APPLICATION NOTE

A1R/AX R Confocal Microscope

Confocal imaging of 3D biological tissues using the **HUMIMIC Chip**

In this application note, we present an intestinal model, a bone marrow model, and a vascular tissue model cultured in TissUse's **HUMIMIC Chips**, which provide a structure that imitates the human body. By visualizing the tissue-specific marker with fluorescence and observing it with a confocal microscope, we confirmed that the tissue created in the chip forms the same structure as the corresponding living tissue. These models have the capability to close the gap between systemic animal models, which are physiological different to human, and standard *in vitro* assays performed in human derived cells, which cannot reproduce the human physiologic conditions.

Experimental overview

An organ-on-a-chip is a device with fine flow paths and organ culture compartments. Since human three-dimensional cell culture is possible on the chip, it is attracting attention as a new evaluation platform for pharmaceutical products. TissUse's **HUMIMIC Chip** is a "Multi-Organ-Chip", meaning it connects different organ models in a common circulating medium flow. As the name suggests, it is possible to imitate the physiological environment of human organ models and simultaneously culture multiple organ models. In the **HUMIMIC Chip2** 96-well, two different organ models are placed in compartment a' and compartment b' (Fig. 1) for possible co-culture with other organ models. Since the microfluidic channels circulating the medium connect the compartments, the tissues establish a homeostatic crosstalk, where metabolites and cytokines are reaching the co-cultured organ model.
Here, 3D cultures of an intestinal model (Fig. 3) and a bone marrow model (Fig. 4) were implemented using compartments a' and b'.
Additionally, a vascular model was implemented using microfluidic channels (Fig. 6). Each experiment was implemented individually.

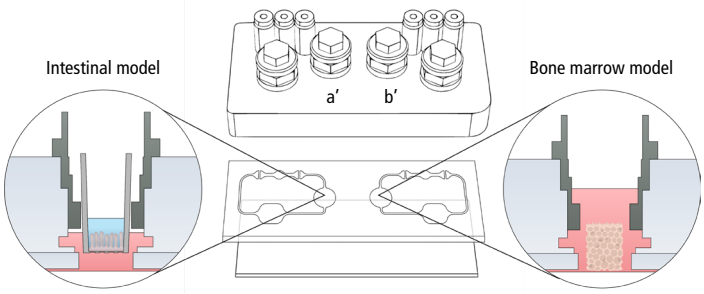


Figure 1. HUMIMIC Chip2 96-well exploded view
An iPSC-derived intestinal model was implemented in one circulation and a primary bone marrow model in the other circulation.

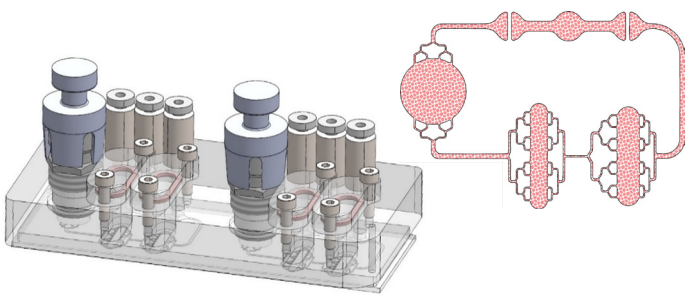


Figure 2. The HUMIMIC Chip2^{vasc} at a glance and an overview of the Chip2^{vasc} microfluidic with iPSC-derived endothelial cells covering the channel on all sides.

3D culture models of the intestine and bone marrow

Intestinal model

iPSC-derived intestinal cells were statically cultured on a collagen cell carrier (Viscofan 500049988), implemented into a **HUMIMIC** Membrane-holder for 9 days, and were then transferred into the **HUMIMIC Chip2** 96-well compartment a'. Then, the cells were cultured for 3 days under dynamic perfusion.
To observe the tissue structure, the cultured tissue was removed from the chip and stained with fluorescent antibodies (Fig. 3). 3D confocal images show that the cultured intestinal tissue formed a closed barrier, and the polarity of the cells can be observed by the staining of the apical tight junction marker ZO-1 (Fig. 3 A and B).

