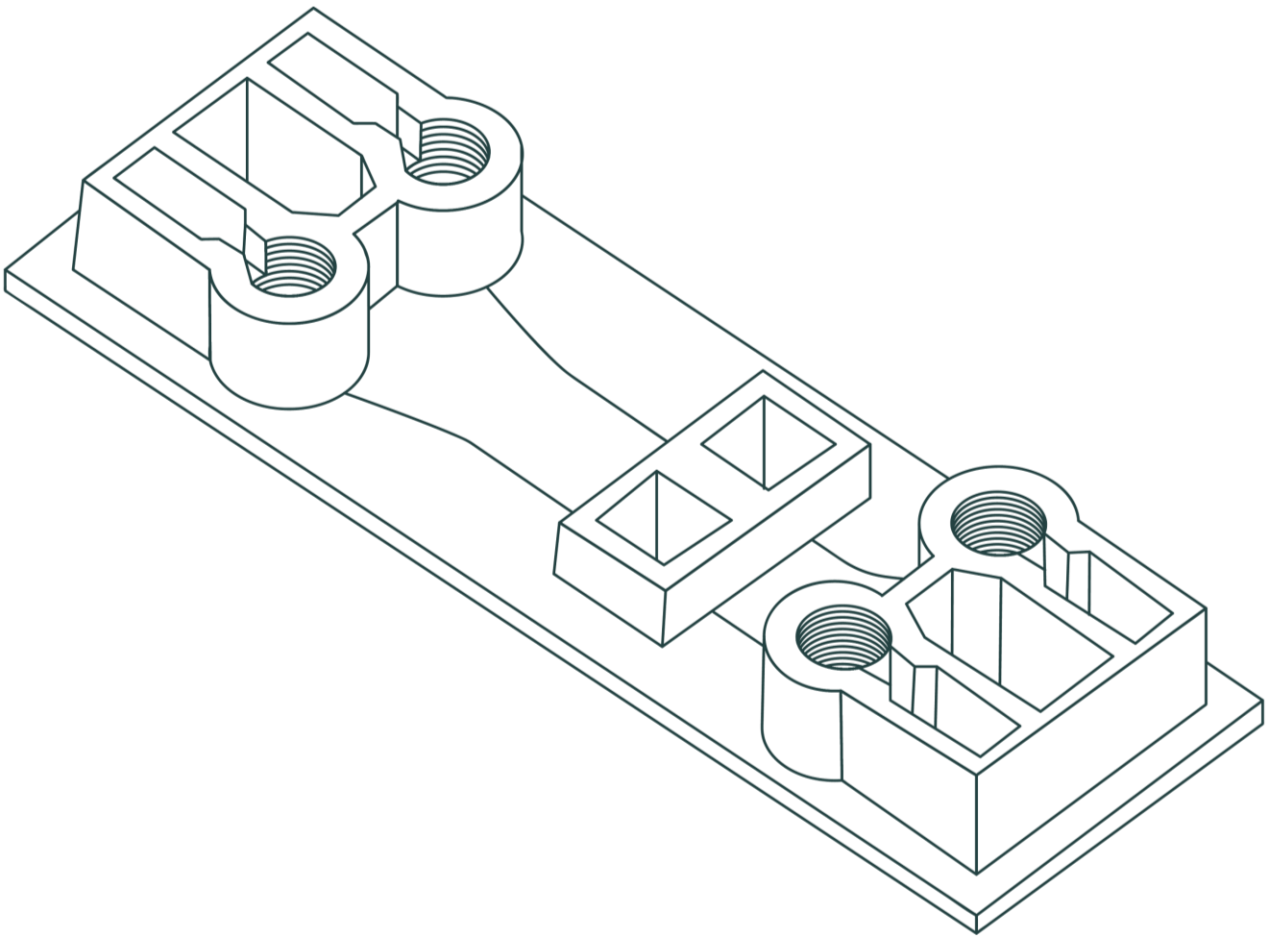
Be-Transflow

Product guide



Beonchip

Organ on chip
made easy.

