PARTNERING OPPORTUNITY

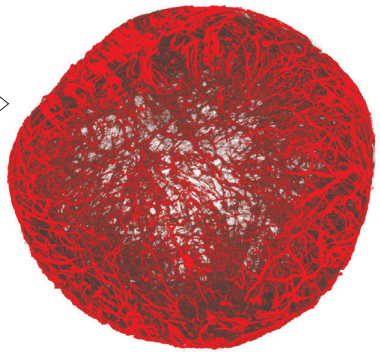
HUMAN BLOOD VESSEL ORGANOIDS FROM PLURIPOTENT STEM CELLS FOR DISEASE MODELING AND DRUG SCREENING

PARTNERING PROPOSAL

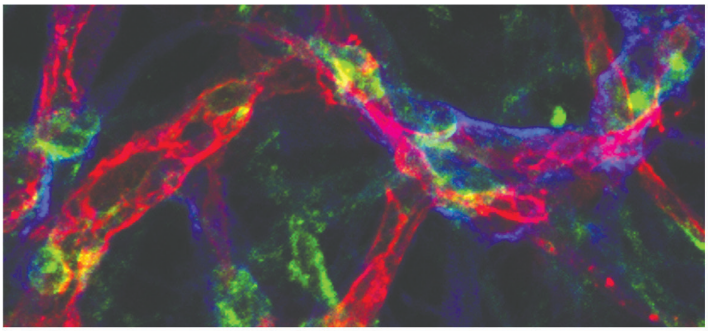
Recent developments in the stem cell field allow the in-vitro generation of complex tissue structures resembling whole organs. These organoids allow for recapitulation of human disorders in culture and in principle provide unlimited amounts of healthy and diseased human tissue for drug screening and disease research. IMBA scientists have successfully generated functional human blood vessels from human pluripotent stem cells (PSCs) in cell culture that can further be transplanted into mice. IMBA is actively seeking industrial partners and/or investors to exploit its human blood vessel technology in customized screening approaches and disease models to develop novel compounds to treat diabetic vascular complications and inherited vascular disorders.

TECHNOLOGY BACKGROUND

Drug development is a long and costly process, to a large extend because promising drug candidates identified in initial in-vitro screens fail to validate in-vivo. This is due to (1) the discrepancy between simplified in-vitro assays and the complexity of the real in-vivo pathologies and (2) differences between animal and human physiology. **New drug screening and validation approaches** are being developed focusing on **organoid technologies using human PSCs**. Recapitulating similar tissue architecture and function of an organ in cell culture, organoids have a great potential to be key intermediators in the drug discovery pipeline. In fact, **organoids could close the gap between primary in-vitro screens and animal models for disease and toxicology research**.



3D reconstruction of the vascular networks of a free floating vascular organoid cultured in a 96 well-plate. Endothelial networks are visualized in red (CD31+)



Mature human capillary grown in vitro. The endothelium (CD31+, red) is covered by pericytes (PDGFR-β^+, green) and enwrapped by a commonly shared basement membrane (Collagen Type IV+, blue).

Recent progress in stem cell research has led to the establishment of efficient differentiation protocols of healthy and diseased iPSCs into endothelial and perivascular cells, such as smooth muscle cells. These protocols have improved our understanding of the vascular differentiation process and are excellent tools to study cell autonomous mechanisms in a simplified setup. In addition, they have raised hopes to be useful for tissue regeneration. However, these simplified culture models do not well recapitulate the architecture and functionality of real in-vivo blood vessels.

IMBA scientists have now **successfully differentiated human iPSCs/ES cells into blood vessels** in cell culture that faithfully **resemble the structure of mature human capillaries**. These capillaries contain a network of lumenized, mature endothelial cells that closely interact with pericytes that are embedded in a commonly shared basement membrane. Expression data as well as ultrastructural analysis further support the notion that in-vitro generated blood vessels from human iPSCs are mature capillaries.

The **human capillaries** can be **efficiently (1) derived from healthy and diseased iPSCs or embryonic stem cell (ESC) lines, (2) cultured as free floating vascular organoids in 96 well plates for drug screening and (3) transplanted into mice, where they develop into a mature vascular tree including arterioles and venules**.

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CURRENT AREAS OF APPLICATION

Growing mature and stable human blood vessels from human iPSCs and ESCs in 3D cell culture allows to study genetic vascular disorders as well as disease associated vascular complications under defined conditions. The following applications are currently being developed:

- **Establishment of screening platform for analysis of diabetic vascular complications:** Human blood vessel organoids can be exposed to hyperglycemia in cell culture for 2-3 weeks which leads to basement membrane thickening and vessel regression as seen in diabetic patients. This generates a platform to screen for novel compounds that specifically prevent basement membrane thickening and finally endothelial cell death before moving drug candidates into time intensive diabetic animal models.
- **In-vivo model for human vasculature:** Furthermore, we established human vascular networks in diabetic (STZ treated) mice that show vessel regression and extensive basement membrane thickening after 3 months of diabetes in contrast to the endogenous mouse vasculature that stays largely unaffected at that time points. This allows to study diabetic vascular complications on a human vasculature under in-vivo conditions.
- **Generation of vascular disease models from patient specific iPSCs:** iPSC cells can be generated from patients with inherited vascular disorders and vascular organoids can potentially be used to gain insight into the disease mechanism. We have reprogrammed iPSCs from CADASIL patients (CADASIL is the most common form of hereditary stroke disorder) and a novel monogenetic form of vasculitis. We will also study defects of the diseased iPSC derived vasculature in-vivo transplants in mice.

FUTURE DIRECTIONS AND DEVELOPMENTS

- **Generation of reporter lines for compound screening:** We are developing various reporters in defined iPSC or ESC lines that will improve screening for novel compounds blocking diabetic basement membrane thickening and endothelial dysfunction.
- **In-vitro perfusion of vascular organoids:** Perfusion of vascular organoids in-vitro will offer the possibility to investigate vessel behavior under various flow conditions. Furthermore, the interaction/consequences of inflammatory cells with blood vessels could be studied.
- **Vascularization of organoids:** We have started to investigate the potential of vascular organoids to vascularize other human stem cell derived organoids that are devoid of blood vessels such as cerebral and intestinal organoids. This would allow to study the interaction of endothelial cells and pericytes with the organoid tissue and the influence of blood vessels on the development of the cerebral or intestinal organoid. In addition, we want to explore the possibility that blood vessels acquire tissue specific phenotypes such as the formation of a blood-brain-barrier in cerebral organoids or fenestrated endothelium in intestinal organoids.
- **Standardization of Good Manufacturing Practice** (GMP)–compliant production of vascular organoids for regenerative medicine applications (e.g. transplantation of vascular organoids into non-healing wounds)

PATENT SITUATION

IMBA filed a European patent application in 2017 followed by a PCT application in 2018 (WO2018/229251). The application claims the method for generating human vascular organoids and their use in modeling diabetic disease.

REFERENCE

Wimmer et al. (2019). Human blood vessel organoids as a model of diabetic vasculopathy.
Nature **565**, 505-510. DOI: 10.1038/s41586-018-0858-8

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