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# Creating a kidney organoid-vasculature interaction model using a novel organ-on-chip system.

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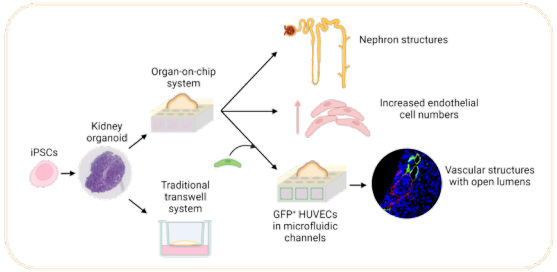
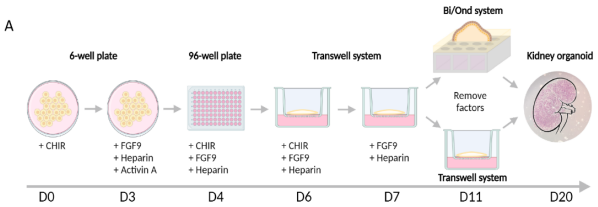
## INTRODUCTION




Kidney organoids are a powerful model to study kidney development and disease, as they recreate main nephron structures including glomeruli, proximal and distal tubuli. However, lack of vascularization implies shortage of nutrients and oxygen supply in the inner layers, resulting in a necrotic core. Although vascularization has been achieved via implantation in animal models, this vasculature derives mostly from the host.

**AIM:** To use the organ-on-chip system designed by Bi/Ond to induce organoid vascularization in a fully human-derived model *in vitro*.
**METHODS:** To culture kidney organoids on chip, generate 3D synthetic vessels by seeding of GFP⁺-HUVECs in the channels and stablish co-culture conditions.
**RESULTS:** We demonstrated our model increased endothelial populations in kidney organoids and induced vessel-like structures in co-culture with HUVECs.

## METHODS



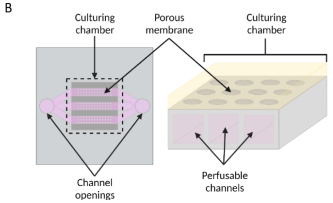




**Figure 1A:** Kidney organoids were generated following traditional transwell protocols until D11, and then either placed on the Bi/Ond chip system or kept in the transwell as controls.
**Figure 1B:** The Bi/Ond chip system consists of three perfusable microfluidic channels that connect with a culturing chamber through a porous membrane on top of which the organoids are placed.

## RESULTS

Organoids were stained for early (MCAM) and late (PECAM) endothelial markers, and an area analysis was carried out to determine the overall percentage of endothelial cells in the tissue. This analysis showed increased endothelial numbers in organoids cultured on chip.

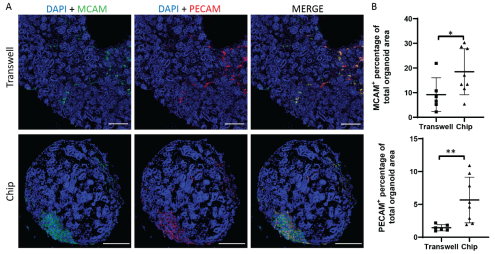



**FIGURE 2:** Organoids cultured on chip show increased endothelial populations.
**(A)** Immunofluorescent images of kidney organoids cultured on transwell and on chip showing expression of early endothelial marker MCAM and late endothelial marker PECAM. Scale bar = 200 µm.
**(B)** Area analysis of endothelial marker expression showing increased endothelial populations in organoids cultured on chip. * = $P < 0.05$, ** = $P < 0.01$.

The channels of the chip system were seeded with GFP⁺-HUVECs to create synthetic perfusable 3D vessels. Moreover, upon exercising fluidic flow, HUVECs acquired a directionality concurrent with flow direction as it is seen in vascular tissue architecture, suggesting flow exercised over these microfluidic channels mimics native vascular conditions.

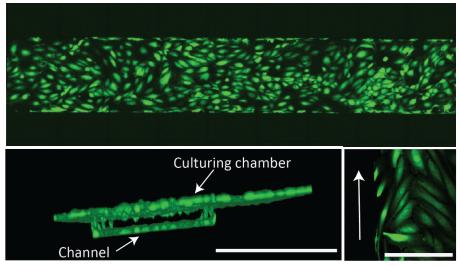



**FIGURE 3:** GFP⁺-HUVECs form 3D vessels upon seeding in microfluidic chip channels.
**(A)** After 48h of static culture the microfluidic channels were covered by a confluent monolayer of GFP⁺-HUVECs. Scale bar = 500 µm.
**(B)** 3D render showing the presence of GFP⁺-HUVECs in all surfaces of the channel and the bottom layer of the culturing chamber. Scale bar = 500 µm.
**(C)** HUVECs showed directionality concurrent with flow direction after 24h of flow. Scale bar = 250 µm.

Kidney organoids were co-cultured with the GFP⁺-HUVECs seeded in the microfluidic channels of the chip under fluidic flow conditions. HUVECs were able to proliferate and migrate through the membrane pores and reach the organoid tissue, where they interconnected with native endothelial cells and formed vessel-like structures presenting open lumens reminiscent of vessels.

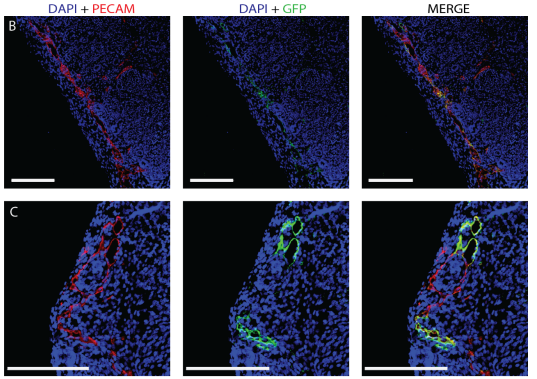



**FIGURE 4:** Immunofluorescent images of kidney organoids co-cultured with GFP⁺-HUVECs. HUVECs (PECAM⁺, GFP⁺) can be observed interconnecting with native organoid endothelial cells (PECAM⁺, GFP⁻). HUVECs formed vessel-like structures with open lumens. Scale bar = 200 µm.

## CONCLUSIONS

- First successful co-culture of kidney organoids and HUVECs in an organ-on-chip system.
- This research establishes the first steps towards *in vitro* functional organoid vascularization.
- Aside from improving organoid culture, other possible applications of our model include (pre-clinical) drug testing from patient derived material.

## CONTACT INFORMATION

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