BEOnChip
Biomimetic Environment On Chip

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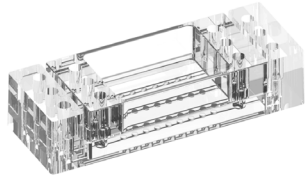


BE-FLOW2D3 is a versatile microfluidic device for cell culture under biomimetic conditions. It allows a combination of a 2D-3D organized coculture with the possibility of establishing flows with or without cells over the epithelium. Our most biomimetic microdevice to copy in vitro different tissue structures.

Examples of applications are immune system in vitro model, Vascular-atheroma plaque formation, Epithelial adhesion.

For further information, please visit https://beonchip.com/be-flow-2d3/ or contact BEONCHIP

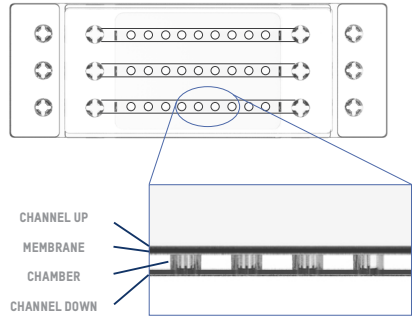
MATERIAL

BE-FLOW2D3 chips are made of biocompatible plastic and are gas-impermeable, for effective gradients of CO_2, O_2, etc. They have excellent optical properties, with high transparency and low auto-fluorescence.

TECHNICAL FEATURES

BE-FLOW2D3 is a combination between Be-Flow and Be-Multiwell so are the same features as in the other devices.

	Channel up	Chambers	Channel down
Quantity	3	27	3
Height	200 µm	700 µm	200 µm
Width	1200 µm	1 mm	500 µm
Volume	6 µL	0,5 µL	3,8 µL
Lenght	24,6 mm	1 mm	37 mm
Area	29,5 mm^2	0,8 mm^2	18,6 mm^2
Total volume	6 µL	9 µL	
Total area	29,5 mm^2	25,7 mm^2	



CONTENT

The product reaches the user sterilized and encapsulated in a Petri dish. There are three BE-GRADIENT devices per Petri dish. It can be stored in dry places which are not exposed to direct sunlight at room temperature (15-25ºC).

CELL CULTURE COATING

BE-FLOW2D3 chips have been treated to obtain a hydrophilic surface that facilitates filling the devices with aqueous solutions and/or gels and promotes cell adhesion.

In case of a certain coating is required, prepare your coating solution (Collagen I, Collagen IV, Fibronectin, Poly-L-Lysine, Poly-D-Lysine…) according to the manufacturer's instructions and apply it into each channel. Aspirate the channel and wash with distilled water to remove excess coating solution by using 5-10 times the volume of the channel.

FILLING AND HANDLING

1. Trypsinize and count cells as usual. A 3-5x106 cells/ml suspension is recommended in order to obtain a confluent layer within 2-3 days.

2. Apply 20-25 µl cell suspension into the upper channel of the microdevice. Await cell attachment; the time required will vary depending on the type of cells.

3. Once cells are completely attached, apply 35 µl into the inlets with fresh medium.

4. Now with the upper channel completely full of medium and cells attached to the membrane, fill the lower channel with fresh medium (70-80 µl).

5. It is recommended to refresh the medium every 24h. For that, aspirate medium with a micropipette and replace it in every channel you are working with.

It is able to perform three independent experiments with different conditions since each channel is isolated from the others in the microdevice.

ASSEMBLY OF THE FLOW SYSTEM

Beonchip has a microfluidic starter kit to set different flow system configurations.

Previous considerations:

1. Set the system in a laminar flow cabinet.
2. Cells must be well adhered to the surface before mounting the flow system.
3. The device should be never left without culture medium inside or in the inlets / outlets.

To assemble the flow system the following will be considered:

1. Fill the inlet area completely with medium so that no air bubbles remain.

2. Prime the system of tubes that reach the inlet before assembling the system.

3. Both inlets and outlets are designed to be able to connect a tube with an outside diameter of 2.4 mm without the need to use connectors. Once this tube system is primed, the tube can be inserted into the inlet. At this point, extreme care must be taken to ensure that no air bubbles enter the system.

4. Finally, the tube is connected to the outlet and thus the system is closed.

5. Check that there are no leaks in the system. To do this, leave the pump running for a couple of minutes before placing the devices in the bioreactor or in the incubator.

To view a video about filling a BE-FLOW2D3 device, please visit https://beonchip.com/be-flow-2d3/

To prevent bubbles from forming during filling, please avoid empty completely tips of pipettes. Hold the plunger firmly while removing the pipette from the inlets so that the negative pressure will not suck the solutions up.

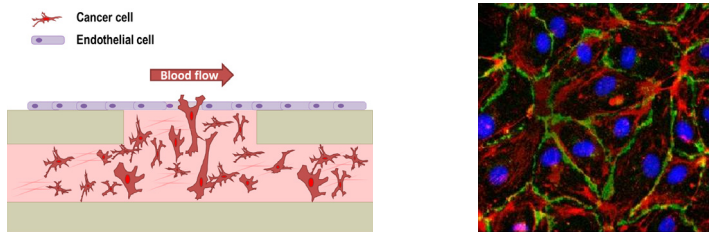
BE-FLOW2D3
INSTRUCTIONS

CELLS SEEDING

Below is an example of using BE-Flow2D3 device for a better understanding of the possibilities of our products:

The design of BE-Flow2D3 consists of three independent channels connected, each of them, by wells with an upper channel. In an assay conducted by our partner, AMB research group from the university of Zaragoza, breast tumor cells embedded in a collagen gel were seeded in the lower part of the device, in such a way that they can grow in a three-dimensional manner. This gel rises right up to the interface with the upper channel, leaving it free and can be used to plant epithelial or endothelial cells that grow in 2D simulating the real disposition in the organism. Through this upper channel, a flow that reproduces the physical stimuli of the tissue can be applied. In this simple way, we can recreate a common blood capillary: a tissue zone (3D cells), a vascular zone (the endothelial cells forming the capillary) and the bloodstream (the upper channel flow). In this experiment it was possible to observe how cancer cells ended up invading the bloodstream simulating a process of tumor methastasis. Cell viability is shown in Figure B.

Outline of cancer cell invasion experiment: (a) illustrative drawing of the experiment (b) image after immunofluorescence.



A B

PREPARATION FOR CELL MICROSCOPY

It is possible to monitor fixed or living cells and also chemical gradients. Most of the monitoring systems used in traditional cell culture can be taken to BEONCHIP microfluidic devices. Common fixatives can be used. Cell viability can be evaluated using different dyes. Moreover, immunofluorescent staining can be performed to identify specific targets. Also, cell cycle fluorescent reporters can be used.

Please contact BEONCHIP for further assistance.

OTHER READOUTS

It is possible to recover cells and perform flow cytometry, RNA extraction (PCR), exosomes…

Please contact BEONCHIP for further assistance.