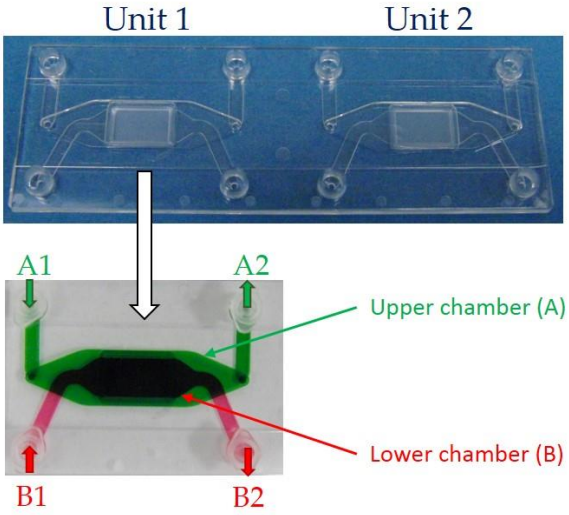

Figure 2: Cross-flow membrane chip Fl 480 for cell-based assays. The chip device contains two functional units (unit 1 and 2). Each unit features two chambers (upper chamber A and lower chamber B) which are separated by a permeable membrane. Both chambers feature Mini Luer interfaces for fluid entry and exit for filling and draining (A1 – inlet upper chamber; A2 – outlet upper chamber; B1 – inlet lower chamber; B2 – outlet lower chamber).

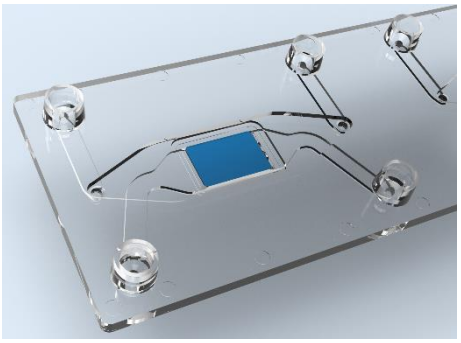

Figure 3: Interaction (membrane) area.

Standardized cell cultivation of adherent cells occurs only on the hydrophilic PET membrane. The rest of the microfluidic chip is hydrophobic unless specially treated. The thin film serving

as cover lid of the lower chamber, see **Figure *1***, can be used as base for adherent cells as well. For the cultivation of adherent cells on this film two strategies are possible, namely:

1. Individual treatment of the surface by the user if special or fresh treatment is required (e.g. collagen, poly-L-lysine or fibronectin)
2. Pre-functionalized by *microfluidic ChipShop*, e.g. with a hydrophilization

Volumes and relevant surface areas of fluidic 480 are summarized in Table 1. Fluidic 480 is available in other configurations regarding material, membrane pore size and surface treatment.

Table 1: Specifications of fluidic 480 – volumes, surface areas, geometrical parameters

Product Code	Description	Chip Material
10000284	Two units forming a double Chamber per device fluidic interface: Mini Luer Chamber volume upper chamber (A): 87,5 µl Chamber volume lower chamber (B): 61,5 µl Ground surface upper chamber (A): 154 mm² Ground surface lower chamber (B): 118 mm² Interaction area (membrane): 36 mm² Chamber height: 500 µm Membrane: PET, offered in 0,2 µm and 8 µm pore size	Topas

3 Assay procedures for dynamic operation

The cross-flow membrane chip represents an advanced cell culture technology tool. It enables the investigation of signaling pathways, to develop therapeutic strategies and to perform toxicity studies by the imitation of in-vivo conditions. It facilitates an effective supply with nutrient medium, discharge of catabolic cell metabolites and defined application of shear stress to cellular monolayers under laminar flow conditions (Gärtner et al. 2015).

Endothelial cells (ECs) have been cultured in a cross-flow membrane chip under dynamic conditions to investigate the role of physiological shear stress regarding cellular behavior. ***Figure 4*** compares the results obtained from visualization of PECAM-1 and vWF expression, which serve as a measure for tight junction between adjacent cells and a measure for physiological behavior of endothelial cells, respectively. The expression of both markers was further increased under flow conditions, simulating flow in blood vessels (Raasch et al. 2015, Gärtner et al. 2015).