Table of contents

About Be-Transflow

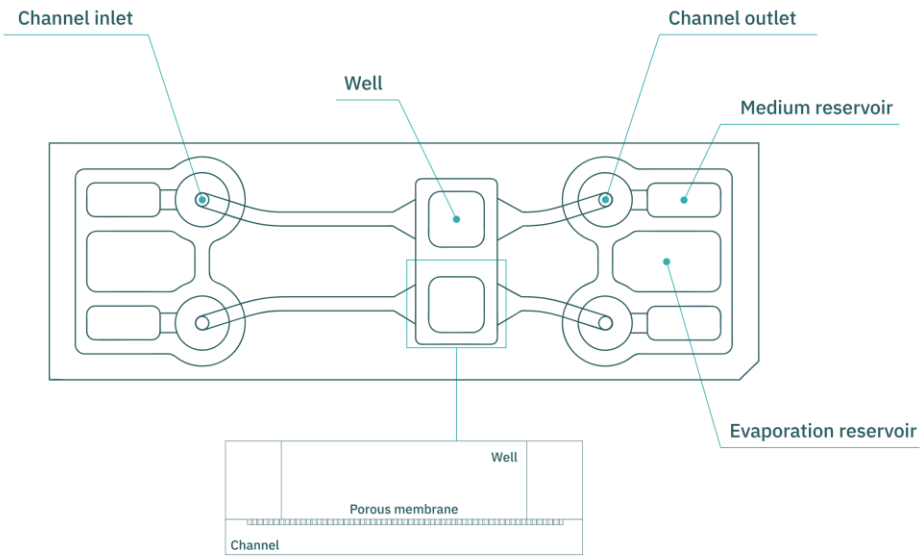
This is our most versatile cell culture platform. It allows the study of complex culture configurations by joining a culture well with a microfluidic channel via a porous membrane. This is the optimal device for Air Liquid Interface (ALI) culture, endothelium /epithelium barrier, and crosstalk studies.

Technical features

The Be-Transflow structure consists of a **culture well** (same volume as one of a 96-well plate) that is **communicated via a porous membrane with a microfluidic channel**. Each chip allows the realization of two independent experiments. It is possible to apply an independent flowrate in both channels connecting any perfusion system with the chip using our patented inlet/outlets that avoid the entrance of bubbles to the channel. Evaporation reservoirs are next to the medium reservoirs to be filled with PBS/water during the incubation before closing the system with tubes.

	Height	Width	Length	Total volume
Channel	375 µm	1,5 mm	45 mm	44 µL
Well	6 mm	5,7 mm	5,7 mm	195 µL
Inlet/Outlet	7 mm	UNF 1/4" - 28		130 µL
Medium reservoir	5 mm	3,6 mm	8,8 mm	185 µL
Membrane pore size	1 µm			

* The volumes presented in the table are theoretical values calculated for the standard products. Changes in the device features of custom chips may modify the exact channel volume.



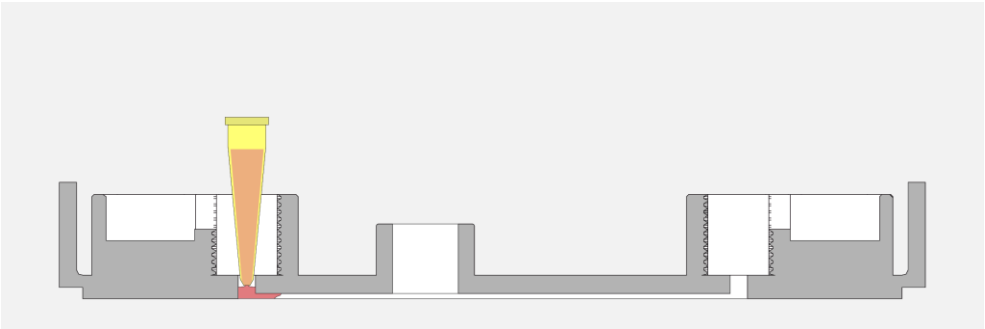
Content

The box contains **10 Be-Transflow** chips that are **individually packaged**. Each chip undergoes a sterilization process before sending the chip to the final user. It can be stored in **dry places** that are **not exposed to direct sunlight** at room temperature **(15-25ºC)**. The devices are **single-use** only, and so not reusable.

Filling and handling

Before seeding, prewarm the device in the incubator overnight to avoid the appearance of air bubbles.

