FROM BARRIER TO BREACH:
A 3D Microvascular Model for Assessment of Barrier Disruption

Leanne de Silva[1], Patrick Kuntschke[1], Katinka Theis[1], Alexander Radüchel[1], Jürgen Groll[1], Matthias Ryma[1]

[1] Department of Functional Materials in Medicine and Dentistry, Würzburg, Germany

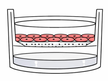
INTRODUCTION

- The endothelium forms a selective barrier controlling molecular transport
- Transition from **barrier → breach** drives leakage, inflammation, and toxicity
- Barrier disruption is still difficult to study *in vitro*

Current *in vitro* models:

2D endothelial monolayers

e.g. Transwell assay



- No 3D vessel structur
- No flow or shear stress
- Barrier function often over-simplified

Static 3D cultures

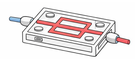
e.g. Hydrogels, spheroids



- No perfusion
- Limited control over transport
- Limited ability to quantify barrier function and leakage

Microfluidic systems

e.g. Organ-on-chip

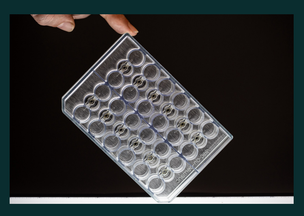


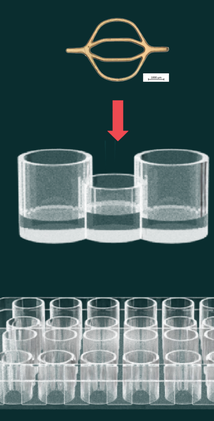
- Complex setup and handling
- Low throughput
- Non-physiological vessel geometry

Need: Perfusable, 3D *in vitro* models with functional readouts of leakage
Gap: No model captures perfusion, 3D structure, and permeability together

TECHNOLOGY

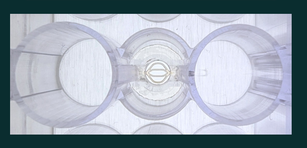
BasicVasc





vasc-on-demand

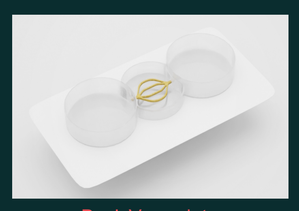
where science meets the pulse of life



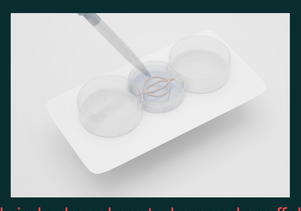
- Perfusable 3D microvascular channel
- Removable sacrificial scaffold defines channel geometry
- Quantifiable barrier function *via* permeability assays

METHODS

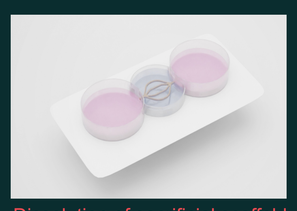
- **Hydrogel fabrication & channel formation**
  Fibrin hydrogel cast around a removable sacrificial scaffold to generate defined microchannels



BasicVasc plate

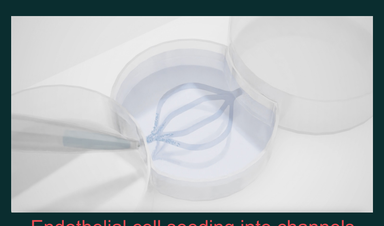


Fibrin hydrogel casted around scaffold



Dissolution of sacrificial scaffold

- **Endothelialisation**
  Channels seeded with HUVECs to form a continuous lining



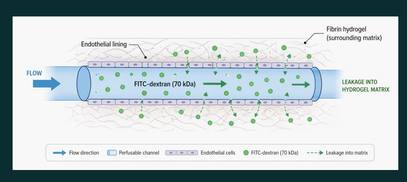
Endothelial cell seeding into channels



GFP-labelled HUVECs

- **Barrier disruption assay**
  Vessels exposed to inflammatory mediators
  - 100U/mL thrombin (acute, 1h exposure)
  - 100 ng/mL TNFα (24 h exposure)
  - 50ng/mL VEGF (24 h exposure)

- **Permeability measurement**
  FITC-dextran (70 kDa) was perfused through channels and leakage into the surrounding matrix was quantified at 0 and 24h



$$\text{Leakage index} = \frac{I_{matrix} - I_{background}}{I_{channel} - I_{background}}$$

- **Statistical analysis**
  Data are presented as mean ± SD; paired t-test comparing baseline (0 h) to post-treatment (1 h for thrombin, 24 h for TNFα and VEGF) (n = 4)