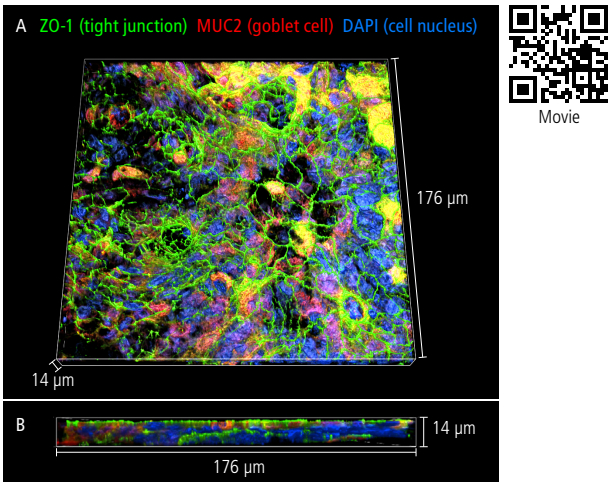
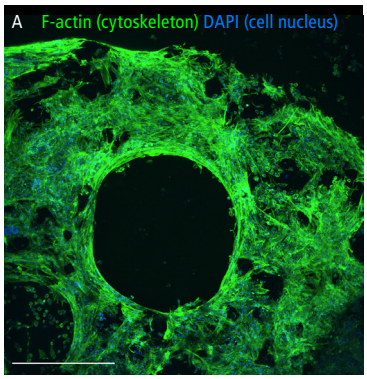


Figure 3. iPSC-derived intestine cultured in the HUMIMIC Chip2
(A) Top view on the surface of intestinal tissue.
(B) Cross section image of A, ZO-1 is polarized on the apical side.
Objective: CFI Apochromat LWD Lambda S 20XC WI

Bone marrow model

A 3D ceramic scaffold (Zellwerk, Sponceram) was placed in the **HUMIMIC Chip2** 96-well compartment b'. Human primary hematopoietic stem, progenitor cells (HSCs) and mesenchymal stromal cells (MSCs) were co-cultured under dynamic perfusion on the scaffold for 35 days.
The 3D ceramics show porous structures and are entirely seeded with elongated MSCs and round HSCs expressing F-actin (Fig. 4 A and B).



Movie

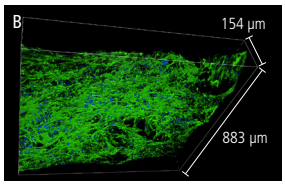


Figure 4. Bone marrow cultured in the HUMIMIC Chip2
(A) MIP image, Scale bar indicates 500 µm. Objective: CFI Plan Apochromat Lambda 10X
(B) Top view on the surface of the scaffold. Objective: CFI Apochromat LWD Lambda S 20XC WI
MSCs and HSCs are evenly distributed on the 3D ceramic.

Construction of vascular structure

Fig. 5 shows the experimental procedure to construct a vascular structure in the **HUMIMIC Chip2^{vasc}**, perform immune staining, and acquire images. With a confocal microscope, the cells are observed through an objective on the bottom side of the chip. The thickness of the glass bottom of the chip is 1 mm, and an objective with a long working distance is required to reach the focal position of the sample. The CFI S Plan Fluor LWD 20XC with a higher numerical aperture (NA = 0.7) and a longer working distance (2.3 mm) was used to observe the vascular structure formed in the microfluidic channels in the chip. **Fig. 6** shows the vascular structure that has formed in the channel of the chip: the endothelial cells form a channel, which splits up and continues into a vascular bed, where they can sprout to build the vascular connection to the organ models.

Culture of iPSC-derived endothelial cells in HUMIMIC Chip2^{vasc}

↓ 3 days

Immunostaining in the chip

↓

Image acquisition

Figure 5. The experimental procedure for constructing vascular structure, immune staining (DAPI, CD31, CD144, vWF and ZO-1), and image acquisition.