When filling the channels up, **place the tip of the pipette completely vertical** into the channel's inlet pinhole. Inject the liquid with a **continuous and constant flow** to avoid backward flow.



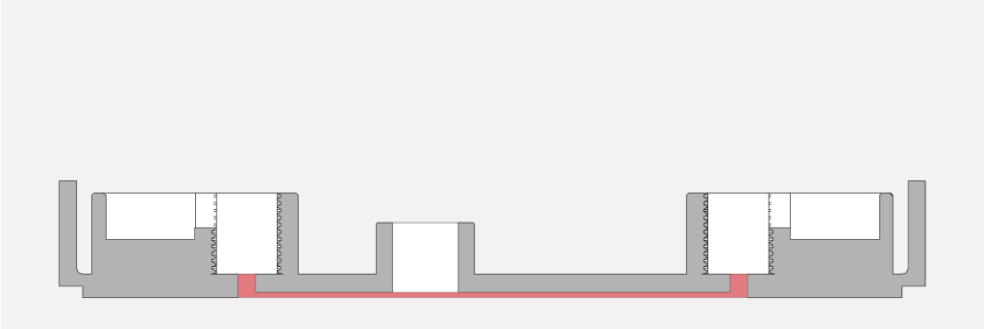
Use the **lateral holders** for easy and safe handling.

Coating

Depending on the cell type(s), one or two different coatings may be necessary. Hinging on the coating you need to apply, applying it inside the channel and in the well might take place at the same time previous to cell seeding.

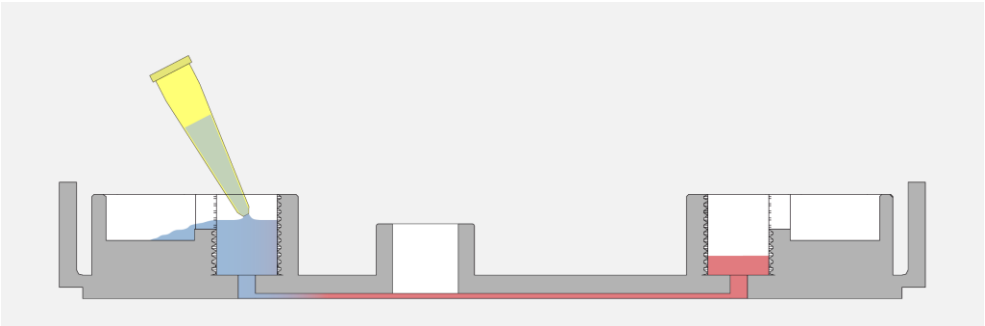
Channel coating

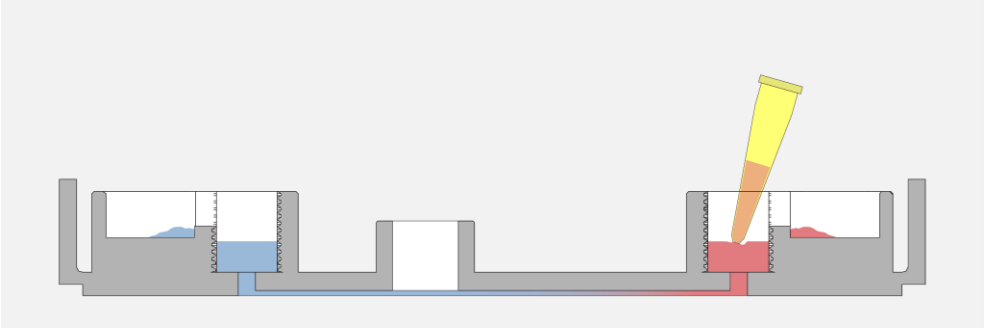
1. Fill the channel volume up with the **coating solution** by pipetting through the pinhole and incubate at manufacturer's conditions.