RESULTS

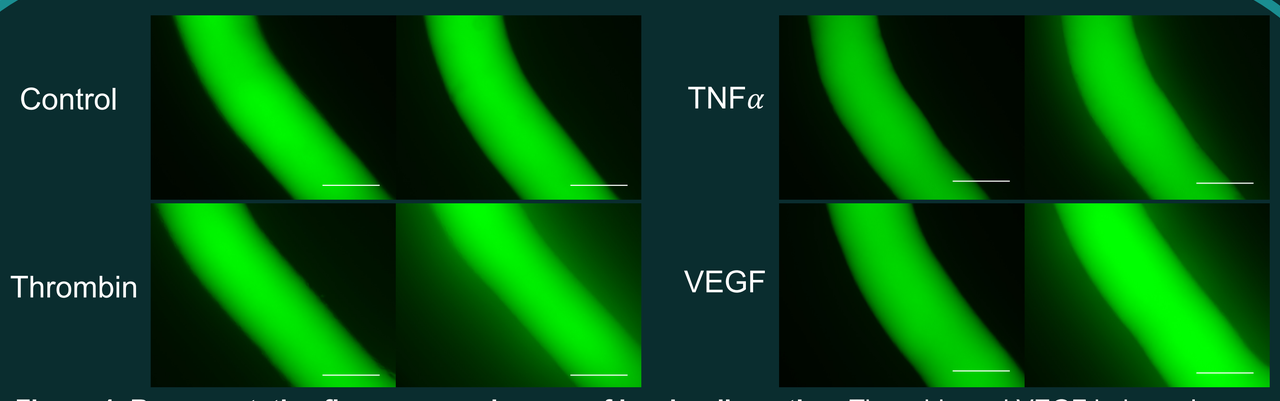


Figure 1. Representative fluorescence images of barrier disruption. Thrombin and VEGF induce clear leakage of FITC-dextran, while TNFα shows a modest effect compared to control. Scale bar= 300 µm

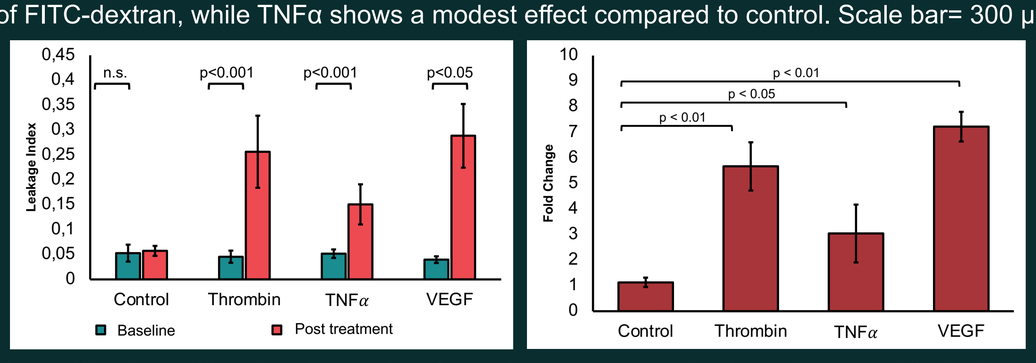


Figure 2. Quantification of barrier disruption following treatment. Leakage index and fold change demonstrate increased permeability after 24 h treatment with thrombin and VEGF, while TNFα shows a modest effect. Data are mean ± SD (n = 4–5), paired t-test.

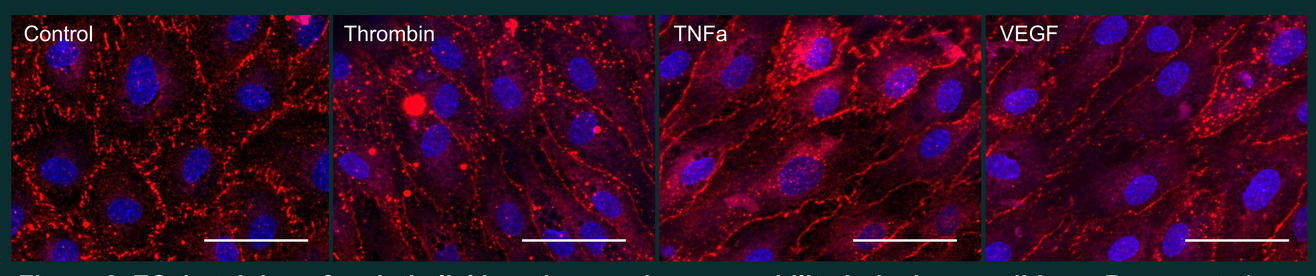


Figure 3. ZO-1 staining of endothelial junctions under permeability-inducing conditions. Representative confocal images of HUVECs stained for ZO-1 (red) and nuclei (blue) in control, thrombin, TNFα, and VEGF-treated groups. Treated conditions show disrupted junctional staining compared to control. Scale bar = 50 µm

DISCUSSION

Barrier disruption
- Thrombin and VEGF induce pronounced permeability increases with clear junctional disruption
- TNFα elicits a more moderate and heterogeneous response

Morphological changes
- Barrier disruption is associated with a transition from cobblestone to elongated endothelial morphology

Structure–function relationship
- Loss of ZO-1 organization corresponds with increased permeability, linking junction integrity to barrier function
- The model discriminates between barrier-disrupting stimuli and enables functional assessment of endothelial dysfunction

CONCLUSION

BasicVasc: perfusable vessel-like structures

- **Controlled flow:** Enables transport within a defined 3D microenvironment
- **Physiological organisation:** Supports endothelial structure within a matrix
- **Quantification:** Allows direct assessment of barrier function
- **Application:** Provides a platform for modelling endothelial dysfunction and drug response

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