Microfluidic slide for 3D-bioprinting applications using the example of a proximal tubule model

Philipp Linke[1,*], Isabelle Schmidt[2], Elin Pernevik[3], Volodymyr Kuzmenko[3], Hanna Hartmann[2], Zeno Guttenberg[1]

[1] ibidi GmbH, Gräfelfing, Germany
[2] NMI Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany
[3] CELLINK Bioprinting AB, Gothenburg, Sweden

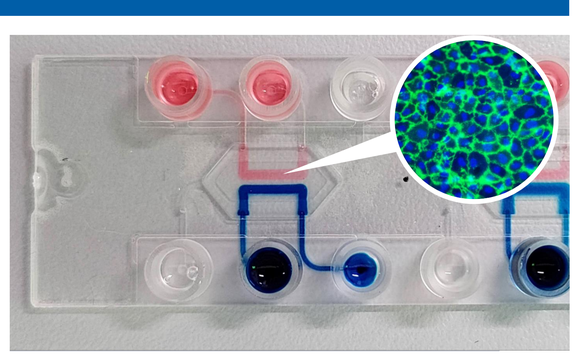
Introduction

Challenge: Bioprinting has come into focus as a method to create specialized organ-on-a-chip models, providing high flexibility and reproducibility of the models. However, realizing such models is usually time-consuming, costly and requires a high degree of expertise.

Our solution: A microfluidic device which makes 3D-printing on a chip accessible and allows perfusion of arbitrary hollow structures.

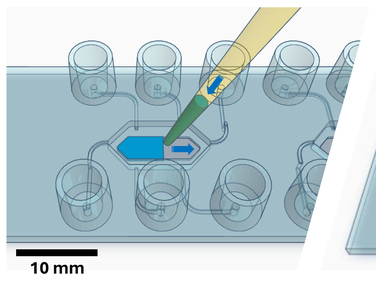
Methods: In collaboration with CELLINK and the NMI, we tested several bioink candidates to develop a proximal tubule model. The rheological properties of all bioinks were assessed. Adhesion and monolayer formation of the cells were tested. The viability of the cells was assessed, and tight junction formation was confirmed by staining. We also measured the diffusion of fluorescent dextran through the layer.

Results: The cells formed a functional monolayer and created a viable model of the proximal tubule.

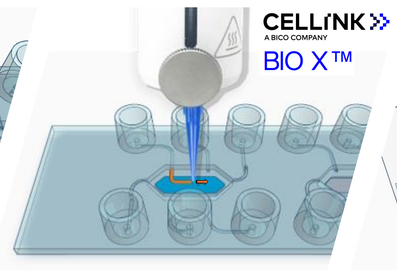


How to create hollow tubes

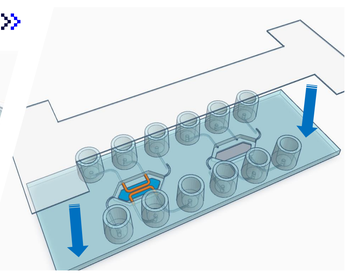
1. Fill base layer with hydrogel.



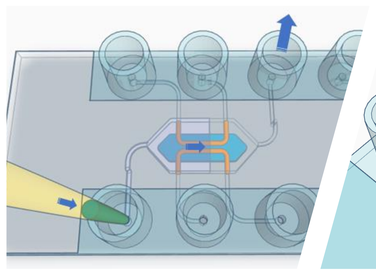
2. Print sacrificial structure.



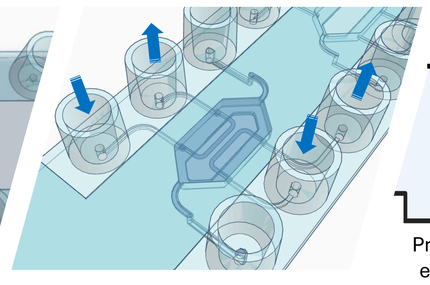
3. Seal chamber



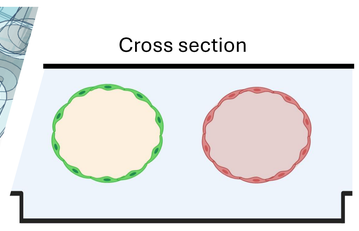
4. Fill chamber with hydrogel.



