BEOnChip
Biomimetic Environment On Chip

BE-GRADIENT

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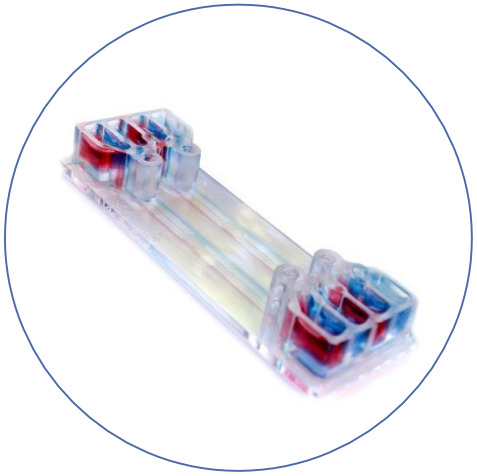
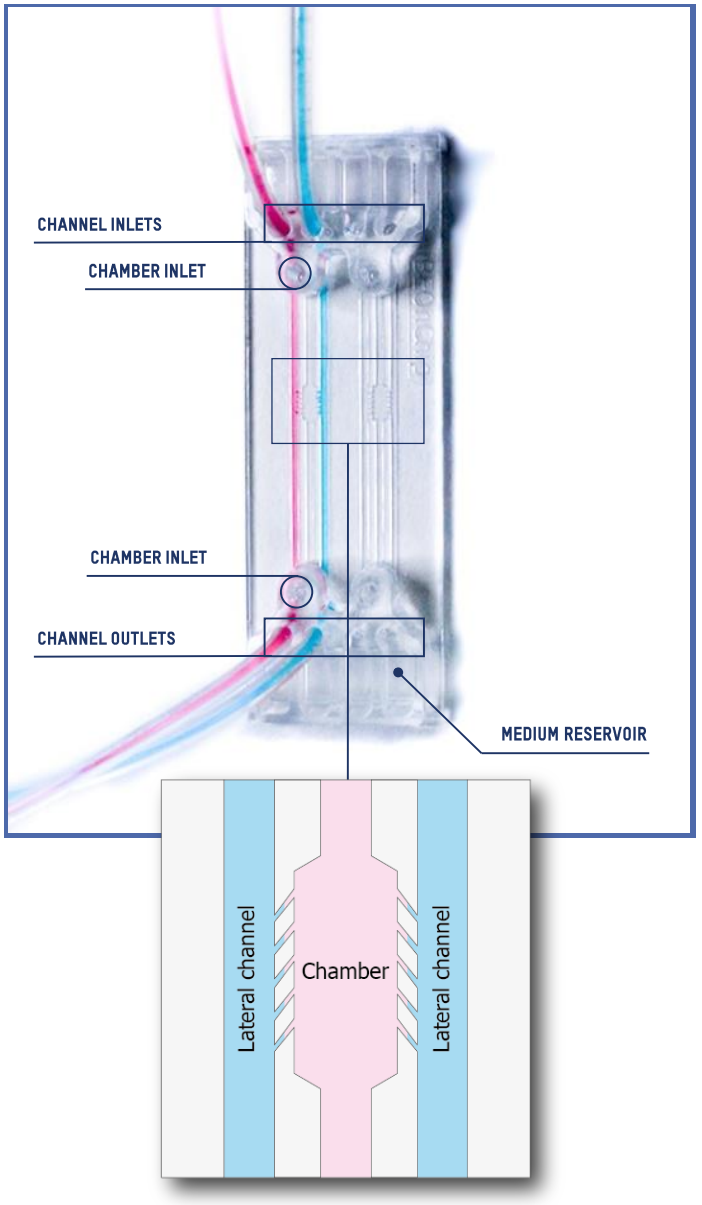
BE-GRADIENT



BE-Gradient is our device designed for the application of **electrochemical gradients to 3D cell cultures**. It is compatible with any type of **optical microscopy** (inverted phase contrast, confocal, fluorescence...). Be-Gradient consists of a **central chamber for cell culture** and two lateral channels connecting to the central chamber through 3 small microchannels. Lateral channels are meant to simulate blood vessels. **2D culture** is also possible for adherent cells not only in the central chamber but also in the lateral channels.