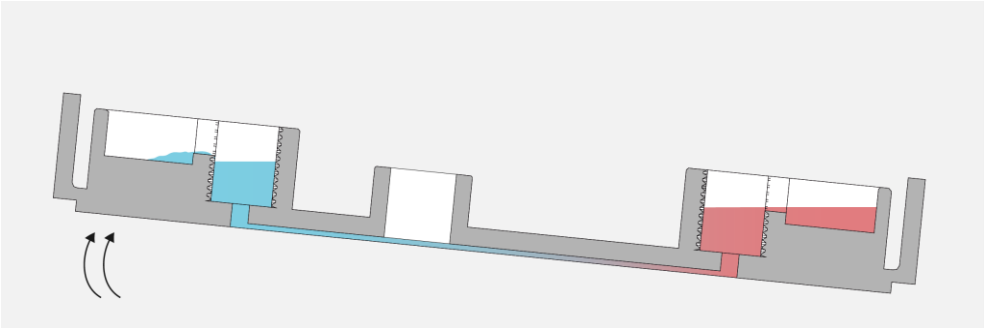
> **Tip**: When seeding a monolayer underneath the membrane, flip the chip downwards and incubate for better coating deposition on the membrane. In this case the volume must be the one that fits in the channel.

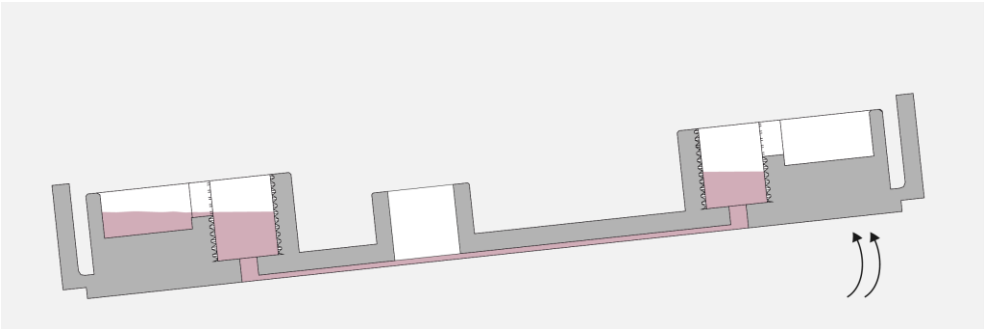
2. Remove the solution from the reservoirs and inlet/outlet wells and **wash the channel** (if needed) by adding the recommended dilution buffer into the inlet well. Remove it at the outlet well with the pipette. **Repeat** this step **as many times as needed**.



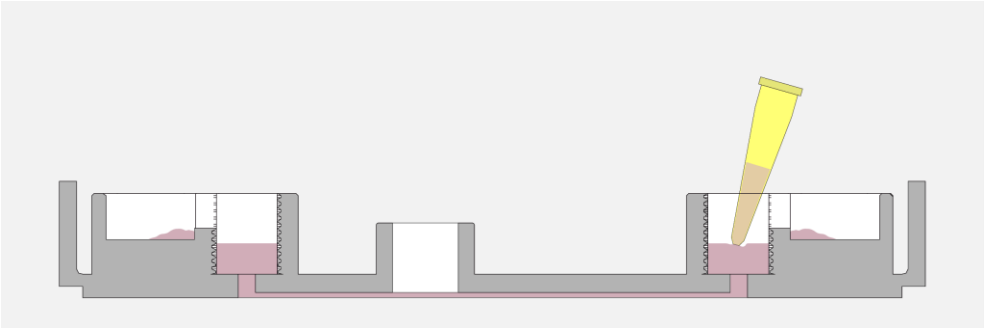


Tip: You may **tilt the device** manually in order to ease the dilution buffer **flow several times** from one reservoir to another before you remove it.





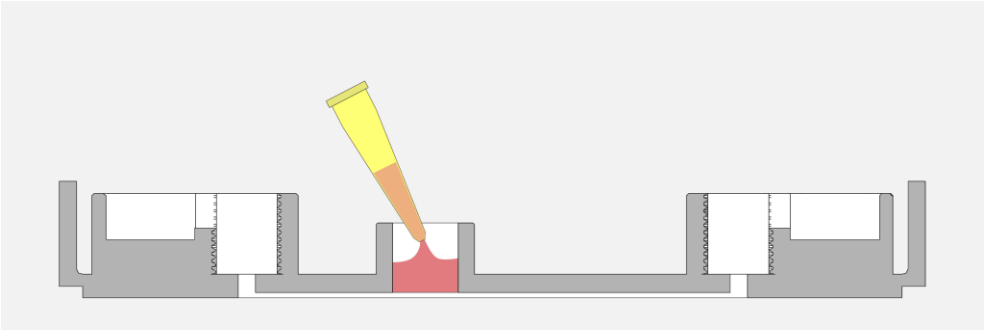
3. Aspirate the dilution buffer completely before seeding.