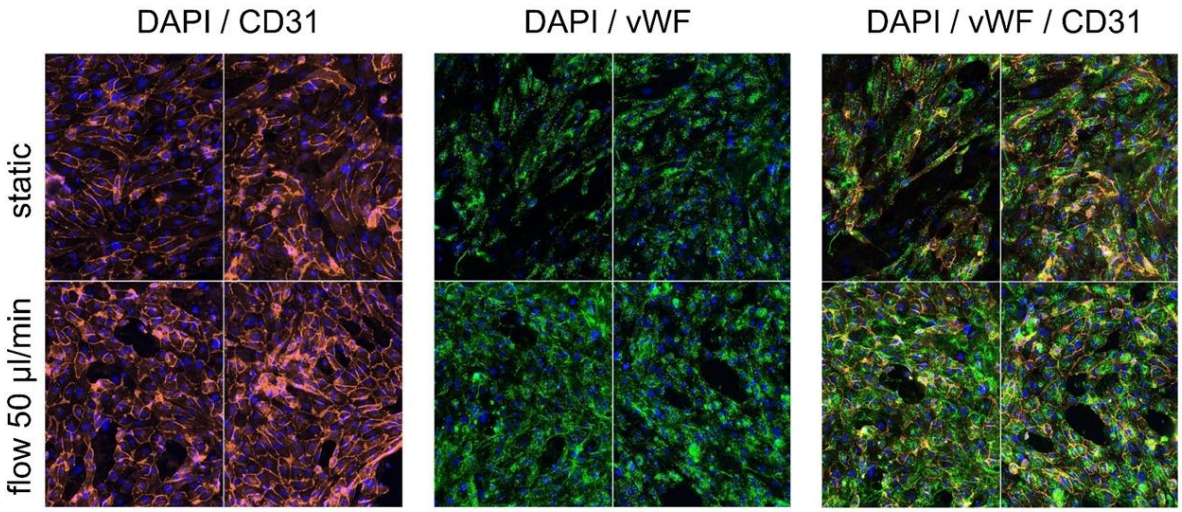

Figure 4: Adaption of endothelial layer to shear stress. Visualization of PECAM-1 expression (left), vWF expression (middle) and overlay (right). The expression of both markers was increased under flow conditions (Gärtner et al. 2015).

The dynamic operation procedure is recommended for co-cultivation, permeability tests, chemotaxis arrays and adhesion assays. The upper and lower chamber were utilized separately. Fluidic operation can be operated manually by pipetting or automated *via* pumps and adequate tubing. Several setups are feasible, depending on the experimental requirements.

3.1 Operation *via* pipetting

3.1.1 Accessories

For the operation of the chip various accessories are at hand:

- Male Mini Luer plugs – low volume displacement (product code: 10000205)
- Male Mini Luer fluid connectors (product code: 10000116)
- Handling frame (product code: 10000041)
- Silicone tube, ID: 0.76, OD: 1.65 mm (product code: 10000031)
- PTFE tube, ID: 0.5 mm, OD: 1.0 mm (product code: 10000032)
- Mini Luer to pipette adapter (product code 10000057)
- Waste container or other appropriate vessels

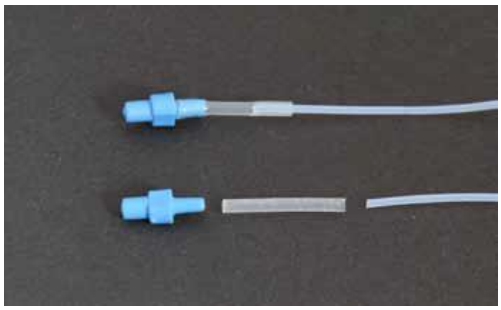

Figure 5: Example for tubing interconnection. Assembly of male Mini Luer fluid connector, silicon tubing (here as sleeve) and PTFE tubing.

3.1.2 Procedure

- **Optional equilibration** of the cavities (recommended, avoids air bubble formation within the growing chamber)
  - For rinsing the upper chamber, seal the in-and outlets of the lower chamber (B1/B2) with plugs and vice versa.
  - For reasons of better understanding this example describes the equilibration of the lower chamber. For the equilibration of the upper chamber please exchange B for A in the protocol.
  - Connect outlet B2 with waste container or similar *via* tubing and Mini Luer fluid connector (see **Figure 4**)
  - Pipet into B1 to rinse the chambers with 70 % ethanol to reduce the formation of air bubbles and to sterilize the chambers and discard through B2
  - Equilibrate the cultivation chamber by pipetting approx. 500-1000 µl cultivation medium into B1 and discard over B2

- **Seeding** of cells in lower/upper chamber
  - **Note:** When working with adherent cells: The lower chamber has to be seeded first and the chip has to be placed upside down in the handling frame (sealed interfaces facing down), to ensure the contact of the cells with the PET membrane. Please give the cells appropriate time to adhere.
  - For seeding cells in the lower chamber, seal the in-and outlets of the upper chamber (A1/A2) with plugs and vice versa.
  - For reasons of better understanding this example describes seeding cells in the lower chamber. For seeding cells in the upper chamber please exchange B for A in the protocol.
  - Connect outlet B2 with waste container or similar *via* tubing and Mini Luer fluid connector
  - Pipet the cell suspension in an appropriate cell concentration (for example 8000 cells per cm² when using HeLa, cell density depends on the kind of cells you use and the duration of cultivation) in inlet B1
  - Remove all connectors and tubing to seal interfaces B1 and B2
  - Incubate the chip at desired conditions

- **Liquid exchange**

Liquid exchange might be necessary for medium exchange, washing or treatment of cells.

- For reasons of better understanding this example describes liquid exchange in the lower chamber. For liquid exchange in the upper chamber please exchange B for A in the protocol.
- Unplug inlet B1 and connect outlet B2 with the waste container
- Pipet the appropriate liquid (medium, washing solution, treatment solution) B1, discard over B2
- Use approximately the 2-fold chamber volume to ensure a complete liquid exchange
- **Note:** Take care of the flow rate, it should be low to prevent damages of membrane and cells
- To minimize evaporation rate and air bubble formation during incubation it is recommended to fill half of the Mini Luer interface with the used liquid
- Seal the interface with a plug and incubate at desired conditions.