TECHNICHAL FEATURES

BE-Gradient chips allow **2 independent experiment** per chip. Be-Gracient has a horizontal structure for **optimal imaging**, having all channels at the same height. The central channel contains **the culture chamber for 3D culture**. The two lateral channels shown in the image above serve for **media perfusion** and are communicated with the central chamber via 100 µm wide microchannels, allowing nutrient supply to the central chamber. Changing the concentration of one element between the channels on both sides of the central chamber results in the creation of a **chemical gradient** of that element in the central chamber. For further instructions regarding proper use of the chip check the following sections.

CONTENT

The box contains **10 BE-Gradient** 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** which are **not exposed to direct sunlight** at room temperature **(15-25ºC)**.

	Height	Width	Lenght	Total volume
Central channel	300 µm	1 mm	39 mm	12,6 µL
Lateral Channels	300 µm	1 mm	50 mm	14,5 µL
Chamber	300 µm	2 mm	4,6 mm	3 µL
Inlet/Outlet	8 mm	Ø = 2,3 mm		18,4 µL
Reservoir	6 mm	3,6 mm	7 mm	151,2 µL

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

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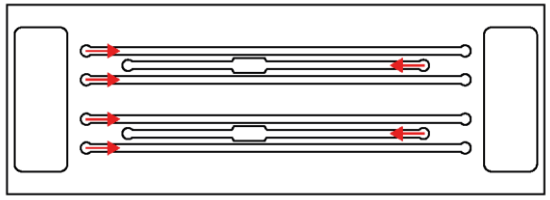
FILLING AND HANDLING

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

Fill the channels using the inlets highlighted in the image bellow, making sure the direction of the flow corresponds with the direction indicated by the arrows (A).



A

The central **culture chamber must be completely dry** in order to confine the hydrogel in it.

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

Important: Note that the channel should never dry up.

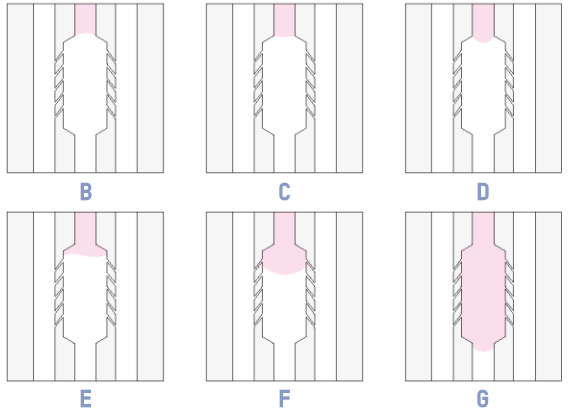
CELL SEEDING/CELL CULTURE

Cell culture without tubing connection

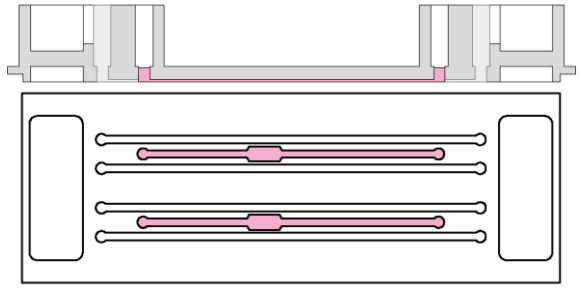
1. Trypsinize and count cells as usual.

2. Inject mixture of cells and hydrogel (25µL to fill the channel up) in the culture chamber by **pipetting through the central inlet**.

3. Inject the solution slowly into the chip (B, C). First, the meniscus will enter into the chamber (D, E). Continue until the chamber is completely filled (F, G).



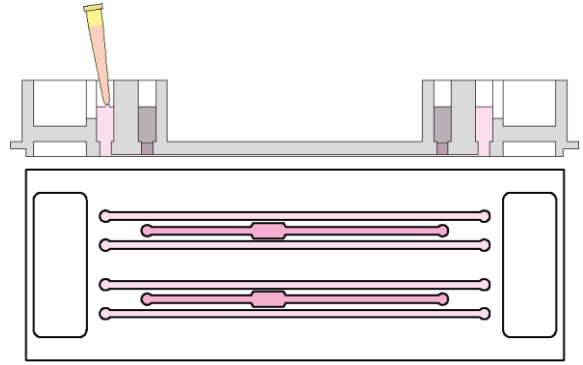
B C D E F G



4. Flip the chip upwards and downwards every 10 seconds for a few minutes for **homogeneous 3D distribution** during the polymerization of the hydrogel. Incubate at manufacturer's conditions.