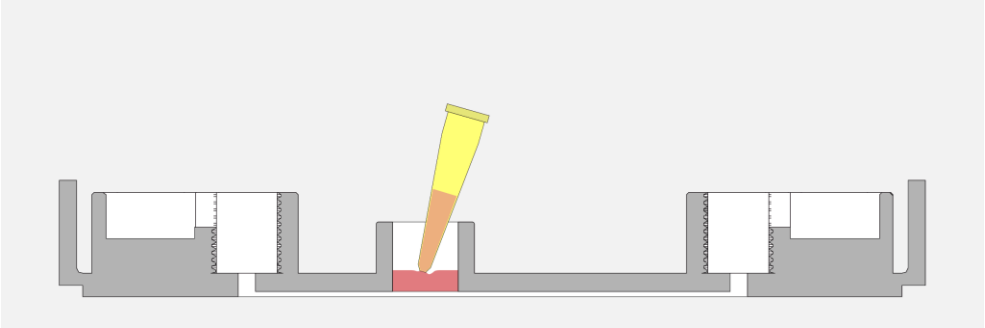
Important: Note that the channel should never dry up. Take incubation times into account and use the evaporation reservoirs as long as possible.

Well coating

4. Add your coating solution to the well and proceed as you would in a culture well.



5. Remove any remaining volume and reserve until needed.



Cell seeding / Cell culture

In case of seeding cells both, inside the channel and in the well, it is recommended to carry out the culture first in the channel and then in the well as follows:

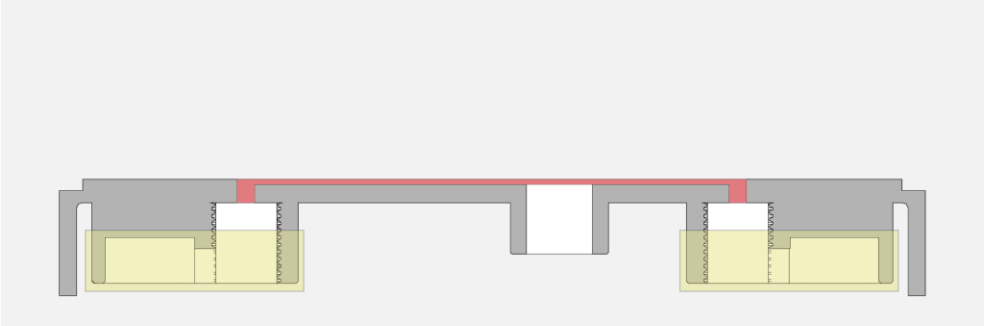
Channel seeding: underneath the membrane

1. Trypsinize and **count cells** as usual. Cell concentration will vary with **cell type** and **channel dimensions**. For 2D culture, it is recommended to seed the cell concentration needed to obtain a **monolayer**.

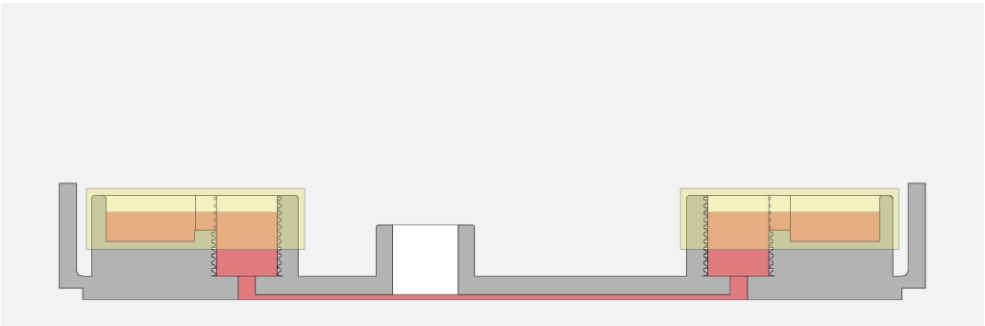
2. Fill the lower channel up with the **exact volume** of the channel by pipetting through the pinhole.

