

QUICKSTART KIT





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Summary

Organ-on-a-chip (OoC) technology aims to mimic human physiology adding controlled cell microenvironments and maintaining tissue-specific functions in cellular cultures. OoC can closely simulate the human microenvironment, synthesize organ-like functional units on a fluidic chip substrate, and simulate the physiology of tissues and organs^{1,2}.

This technology allows to improve cell and tissue engineering from primitive 2D monocultures to complex 3D co-culture systems, overcoming the traditional drawbacks of 2D (flat) cell-culture technology *in vitro* and *in vivo* animal trials, neither of which generate completely reliable results when it comes to the actual human biology^{1,2} (figure 1).

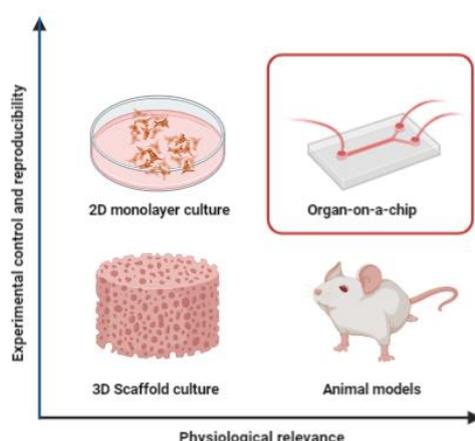


Figure 1: Comparison of current biological experimental conditions in terms of control and reproducibility and physiological relevance. 2D allows a fast and replicable way to analyze drug testing but without a suitable microenvironment. 3D in hydrogels enables culture in a suitable tissue microenvironment but does not provide a proper simulation of the physiology and pathology observed *in vivo*. Animal models allow *in vivo* analysis, but there are physiology and complexity differences with humans. Whereas, organ-on-a-chip (OoC) allow controllable cell culture, with a more physiologically-like microenvironment that permits a clearer study of the organotypic characteristics of human biology (Modified from Chen et al. (2021))³.



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It will become an increasingly important platform for in vitro drug development and screening. It is expected that organ chips will allow huge reductions in the incidence of failure in late-stage human trials, thus reducing the cost of drug development and speeding up the introduction of drugs that are effective^{1,3,4}.

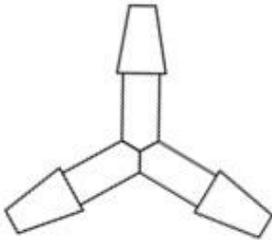
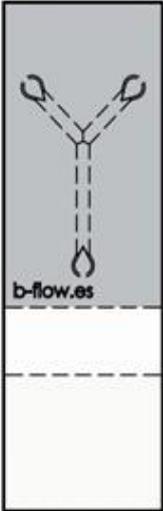
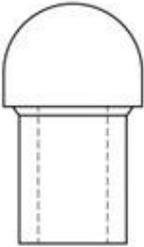
One important barrier for the implementation of OoC in the laboratory routine is its specific experimental set up, which is currently away from the traditional assays in cell culture plates. BFlow's Quickstart includes all the needed material and information to start OoC in laboratories with experience with cell culture models. Chips in this kit allowed to collect high quality scientific information such as

- Accurate reproduction of physiological flow conditions⁵,
- Differential interaction of circulating tumour cells with the endothelium depending of the vascular geometry⁶ or
- Increased local endothelial phagocytosis of erythrocytes derived from diabetic conditions⁷.



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Content of the kit

 <p>x2 REF: B02_0001</p>	 <p>x1 REF: B02_0003</p>	 <p>x5 REF: B01_0007</p>
 <p>x3 REF: B02_0006</p>	 <p>x3 REF: B02_0010</p>	
 <p>x2 REF: B02_0009</p>	 <p>Cell Culture Protocol</p>	 <p>Technical Support</p>



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Additional materials (not provided)

- **Cell culture room fully equipped**, including cell culture incubator and laminar flow hood, autoclave, cell culture incubator for standard human cell conditions (37°C temperature, 90% humidity and 5% CO₂), temperature-regulated water bath, inverted microscope with phase contrast, system to count the cells and centrifuge.
- **Cell culture reagents and materials**, including endothelial cells, cell culture medium, trypsin, cell culture material including flask, plates and/tubes, sterile PBS and centrifuge tubes.
- **Reagents and equipment for fluidics**, including fibronectin, forceps, tubing with stoppers, male leuc lock and peristaltic or syringe pump.