3.2 Fluidic operation *via* syringe pumps

Filling, equilibrating and seeding is easiest by pipetting. In case a very slow and steady flow is necessary, syringe pumps may be convenient. When syringe pumps are used for long term experiments, exchange the syringe and media regularly to avoid contamination.

3.2.1 Accessories

For the operation of the chip various accessories are at hand:

- Male Mini Luer plugs – low volume displacement (product code: 10000205)
- Male Mini Luer fluid connectors (product code: 10000116)
- Handling frame (product code: 10000041)
- Silicone tube, ID: 0.76, OD: 1.65 mm (product code: 10000031)
- PTFE tube, ID: 0.5 mm, OD: 1.0 mm (product code: 10000032)
- Waste container or other appropriate vessels
- Syringe pumps and appropriate syringes

3.2.2 Procedure

- Upper and lower chamber were utilized separately and their inlets connected to an appropriate syringe pump each (***Figure 6***)
- **Note:** For Installation of the syringe pumps and syringes follow the manufacturers instructions
  - **Optional equilibration** of the cavities (recommended, avoids air bubble formation within the growing chamber)
    - Follow the instructions given in *3.1.2 Procedure*

- **Seeding** of cells in upper chamber
    - **Note:** When working with adherent cells: The lower chamber has to be seeded first and the chip has to be placed upside down in the handling frame (sealed interfaces facing down), to ensure the contact of the cells with the PET membrane. Please give the cells appropriate time to adhere.
    - For reasons of better understanding this example describes the seeding in the lower chamber. For seeding cells in the upper chamber please exchange A for B and B for A in the protocol.
    - For seeding cells in the lower chamber, seal the interfaces of the upper chamber with Mini Luer plugs.
    - Connect outlet B2 with waste container or similar *via* tubing and Mini Luer fluid connector
    - Pump an adequate volume of cell suspension in a defined cell concentration into inlet B1
    - Remove all connections and seal interfaces B1 and B2 with Mini Luer plugs
    - Incubate the chip at desired conditions

- **Liquid exchange, continuous fluid supply, long term assays**

Note: Liquid exchange might be necessary for medium exchange, washing or treatment of cells.

- For reasons of better understanding this example describes liquid exchange in the lower chamber. For liquid exchange in the upper chamber please exchange A for B and B for A in the protocol.
- Connect B1 with the appropriate syringe pump *via* tubing and Mini Luer connector
- **Note:** Avoid air bubble formation in the tubing connections. Fill the tubes with liquid before connecting
- Connect B2 with the waste container
- Pump the appropriate liquid (media, washing solution, treatment solution) *via* B1
- Use approximately the 2-fold chamber volume to ensure a complete liquid exchange
- **Note:** Take care of the flow rate, it should be very low, in order to prevent damages of membrane and cells
- To minimize evaporation rate during incubation, the filling of half of the Mini Luer interface with the used liquid is recommended
- Seal the interface with a plug and incubate at desired conditions.

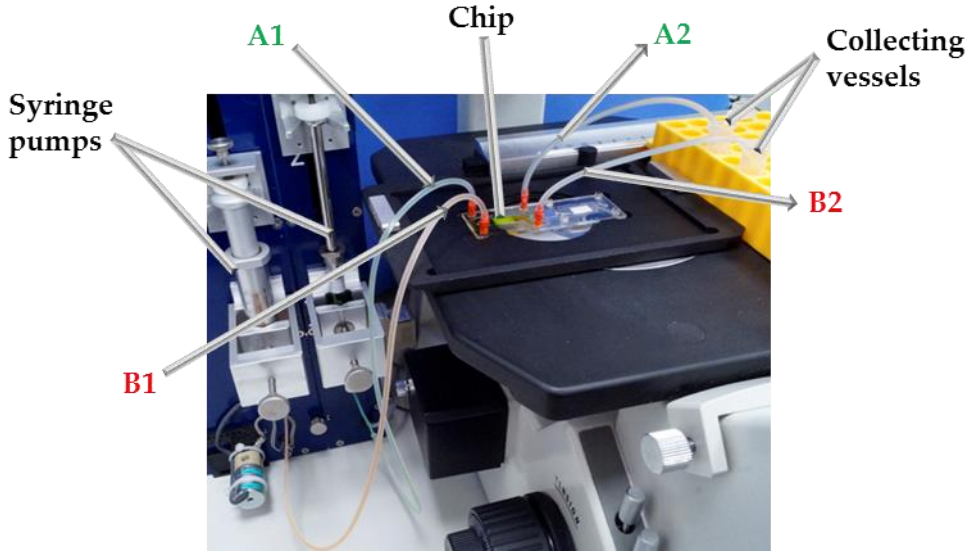

Figure 6: Experimental set-up for dynamic operation. The chip is placed on a microscope and connected to two syringe pumps *via* the left tube, A1 and B1 and to two collection vessels *via* the right tube, A2 and B2. A1 and A2 rinse the upper chamber while B1 and B2 serve the lower chamber.