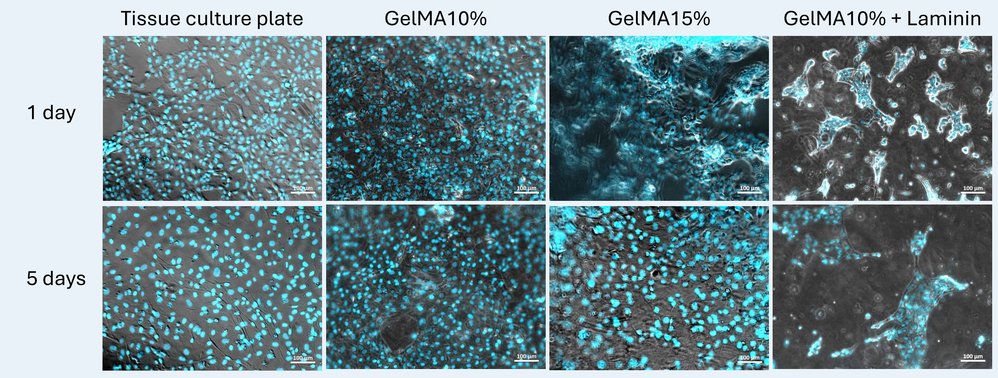
5. Remove sacrificial material

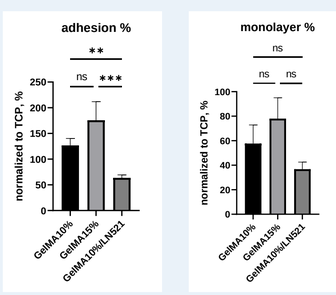


6. Seed cells into channels



Adhesion and monolayer formation on 2D hydrogel





Endothelial cell adhesion was tested on hydrogel candidates:

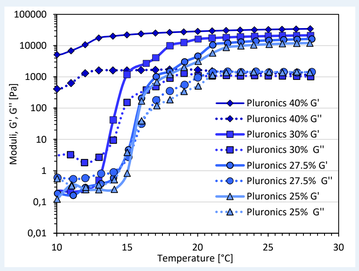
- Gelatin methacrylate 10 wt%
- Gelatin methacrylate 15 wt%
- Gelatin methacrylate 10 wt% + Laminin (LN)

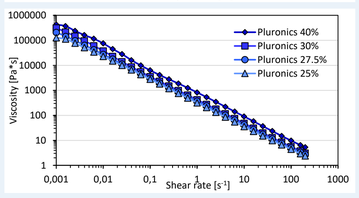
Significance tested with Kruskal-Wallis test, error bars: SEM, *p < 0.05

Results: Adhesion of cells was significantly better on pure GelMA compared to GelMA + LN, the concentration did not have significant influence. Monolayer formation was similar on all tested hydrogels.

Viscosity and elasticity of the bioinks

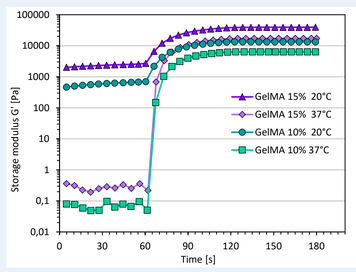
Sacrificial ink (Pluronics)

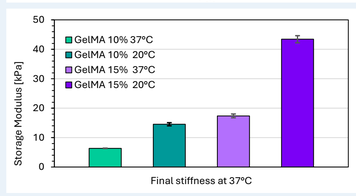




Pluronics 30 % provides sufficient viscosity for good printing fidelity, and a melting point at about 13 °C so that it can be printed at room temperature and easily be melted by applying cold PBS.