> **Important** Adding the correct volume into the channel during seeding helps attaching cells to the lower side of the membrane.

3. Cover the inlets, **flip the chip downwards** and **incubate until the cells attach** (time may vary depending on the cell type).



4. After cell attachment, **add more medium** to the medium reservoirs. **Cover and incubate** until needed.



Remember: Beonchip membranes are not impermeable, they are porous. During 2D culture, if the monolayer underneath the membrane is not completely confluent, medium may go through the membrane.

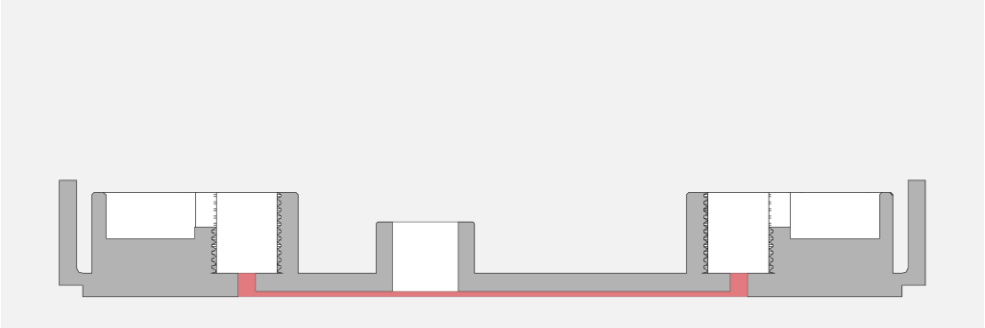
For optimal seeding inside the channel, we recommend a constant renewal of the medium once the cells have adhered to the surface of the device (from two to six hours after seeding).

Culture well seeding

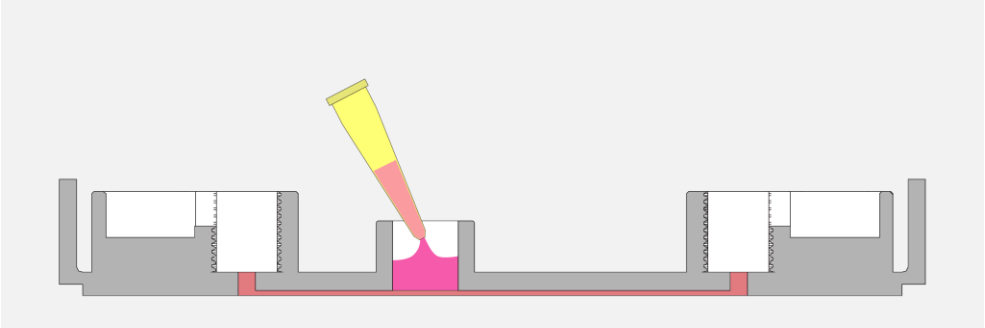
2D culture

When coating on the membrane just before cell seeding, we recommend performing the following protocol:

5. Remove the medium almost completely from the reservoirs leaving a very small volume in the inlet/outlet. This will prevent the medium from going from the channel to the well when adding the coating to the well.



