A NEW VERSATILE 3D ORGANS-ON-CHIP MODEL ALLOWING AIR-LIQUID INTERFACE, BLOOD-TISSUE BARRIER RECREATION AND MULTICOMPARTMENT CONNECTION

P3.050

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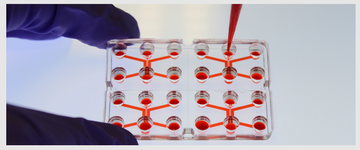
BACKGROUND

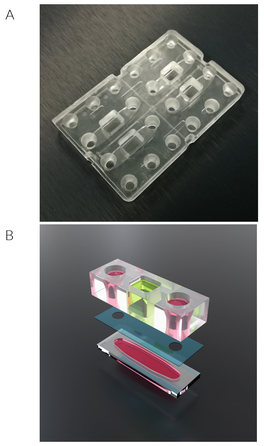


The creation of versatile and reproducible functional 3D tissue represents a current challenge. The culture of organoids or complex tissue requires an adequate supply in oxygen and essential nutrients through blood vessels. Cell culture inserts may represent relevant models to achieve co-culture on both sides of a membrane, however they may have limitations specially to create blood vessels structures close to the physiology. NETRI's devices use the concept of compartmentalization to mimick the actual anatomy. NETRI develops versatile microfluidic devices allowing the 2D co-culture of different cell types including especially human derived iPs neurons.
Here we present the development of our new multifluidic line called Duplex Well allowing 3D co-culture.

RESULTS

DESIGN AND TECHNICAL SPECIFICATIONS

We developed Duplex Well, a 3D microfluidic device with two compartments: a large channel in the middle and an open well, placed on top, which allow an air-liquid interface. Both compartments are separated by a membrane. To improve the integration of the membrane and scale up the industrialization of our devices, the Duplex Well microfluidic devices consist in a hybridization based on a polystyrene bottom part and a PDMS top part.



Duplex Well chips. A. Final product (four Duplex Well chips) B. Virtual representation of our Duplex Well chip which consists of 3 parts: 1/Open well + inlet + outlet reservoir made in PDMS on the top, 2/ the membrane (in blue) and 3/ the bottom channel made in polystyrene.

Channel.

- Suitable for endothelial cells
- Possibility to add immune cells/cytokines
- Possibility to apply shear stress

Open well.

- Air-liquid interface
- Insert explant, organoids…
- Various epithelia types
- Volume & Surface ~ P96 well

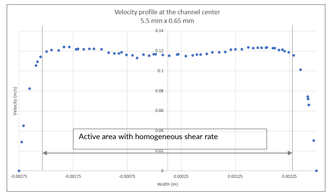
Membrane.

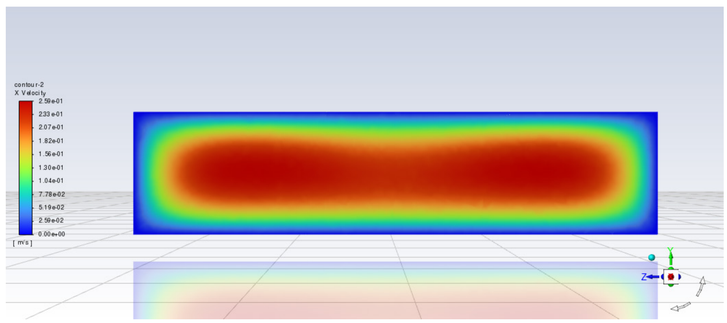
- Transparent
- Polyéthylène téréphtalate (PET)
- 12µm thick, 4.106 pores/cm²
- Allow passage of secreted factors like cytokines

MICROFLUIDIC CHARACTERIZATION

Duplex Well.

- Shear stress is applied into our chip to ensure the functionalization of endothelial cells and to mimic the blood flow.
- Our device has been optimized to have a homogeneous shear rate in the bottom channel at the interface with the open well
- Our device can support ≈ 3 dyn/cm² with lateral rocking (15° - 50 rpm).
- The device development was helped with CADFEM® simulations with Fluent®
- The integrity of the membrane has been also characterized.



