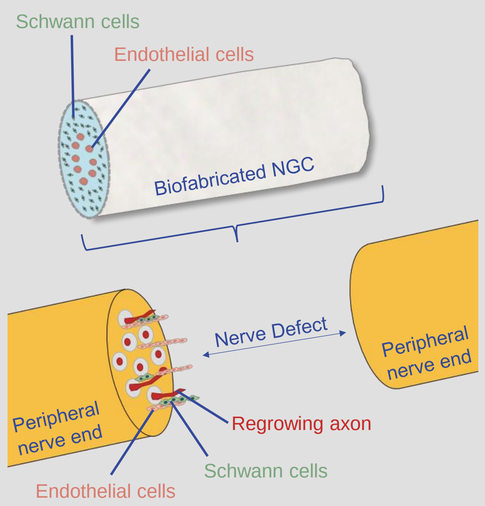
Evaluation of Schwann and endothelial cell compatibility in relation to a biofabricated nerve guidance conduit

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Introduction

Nerve guidance conduits (NGCs) are used to treat peripheral nerve injuries (PNI), but current NGCs are effective only for short defect distances [1]. **Our aim** is to develop an enhanced NGC capable of overcoming these size limitations. To achieve this, we integrate preformed human umbilical vein endothelial cell (HUVEC) strands with rat Schwann cells (RSC96) using a complex biofabrication process, creating a defined microvascular structure to guide ingrowing axons. **In this study**, we evaluate different hydrogels for 3D bioprinting with HUVEC and RSC96 cells, correlating cell compatibility with the mechanical properties of the materials.



Methods and Materials

Hydrogels:

- Type I collagen (rat tail)
- Alginate (from alginic acid from brown algae)
- Cultrex® basement membrane extract
- Composite hydrogels 1:1 collagen-alginate blends

Rheological characterization was performed using a rotational rheometer with a cone-plate geometry. Shear rate was varied from 10 to 5000 s^{-1} to determine viscosity.

After 2 days of incubation, cell viability of RSC96 cells within the hydrogel was assessed using a Live/Dead assay (calcein AM/ ethidium homodimer-1), while cell morphology was quantified by measuring circularity using ImageJ.

A tube formation assay was performed to evaluate the compatibility of the hydrogels with HUVECs and to assess their tube-forming capacity (Fig 1).

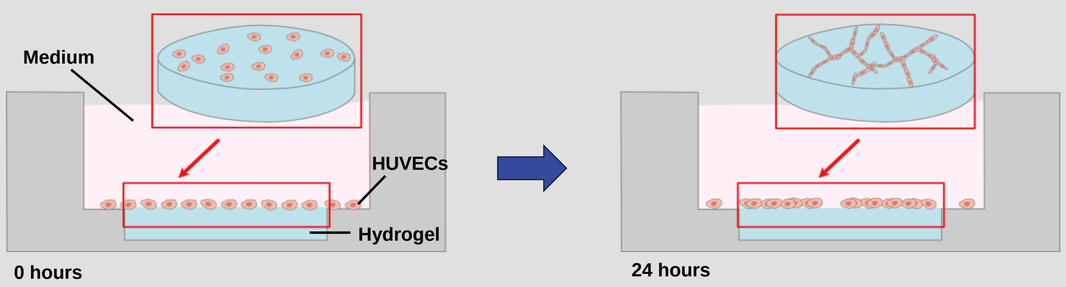


Fig. 1: Schematic representation of the tube formation assay with HUVECs cultured on a hydrogel in a µ-Slide 15 Well 3D (Ibidi). Left: 0 h after seeding. Right: Formation of a capillary-like network after 24 h.

Results

Rheological characterization

- Collagen I, alginate, Cultrex and composites exhibit shear-thinning behavior → useful for bioprinting applications
- Increasing polymer concentration leads to higher viscosity in all materials
- Viscosity for composite hydrogels increases progressively with rising alginate content → easy and controlled adjustment of viscosity

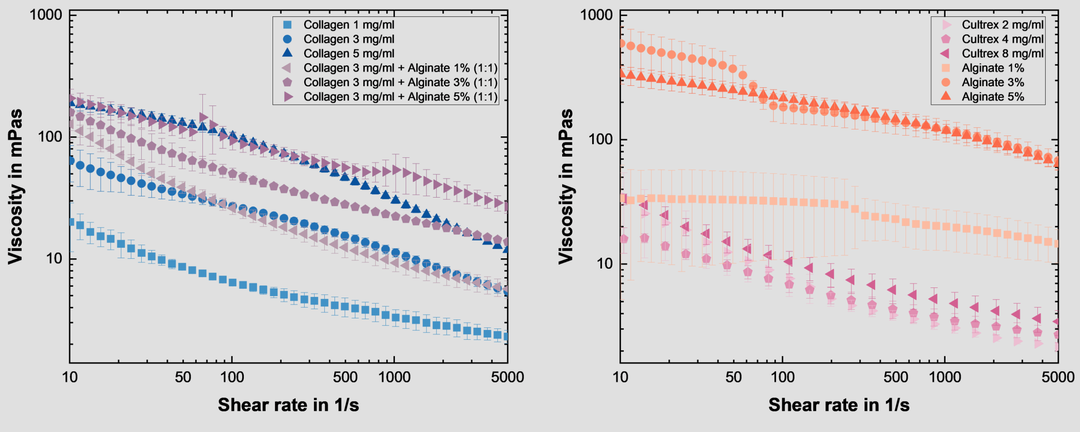


Fig. 2: Viscosity vs. shear rate for representative materials, n = 3 biological and technical replicates per material.