5. Add medium to the central chamber inlet and outlet to avoid hydrogel evaporation.

6. Add medium to the reservoirs to fill the lateral channels. **Cover and incubate** until needed.



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CELL SEEDING/CELL CULTURE

Cell culture without tubing connection

7. Refresh medium according to cellular requirements. You may refresh medium manually at different time points or constantly with a rocker.

Remember that COP has very low gas permeability so oxygen is only supplied through lateral channel medium.

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.

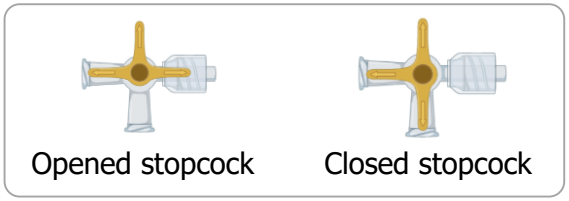
Refreshing medium is required to avoid oxygen and nutrients depletion in the media close to the culture chamber.

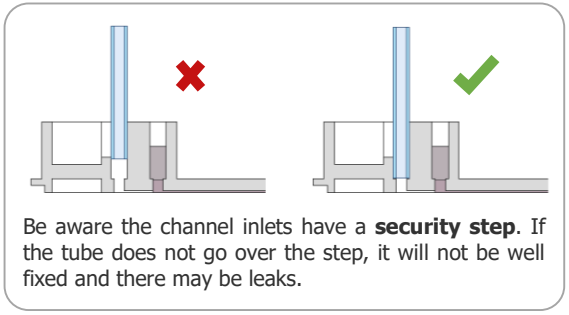
Important: Note that the channel should never dry up.

CELL SEEDING/CELL CULTURE

Cell culture with tubing connection

1. Before seeding. **Pre-warm the device and the tubes** overnight in the incubator.





Be aware the channel inlets have a **security step**. If the tube does not go over the step, it will not be well fixed and there may be leaks.

Set the tubes and stopcocks within inlets/outlets of lateral channels **before hydrogel injection**. *(When possible, pre-warm the device already connected over the previous night).*

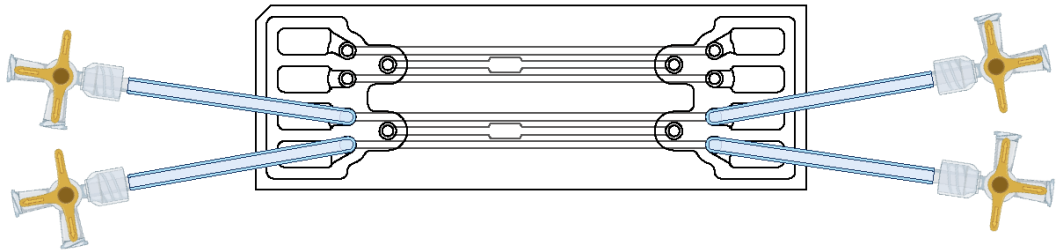
2. Inject mixture of cells and hydrogel (25µL to fill the channel up) into the central chamber. Make sure that it reaches the outlet; otherwise, bubbles may appear.

Make sure the **stopcocks are closed** before injecting, blocking the lateral channels. This will prevent hydrogel leaking and assure complete filling until the outlet.

3. Flip the chip upwards and downwards every 10 seconds for a few minutes for homogeneous 3D distribution during the polymerization of the hydrogel. Incubate at manufacturer's conditions with **opened stopcocks**.

Important: If the system is closed, the air from the lateral channels will expand with temperature and **hydrogel integrity is compromised.**

4. Close central channel inlet and outlet with **parafilm**.



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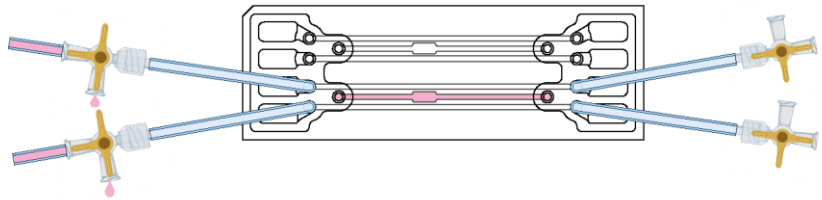
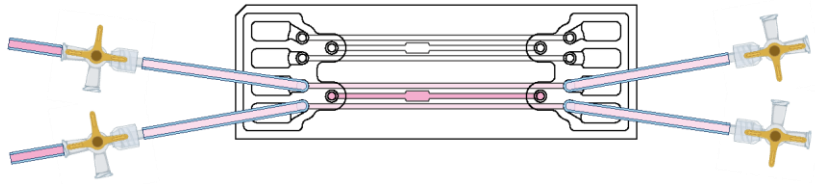
CELL SEEDING/CELL CULTURE