- Note: For long-term experiments or continuous fluidic operation over a longer period of time, the tubing should be selected in a way that the chip can be stored in the CO_2 cell incubator for incubation. The amount of media used must be calculated accordingly.

For most convenient handling: Our *microfluidic ChipShop* LoC-CCI1-device allows the storage, incubation and fluidic management of the chip while simultaneous observation on the tray of an inverted microscope is possible (**Figure 7**).

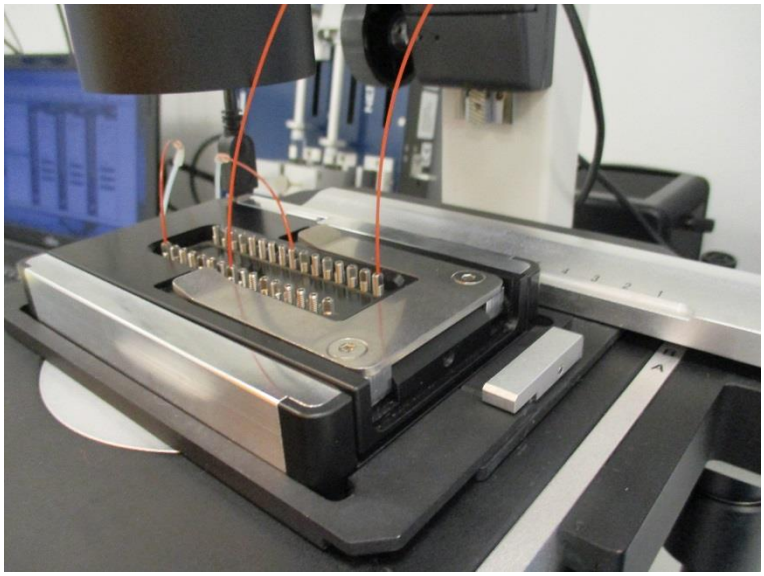

Figure 7: Experimental Set-up with LoC-CCI1 device

4 Assay procedure for cell sorting, cell separation and suspension cell culture (mammalian, yeast & bacteria)

This operation mode is based on the well-established membrane filtration method. The separation performance depends on the pore size of the integrated membrane and the size of particles or cells to be investigated.

In **Figure** *8* the filtration effect of fluorescent beads and HeLa cells is demonstrated. Using a membrane with 3 µm pore size, 15 µm beads and HeLa cells were trapped at the membrane in the lower chamber, while the majority of the 1 µm beads passed the membrane, flowing through the upper chamber and were finally collected in an external chamber.

Additional flow through steps (washing steps) with particle free liquid solution led to a nearly complete removal of the 1 µm beads from the lower chamber. In this case the flow moves enters the chip through the upper chamber and leaves it through the lower or vice versa.

This procedure is recommended for cell sorting, cell separation and suspension cell culture of mammalian cells, yeast and bacteria. Fluidic operation can be done manually by pipetting or automated *via* pumps.

For cell sorting operations a diagonal flow must be applied, entering through one chamber and leaving through the other. For Co-culture, a parallel flow can be applied. For the parallel flow, please follow the instructions in chapter 3. For diagonal flow, please read the instructions in this chapter.

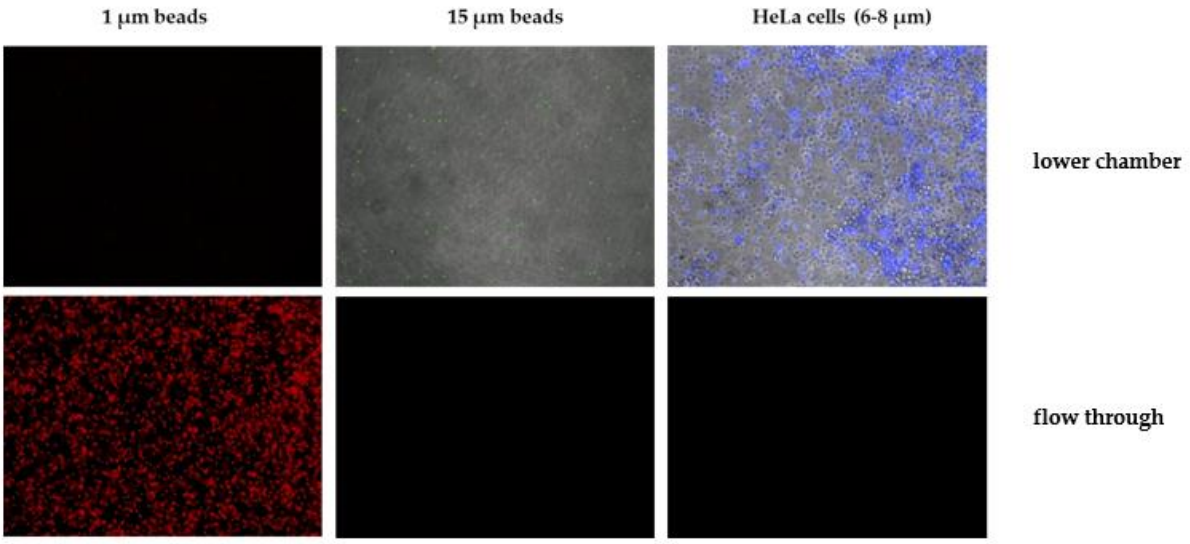

Figure 8: Particle separation via cross flow membrane module. The 15 µm beads and HeLa cells were trapped by a 3 µm pore size membrane, while 1 µm beads passed this barrier.