Surrounding ink (GelMA)

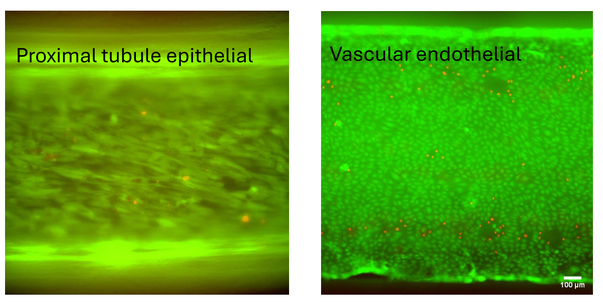




The final stiffness is dependent on the polymer concentration and thermal gelling before photocrosslinking. The difference is significant between final stiffness for all conditions tested by *t*-test with $p < 0.005$.

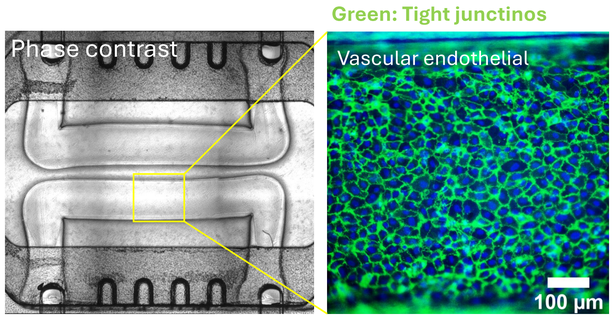
Immunostaining inside tubes

Viability Green: alive Red: dead

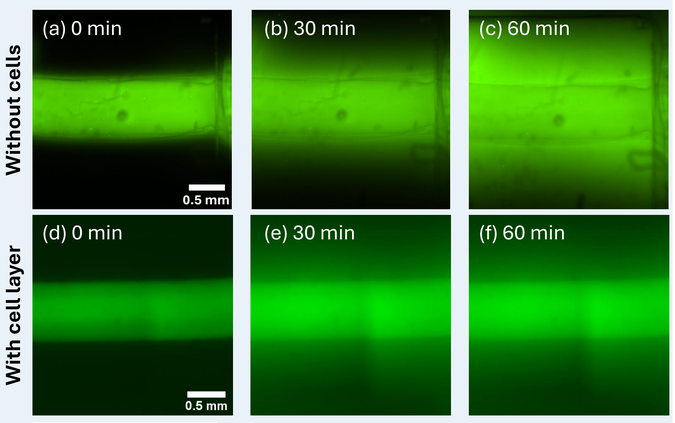


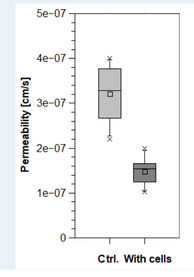
Tight junctions

Blue: Nucleus
Green: Tight junctinos



Diffusion through cell layer





Fluorescent dextran was filled into the channels with and without cells.
Intensity outside and inside was measured to calculate the permeability.

Result: The reduced permeability in the cell-covered channel indicates a dense network and functional barrier formation.

Summary

Results: GelMA 10 % was chosen to be used as a surrounding ink, since it facilitates optimal adhesion and monolayer formation of the cells. The printability of Pluronics as sacrificial material was characterized and fine-tuned. After creating the channels and seeding cells, their viability was shown to be sustained, and tight junction formation was clearly visible. Finally, perfusion with a fluorescent dye showed the effective barrier function of the cell layer, indicating that that the cells formed a viable model of the proximal tubule.

Outlook: The universal approach of 3D-printing allows for printing of arbitrary structures, which can be adapted to mimic specific geometries of tissue models, opening a large field of applications. We believe that the benefits of our platform will enable scientist to develop tissue models more quickly and perform pharmaceutical studies on a large scale.

Acknowledgments

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