6. Add your **coating solution** to the well and proceed as you would in a regular culture well.

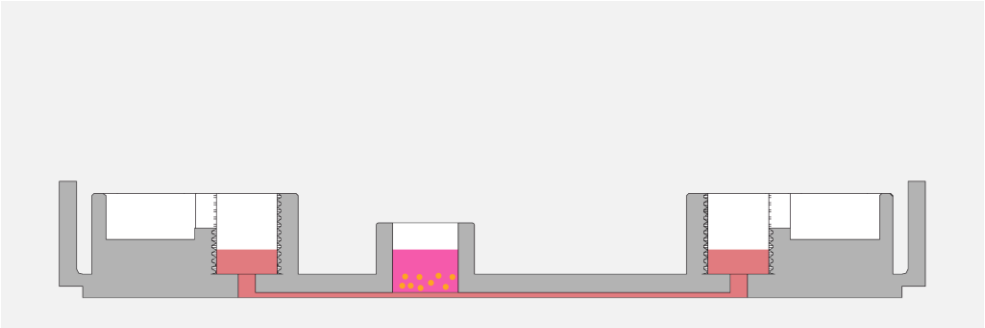


7. Pippete the cells into the culture well.

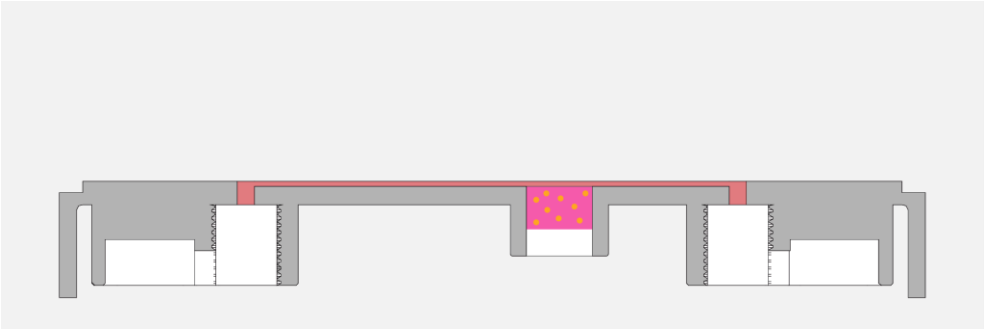
8. After cell attachment, **add more medium** to the medium reservoirs.

3D culture

9. In case of **seeding cells embedded in a hydrogel** in the culture well, **flip the chip upwards and downwards every 10 seconds** for a few minutes (depending on the hydrogel polymerization time) for homogeneous **3D distribution** and proceed according to the specific hydrogel protocol.

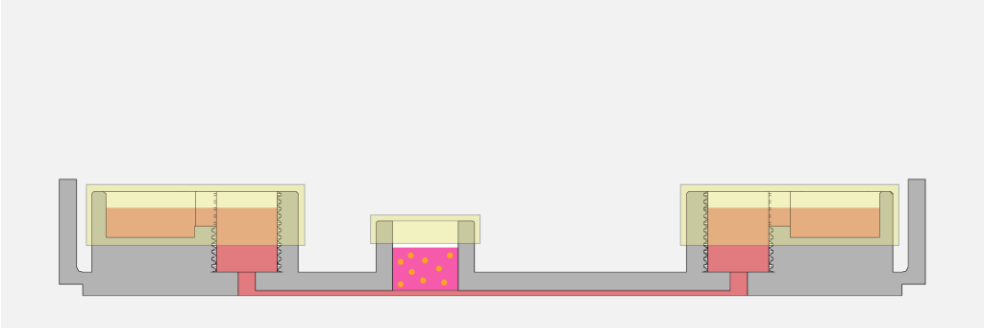


Make sure that all the medium **has been depleted** from the reservoirs and inlet/outlet to avoid spilling medium when flipping the device.



10. Add more medium to the reservoirs and culture well and PBS or water to the evaporation reservoirs. Cover the device and **incubate until needed**.

11. Refresh the medium according to cellular requirements.



Important: Do not remove the medium inside the channel during medium refreshing. This will prevent the entrance of air bubbles. Thus, when removing the solution, **place the tip of the pipette away from the pinhole** to avoid emptying the channel.

When using Be-Transflow in long-lasting experiments we recommend using a **flow control system** of your choosing (rocker, syringe, peristaltic, pressure-based..) to **avoid the depletion of nutrients** in the media beneath the well.

Flow set up

Before setting the flow up:

- **Sterilize and prewarm** the tubes and the fluidic elements overnight at 37°C.
- Set the system in a **laminar flow cabinet**.
- **Cells must be well adhered** to the surface before mounting the perfusion system.
- The channels and inlet/outlet wells should **never be depleted of culture medium**.
- Both inlets and outlets are designed to be for **screw connectors (1/4'' - 28)**.

For more information regarding connecting a microfluidic flow system check our website. Link.