4.1 Operation *via* pipetting

4.1.1 Accessories

For the operation of the chip various accessories are at hand:

- Male Mini Luer plugs – low volume displacement (product code: 10000205)
- Male Mini Luer fluid connectors (product code: 10000116)
- Handling frame (product code: 10000041)
- Silicone tube, ID: 0.76, OD: 1.65 mm (product code: 10000031)
- PTFE tube, ID: 0.5 mm, OD: 1.0 mm (product code: 10000032)
- Mini Luer to pipette adapter (product code 10000057)
- Waste container or other appropriate vessels

4.1.2 Procedure

- **Instructions for optional equilibration** and **seeding of the cells** are identical to chapter 3.1.2

- **Liquid exchange**

  Media can be exchanged with two different protocols. **Either** a diagonal flow is applied leading the media through the membrane **or** a flow parallel to the membrane is applied. Or the parallel flow, please follow instructions in chapter 3.

  - For reasons of better understanding this example describes liquid exchange from the lower chamber to the upper chamber. For liquid exchange in the opposite direction please exchange B for A and A for B in the protocol.
  - Open interfaces B1 and A2 and connect A2 with the waste container
  - Pipet the appropriate liquid (medium, washing solution, treatment solution) into B1
  - Use approximately the 2-fold chamber volume to ensure a complete liquid exchange
  - Seal the interfaces with plugs and incubate at desired conditions
  - **Note:** Take care of the flow rate, it should be low to prevent damages of membrane and cells
  - **Note:** To minimize evaporation rate during incubation, the filling of half of the Mini Luer interface with the used liquid is recommended

4.2 Fluidic operation *via* syringe pumps

Filling, equilibrating and seeding is easiest by pipetting. In case a very slow and steady flow is necessary, syringe pumps may be convenient. When syringe pumps are used for long term experiments, exchange the syringe and media regularly to avoid contamination.

4.2.1 Accessories

For the operation of the chip various accessories are at hand:

- Male Mini Luer plugs – low volume displacement (product code: 10000280)
- Male Mini Luer fluid connectors (product code: 10000094)
- Handling frame (product code: 10000041)
- PTFE tube, ID: 0.5 mm, OD: 1.0 mm (product code: 10000032)
- Silicone tube, ID: 0.5, OD: 2.5 mm (product code: 10000033)
- Waste container or other appropriate vessels
- Syringe pumps and appropriate syringes

4.2.2 Procedure

- Upper and lower chamber can be utilized simultaneously or in sequence (***Figure 9***)
- **Note:** For Installation of the syringe pumps and syringe follow the manufacturers instructions
- **Optional equilibration** of the cavities (recommended, avoids air bubble formation within the growing chamber)
    - Follow the instructions given in *3.1.2 Procedure*

- **Seeding** of cells in upper/lower chamber
    - **Note:** When working with adherent cells: The lower chamber has to be seeded first and the chip has to be placed upside down in the handling frame (sealed interfaces facing down), to ensure the contact of the cells with the PET membrane. Please give the cells appropriate time to adhere.
    - For reasons of better understanding this example describes the seeding in the lower chamber. For seeding cells in the upper chamber please exchange A for B and B for A in the protocol.
    - For seeding cells in the lower chamber, seal the interfaces of the upper chamber with Mini Luer plugs.
    - Place the chip in a handling frame
    - Connect outlet B2 with waste container or similar *via* tubing and Mini Luer fluid connector
    - Pump an adequate volume of cell suspension in a defined cell concentration into inlet B1
    - Remove all connections and seal interfaces B1 and B2 with Mini Luer plugs
    - Incubate the chip at desired conditions

- **Liquid exchange**

Note: Liquid exchange might be necessary for medium exchange, washing or treatment of cells

- For reasons of better understanding this example describes liquid exchange from the lower chamber to the upper chamber. For liquid exchange in the opposite direction please exchange B for A and A for B in the protocol.
- Connect B1 with the syringe pump *via* tubing and Mini Luer connector
- **Note:** Avoid air bubble formation in the tubing connections. Fill the tubes by pumping the liquid up to its end before connection
- Open outlet A2 and connect it with the waste container
- Pump the appropriate liquid (medium, washing solution, treatment solution) into B1
- Use approximately the 3-fold chamber volume to ensure a complete liquid exchange
- Remove all connectors and tubes from the interfaces
- Seal the interfaces with Mini Luer plugs and incubate at desired conditions
- Note: For long-term experiments or continuous fluidic operation over a longer period of time, the tubing should be selected in a way that the chip can be stored in the CO_2 cell incubator for incubation or utilize MFCS LoC-CCI1 (see Figure 6). The amount of medium needed must be calculated accordingly.
- **Note:** Take care of the flow rate, it should be low to prevent damages of membrane and cells
- To minimize evaporation rate during incubation, the filling of half of the Mini Luer connection with the used liquid is recommended