Live/Dead assay and cell morphology

Cell viability exceeded 90% in all tested materials (Collagen I 1-5 mg/ml, alginate 1-5%, Cultrex 2-8 mg/ml, and collagen-alginate blends: Collagen 3 mg/ml mixed 1:1 with alginate 1-5%)

Cell morphology varied (Fig. 3): Cells are roundest on alginate, spindle-shaped on Cultrex, and become increasingly rounded with higher hydrogel concentration due to increased stiffness.

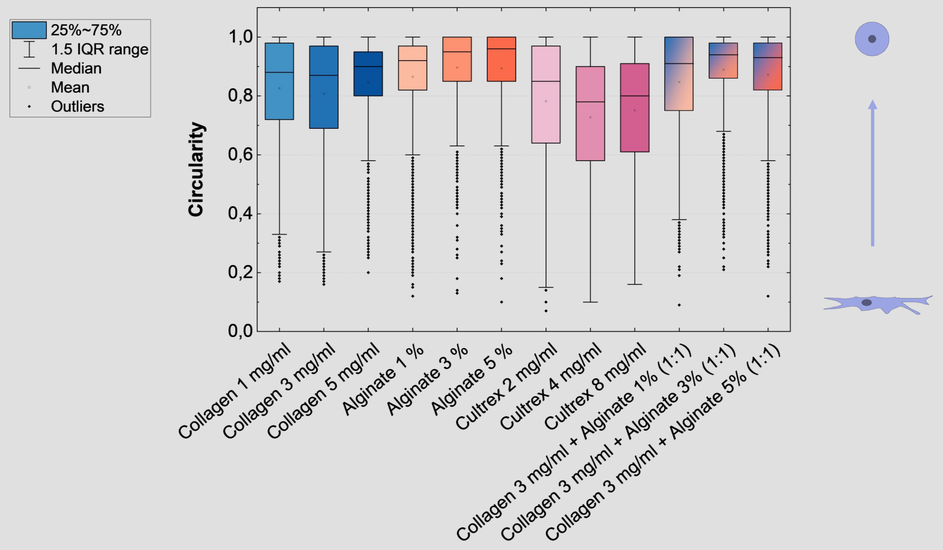


Fig. 3: Box plot showing cell morphology (quantified by circularity) on different materials.

Tube formation assay

Tube formation was observed exclusively on Cultrex; no tubes were formed on any other tested substrates (Fig. 4).

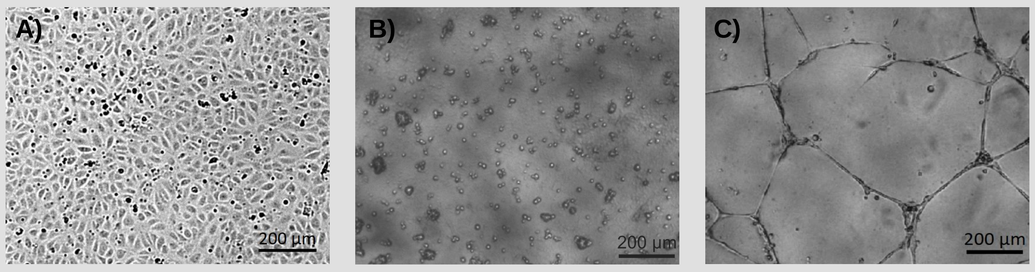


Fig. 4: Representative microscope images of HUVECs after 24 h on collagen type I with cobblestone-like morphology (A), on alginate with rounded cell morphology (B), and on Cultrex showing tube formation (C).

Summary

Material	Viscosity	HUVECs tube formation	Morphology of RSC96
Alginate	High	✗	
Cultrex	Low	✓	
Collagen type I	Medium	✗	
Collagen-alginate composites	Medium - High	✗	

However, reported lumen formation in 3D sandwich models using collagen type I [2] highlights its potential as a promising material and supports its selection for further investigation in this study.

→ **Overall this study provides a comprehensive characterization of hydrogels suitable for 3D bioprinting with endothelial and Schwann cells.**

References

[1] D. Grinsell et al., "Peripheral nerve reconstruction after injury: a review of clinical and experimental therapies," BioMed research international, 2014
[2] K. Tröndle et al., "Bioprinting of high cell-density constructs leads to controlled lumen formation with self-assembly of endothelial cells," Journal of tissue engineering and regenerative medicine, 2019

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