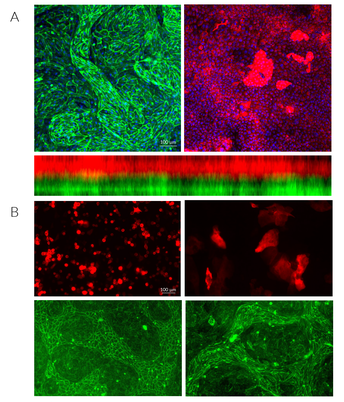


BIOLOGY

Our Duplex Well devices aim to create physiological barriers with porous membrane such as epithelial or endothelial, blood-brain-barrier or organoids culture.

Keratinocytes and endothelial cells.

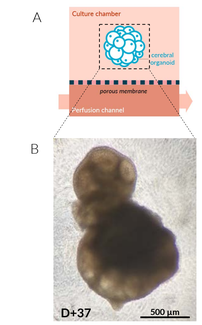
- Expression of **standard markers and morphology.**
- Cell-cell interaction: at the level of the membrane, we observed **modifications of cells arrangement**, **cells morphology** and some **differentiation markers** demonstrating an **interaction between cells and a passage of molecules** from one compartment to the other.



Co-culture of Normal Human Epidermal Keratinocytes (NHEK) and Human Dermal Microvascular Endothelial Cells (HDMEC) in Duplex Well chip A. Co-culture with NHEK stained for K14 (red, right picture) and HDMEC stained for CD31 (green, left picture), side illustrate the physical separation by the membrane (bottom picture). Top pictures counterstained with DAPI. B. Illustration of NHEK change in differentiation with K10 staining in absence (top left picture) or presence (top right picture) of HDMEC. Illustration of HDMEC change in morphology with CD31 staining in absence (bottom left picture) or presence (bottom right picture) of NHEK.

Cerebral organoids.

- 3D **self-organized** cellular aggregates as **relevant *in vitro* human brain models.**
- **Robust microfluidic-based anti-adherence** approach to prevent organoids from adhering to the porous membrane.
- Morphological observations, viability analyses, supernatant analyses, immunostaining, or transcriptomics analyses.



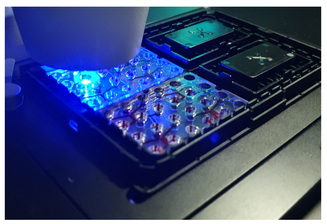
Brain Organoids in microfluidic device (Castiglione *et al.*, 2022)
A. Representation of a Duplex Well device. B. Image (5X) of a cortical cerebral organoid maintained in Duplex Well for 37 days. Protocol adapted from Xiang *et al.*, 2017, 2019.

CONCLUSION & PERSPECTIVES

Our compartmentalized and fully human organs-on-chip devices are used to accelerate preclinical phases, decrease the rate of clinical failure and minimize animal testing.

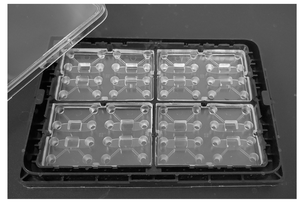
Thanks to the industrial production and a strict quality control, NETRI provides repeatable and reproducible devices in a high-throughput (HT) format, NeoBento™, and are therefore compatible with:

- HT MicroElectrode Array (MEA) systems.
- High Content Screening (HCS) imaging systems & microscopy.
- Liquid handling robots.



The versatility of our devices allows multiple evolution by:

- Connecting other compartments to interconnect open well to neurons by microchannels (Duplex Well Link).
- Connecting the chips together to create a multi-organ model.
- Adding electrodes (MEA) for electrophysiology.
- Developing specific equipment to increase the shear stress and allow multiorgan connection.



Duplex Well Link (prototypes) in NETRI's NeoBento™ format (SBS compatible)

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