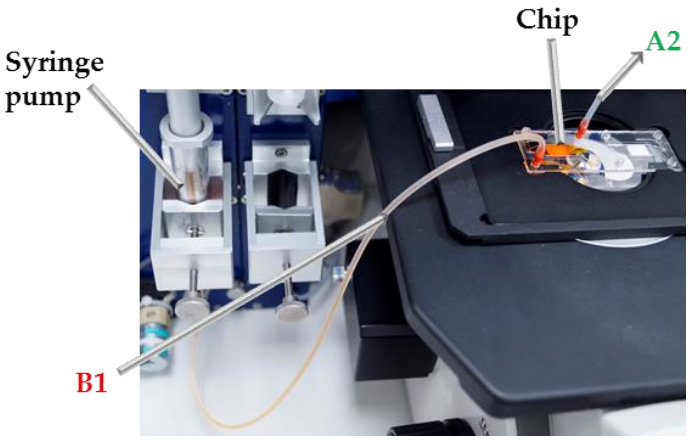

Figure 9: Experimental setup for diagonal flow. The chip is placed on a microscope tray and connected to a syringe pump through inlet B1 and to a waste container through outlet A2. Through the use of B1 and A2, the fluid fills the lower chamber, rinses the membrane and leaves the chip through the upper chamber afterwards.

5 Co-cultivation

Co-cultivation of different cell types per unit is possible for the following combinations (Table 2). Please note the recommendations for the single applications as given below in Table 2.

Table 2: Co-cultivation

Cell type and growing chamber	Adherent cells, upper chamber	Adherent cells, lower chamber	Suspension cells, upper chamber	Suspension cells, lower chamber
Adherent cells, upper chamber		x		(x)
Adherent cells, lower chamber	x		(x)	
Suspension cells, upper chamber		(x)		-
Suspension cells, lower chamber	(x)		-	

"**-**": not possible

"**X**": recommended

- For adherent cell-types, follow the instructions of the chapter *"**3 Assay procedures for dynamic operation**"*.

"**(X)**": recommended with limitations

- For one adherent and one suspension cell-type follow the instructions as given in "**4 Assay procedure for cell sorting, cell separation and suspension cell culture (mammalian, yeast & bacteria)**".
- **Note:** Adherent cell-line has to be seeded first. After a cell-line dependent adhesion and growing time, suspension cell-line can be seeded. Liquid exchange has to be conducted for the suspension cell culture first, followed by the adherent cell line.

6 Incubation

Cultivation can be conducted at a standard CO_2 humidified incubator or equivalent incubation chambers. To reduce the risk of evaporation effects, the chip can be placed in a wet chamber during incubation. In general, it is recommended to fill the Mini Luer interface halfway with the appropriated liquid to prevent evaporation. It also prevents bubble formation/inclusion when closing the ports with plugs or utilizing connectors. Medium exchange depends on cell-line and metabolism but is necessary every 24h, in general. To increase humidity, the chip may be placed on a wet tissue.