Connecting a perfusion system

- **Remove the medium** from the reservoirs.
- **Screw the outlet connector** and ensure that the tube is perfectly fixed. **Remove the displaced medium** from the reservoir.
- **Prime the tube** that will **connect to the inlet** before assembling. This will **prevent air bubbles** from entering the device.
- **Screw the inlet connector** and remove the displaced medium from the reservoirs.
- Once the system is closed, **switch the flow on.**

Check that there are no leaks in the system by leaving the pump running for a couple of minutes before placing the device in the incubator. Medium reservoirs will be filled up if the connections are not completely closed.

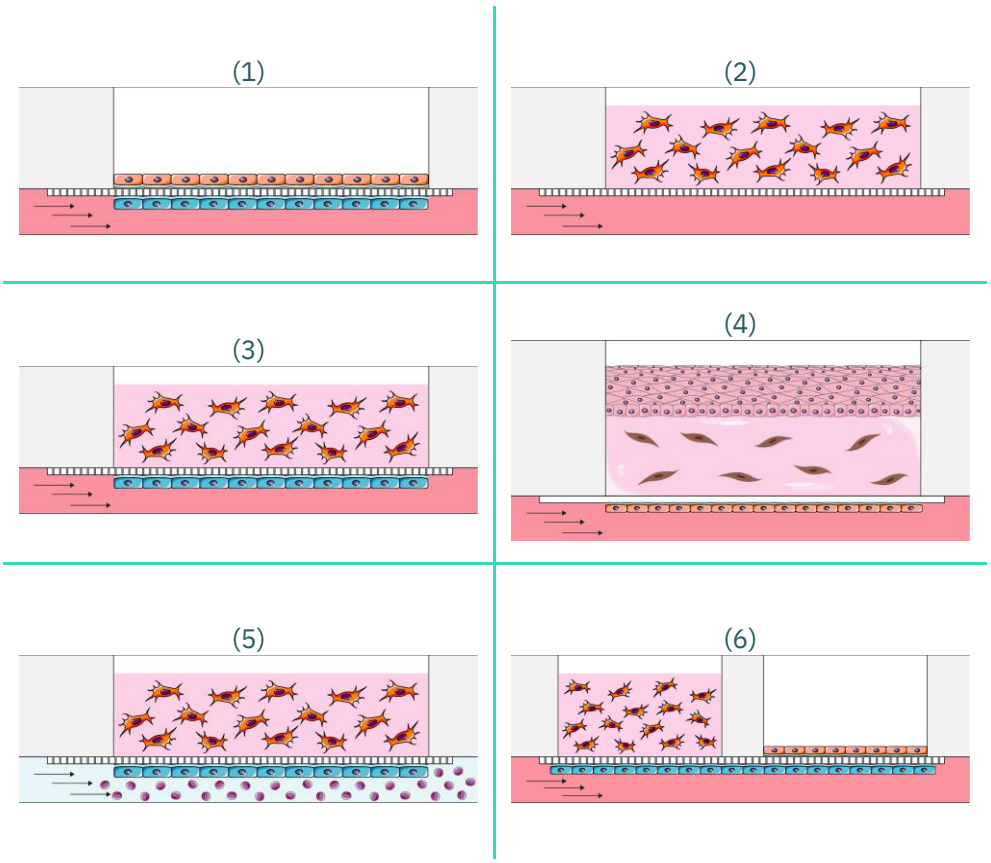
Culture models

Find several of the most frequently used culture models on the Be-Transflow below.

Depending on the assay, either monoculture or coculture can be performed. **Seeding a monolayer** over and/or below the membrane with **different cell types (1)**. For more realistic assays it is possible to **add a hydrogel for 3D cell culture (2) or combine 2D and 3D (3)**.

Be-Transflow device is ideal for developing **air-liquid interface (ALI) models**, exposing the cells of the well to air and maintaining the supply of medium through the flow in the channel **(4)**.

Other models include **circulating particles** such as cells of the immune system **(5)** or **crosstalk assays** by connecting in series the two experiments that the device provides **(6)**.



Contact and support

Our team of technicians will be happy to help you with any questions about the operation of our devices. **You can contact us on the following channels:**

info@beonchip.com
+34 655 16 16 91
www.beonchip.com

Beonchip S.L.
Campus Río Ebro
Mariano Esquillor s/n
Edificio CEMINEM
50018, Zaragoza, Spain

Beonchip