General considerations

- Chips were tested with the indicated reagents and cellular culture. The conditions have to be adapted to other experimental settings and cells.
- All material used for cell cultured has to be sterile. Chip plugs and tubes for the planned experiments have to be autoclaved in advance.
- Avoid forming bubbles into the channels. These bubbles can affect the integrity of the adhesive cell culture.
- To avoid cell damage, the injection of any cell suspension solution should be performed slowly and allowing the re-equilibration of the internal pressure of the circuit.
- The following protocol is based in HUVEC culture from Rodiño-Janeiro et al⁸.



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Protocol of Vessel-on-a-chip cell culture

- Autoclaved 1mm channels are filled with 110-120 μ L fibronectin (5 μ g/ml) in gelatin 0.02% from bovine skin avoiding the formation of bubbles* to coat the surface. Close the inlets and the outlets with autoclaved plugs and incubated overnight in the cell culture incubator (37 °C).

*It is very important to avoid the formation of bubbles at this time because if bubbles remain that area of the channel will not be filled with medium or cells in the following steps!

- Remove fibronectin (and wash with EGM-2 medium -not required).
- Detach HUVEC at confluence with trypsin, pellet and resuspend in complete medium at a density of 1×10^6 per mL and seed them into the chip pipetting between 110-120 μ L in a 1mm channel. Close the inlets and the outlets with autoclaved plugs.
- Cells are incubated into the device for 4 hours. [\(Video\)](#)
- Repeat the cells' seeding procedure with cells for the other side of the channel. Close the inlets and the outlets with autoclaved plugs. Incubate the chip upside-down other 4 hours to cover the whole channel surface. At the end change medium and left 12-16 hours in the cell culture incubator at 37°C (normally overnight).
- One end of the silicon tube for the inlet is introduced in the medium reservoir through the septum with a needle without dipping into the cell culture medium. The other end of the tube is connected to the stopper to be mounted in the peristaltic pump.
- Other tube is inserted in the reservoir with a needle, being sure that is dip into the cell culture medium. This tube is primed with cell culture medium (until one drop start to form in the connector) and then, the tube is inserted in the inlet of the chip (using a forceps). [\(Video\)](#)



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- The silicon tubes for the outlets are inserted in the corresponding outlets of the chip and the other end connected with the stopper (shared with the inlets if the model has recircularization). The tubes are clamped close to the chip to avoid any flow in the next step.
- Install the number of channels that are needed for the experiment.
- The full system is placed in an incubator (if the incubator has an outlet port, the pump can be placed outside). Once placed, remove the clamps.
- The pump is turned on at the desired flow rate: 0,12 $\mu\text{L}/\text{min}$ for 4 hours and then increased directly to the maximum flow 0,75 $\mu\text{L}/\text{min}$.
- Add the treatment the experiment requires and maintain the flow at the desired conditions for the duration of the experiment (in this particular case during 48h).



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Bibliography

1. Yan, J., Li, Z., Guo, J., Liu, S. & Guo, J. Organ-on-a-chip: A new tool for in vitro research. *Biosens Bioelectron* **216**, 114626 (2022).
2. Leung, C. M. *et al.* A guide to the organ-on-a-chip. *Nat Rev Methods Primers* **2**, 1–29 (2022).
3. Ma, C., Peng, Y., Li, H. & Chen, W. Organ-on-a-Chip: A New Paradigm for Drug Development. *Trends in Pharmacological Sciences* **42**, 119–133 (2021).
4. Franzen, N. *et al.* Impact of organ-on-a-chip technology on pharmaceutical R&D costs. *Drug Discov Today* **24**, 1720–1724 (2019).
5. Otero-Cacho, A. *et al.* Determination of hemodynamic risk for vascular disease in planar artery bifurcations. *Scientific Reports* **8**, 2795 (2018).
6. Casas-Arozamena, C. *et al.* Haemodynamic-dependent arrest of circulating tumour cells at large blood vessel bifurcations as new model for metastasis. *Sci Rep* **11**, 23231 (2021).
7. Turpin, C. *et al.* Impact of Enhanced Phagocytosis of Glycated Erythrocytes on Human Endothelial Cell Functions. *Cells* **11**, 2200 (2022).



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Notes:

A series of horizontal dashed lines spanning the width of the page, intended for taking notes.



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