7 Available cross-flow membrane chips

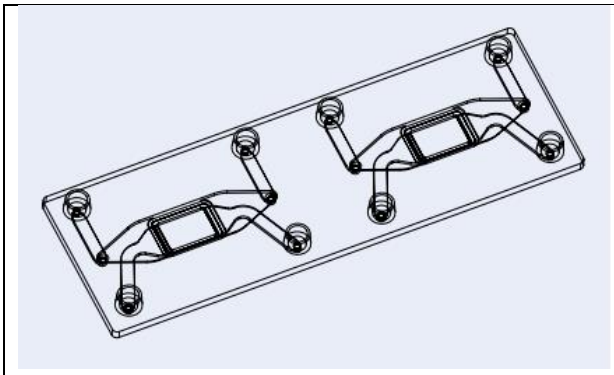

Figure 10: Cross-flow membrane chip 480

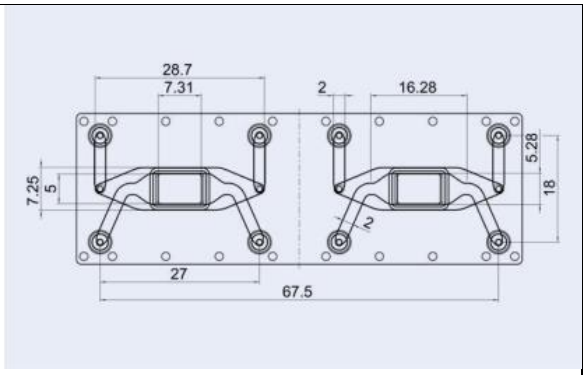

Figure 11: Cross-flow membrane chip 480 with dimensions

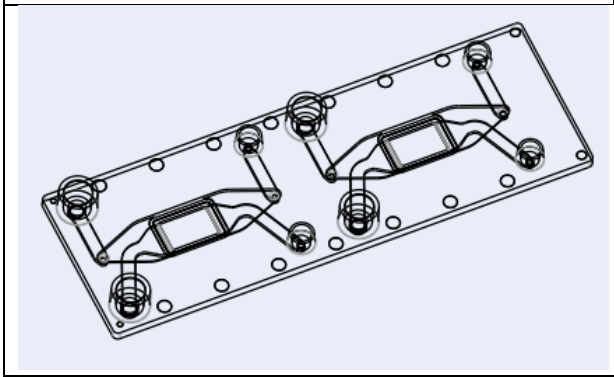

Figure 12: Cross-flow membrane chip 568

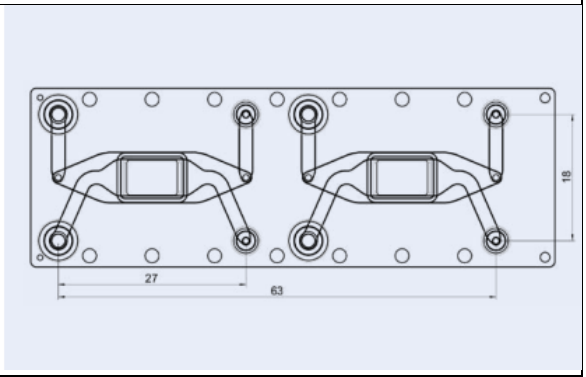

Figure 13: Cross-flow membrane chip 568 with dimensions

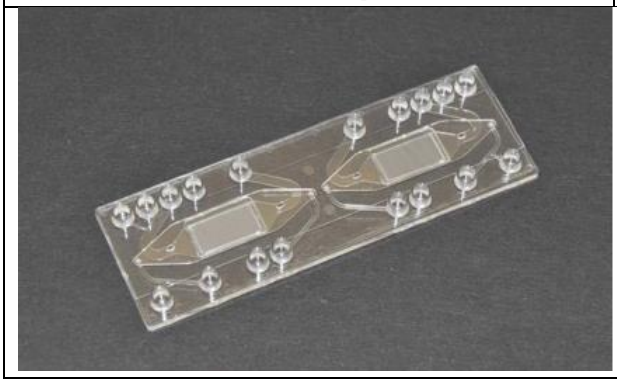

Figure 14: Cross-flow membrane chip 653

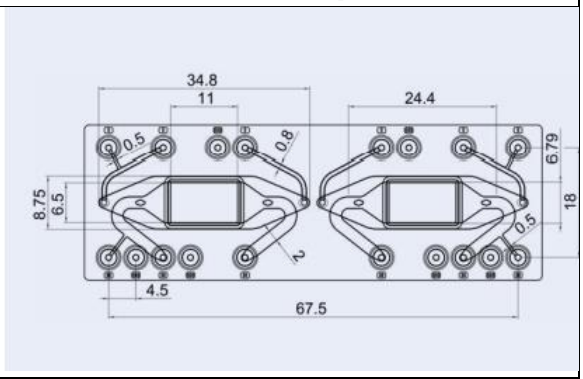

Figure 15: Cross-flow membrane chip 653 with dimensions

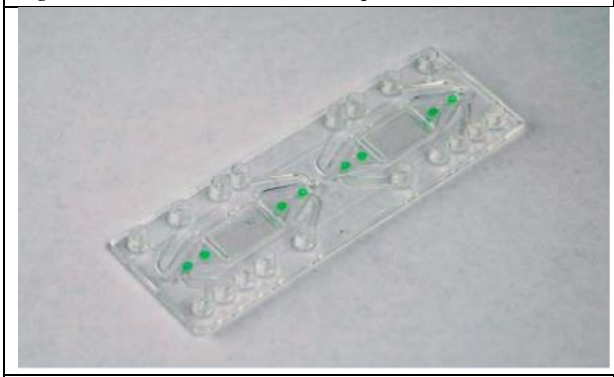

Figure 16: Cross-flow membrane chip 653 with oxygen-sensor spots

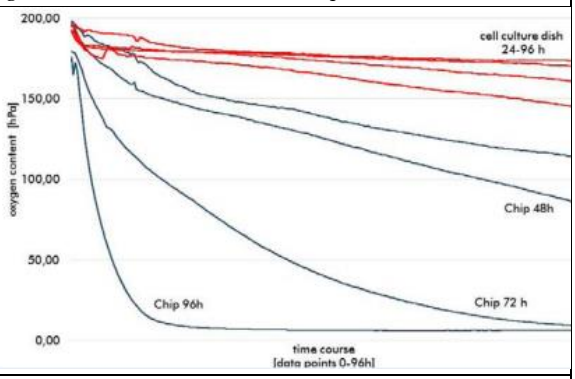

Figure 17: Oxygen consumption in cell-culture disc versus oxygen consumption on chip measured with integrated oxygen sensors on chip. The curves show the oxygen concentration over 24 h intervals.