Cell culture with tubing connection

5. Connect the **inlet stopcocks to the medium reservoir/flow system** and set the flow to **prime the tubes**. Inlet **stopcock should be closed** to let the medium evacuate and avoid air entrance into the system.



6. When medium has filled the tube from the perpendicular entrance in both inlet stopcocks, **stop the flow**, **open the inlet and outlet stopcocks** and reactivate the flow to let the medium pass through the lateral channels.



Important: Note that the channel should never dry up.

Cell culture with tubing connection may be performed without three-way stopcocks (**Be-Gradient: Cell culture**). Using three-way stopcocks is optional but they prevent most common issues:

- Moving the device easier from the hood to the incubator before connecting to the flow system; or microscopy.
- Priming the system without bubbles.
- Achieving simultaneous flow entrance into both lateral channels

Both inlets and outlets are designed to be connected to a flexible tube with an outside diameter of 2.4 mm.

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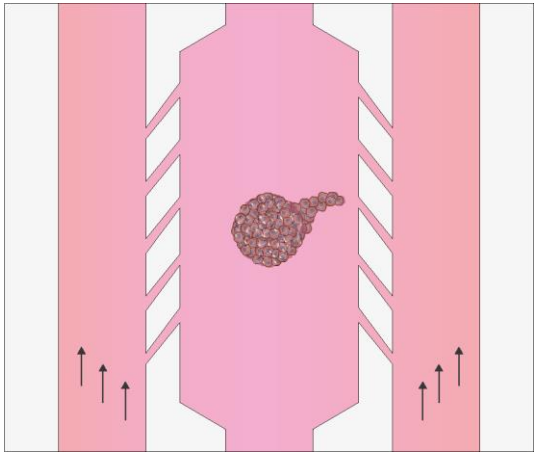
CULTURE MODELS

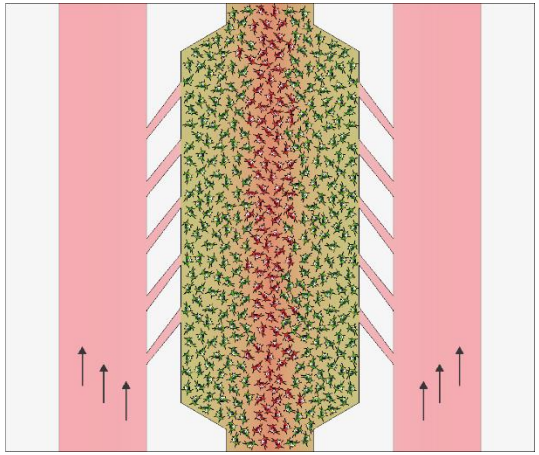
Cell/spheroid **invasion and migration** by chemotaxis stimuli (1)

Ischemia model. Depending on cell type metabolism, cell density, flow conditions, nutrients & oxygen availability, tumour and ischemia models can be performed (2)

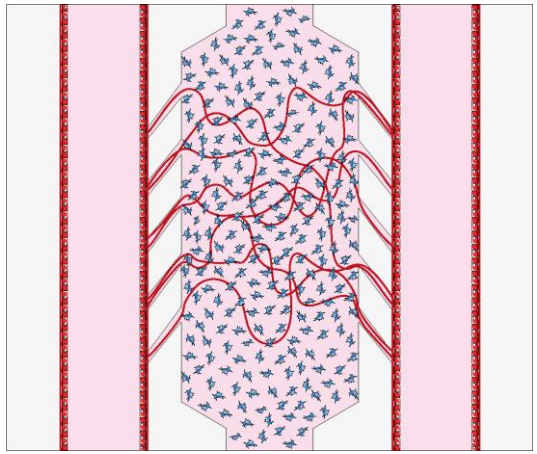
Angiogenesis and **endothelization** (3)



(1)



(2)



(3)

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