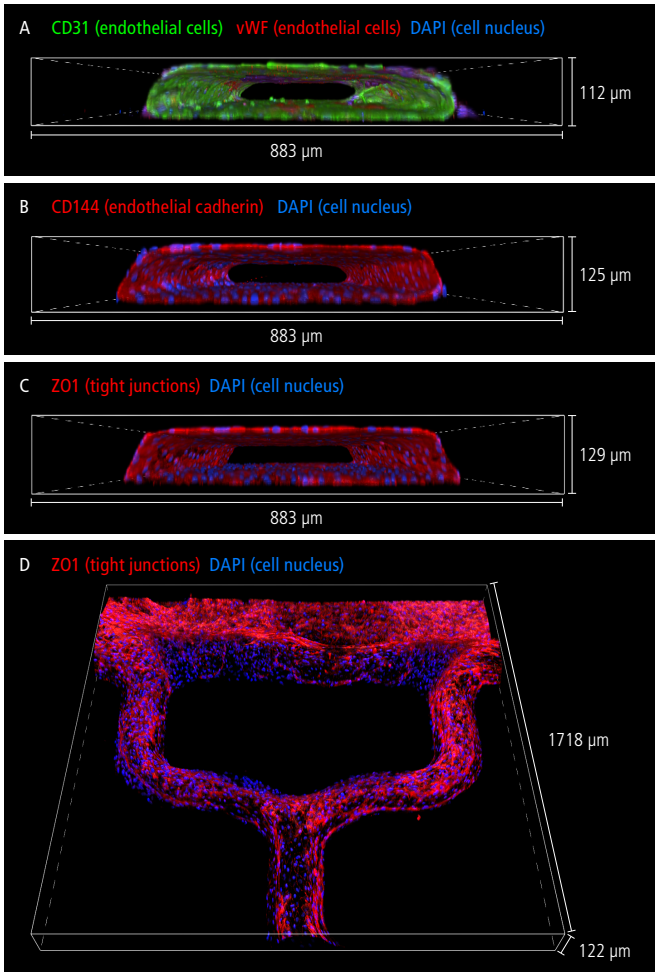


Figure 6. Vascular structure cultured in the channel of the Chip2^{vasc}
(A, B) Endothelial cells cover all sides of the channels to form a tube and (C) express tight junctions. (D) The flow splits up and continues into a vascular bed. Objective: CFI S Plan Fluor LWD 20XC

Movie

Summary

In this application note, it was shown that **HUMIMIC Chips** can be used to form cultured tissue with a structure similar to that of human living tissue.
By selecting the appropriate image acquisition method according to each organ model, 3D structures and localizations of markers specific for each tissue were confirmed.

AUTHOR INFORMATION

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Product information

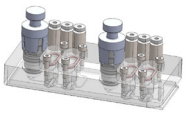
HUMIMIC Starter

Pumping pressure can be continuously adjusted then set automatically by the **HUMIMIC Starter**. This product is also equipped with a USB-port for easy management of pressure profiles and transfer of data. The **HUMIMIC Starter** is compatible with **HUMIMIC Chip2**, **Chip3**, **Chip3plus** and **Chip4**.

HUMIMIC Chip2

The **HUMIMIC Chip2** allows the simultaneous culture of two different organ models. Cells and tissues can be used to emulate biological barriers like the intestine, lung or skin, as well as to grow spheroidal and matrix-supported cultures for mimicking the three-dimensional environment of organs like the liver.

HUMIMIC Chip2^{vasc}



The **HUMIMIC Chip2^{vasc}** is designed for endothelial cells to spread and cover all sides of the microfluidic channels. These channels are connected to a vascular bed where they can sprout to build the vascular connection to the organ models.

AX/AX R Confocal Microscope

These microscopes achieve high resolution images of 8K x 8K pixels, which is four times that of conventional models. A large FOV with a diagonal of 25 mm allows acquisition of a large area of samples in a single scan, reducing phototoxicity. The AX R's resonant scanner achieves a high resolution of 2K x 2K pixels, allowing acquisition of live sample dynamics with high-speed imaging of up to 720 fps (2048 x 16 pixels).