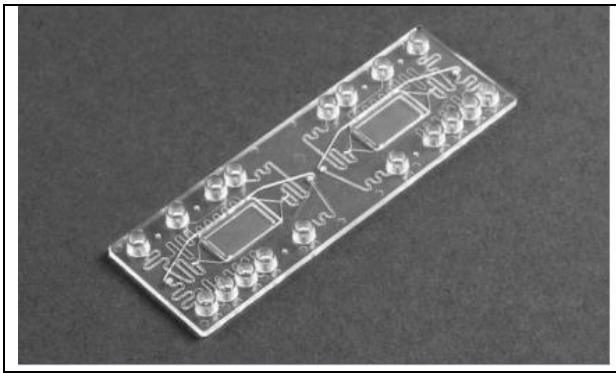

Figure 18: Cross-flow membrane chip 694 with additional in- and outlet channels

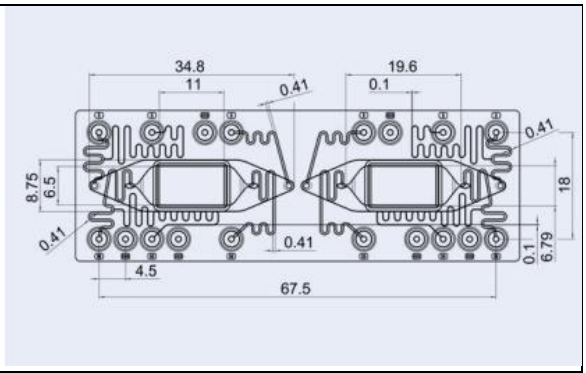

Figure 19: Cross-flow membrane chip 694 with dimensions

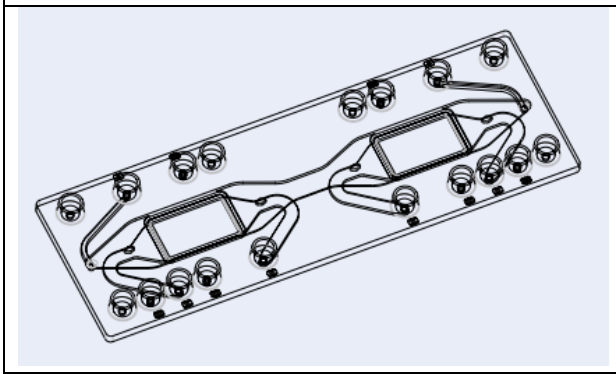

Figure 20: Cross-flow membrane chip 747 with connected cross-flow membrane units

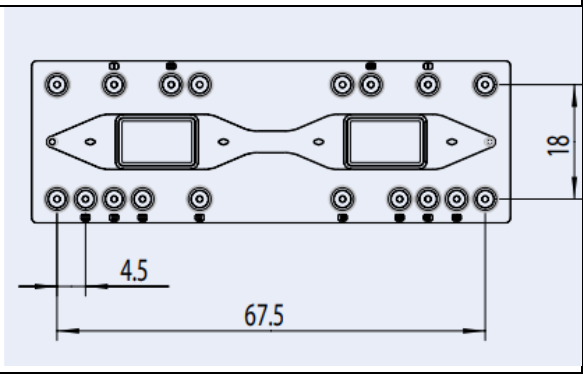

Figure 21: Cross-flow membrane chip 747 with dimensions

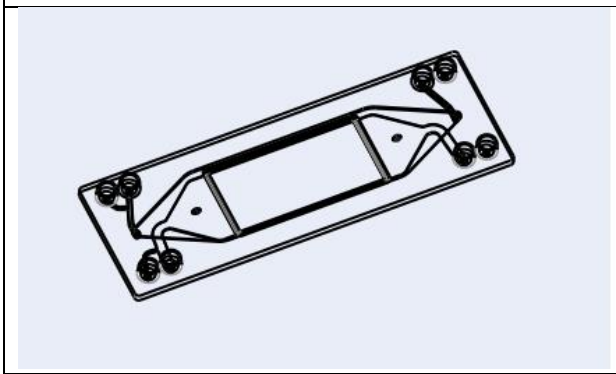

Figure 22: Cross-flow membrane chip 846

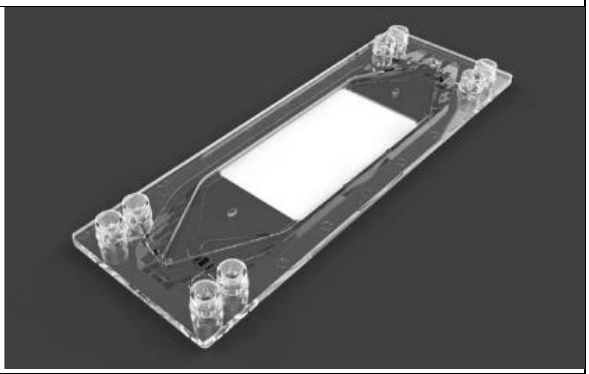

Figure 23: Cross-flow membrane chip 846

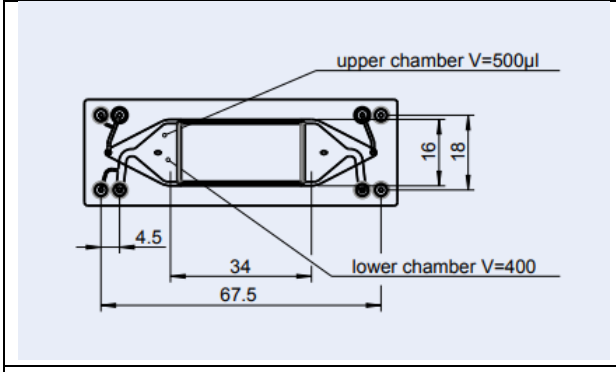

Figure 24: Cross-flow membrane chip 846 with dimensions

8 References

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