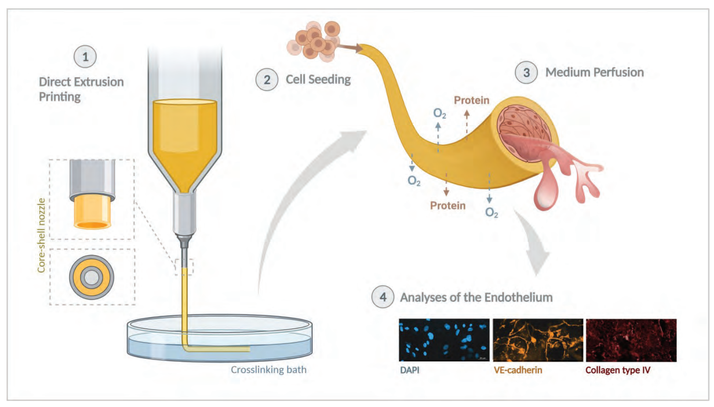
Franziska Ullm, Michaela Schröpfer, Caroline Seidel, Claudia Krumbiegel, Tina Hille, Frank Sonntag, Stephen Behrens, Florian Schmieder, Michael Meyer, Ina Prade

FILK Freiberg Institute

FREESTANDING COLLAGEN HOLLOW FILAMENTS – A TOOL FOR VASCULARIZATION OF IN VITRO 3D TISSUE MODELS

Introduction

Microfluidic cultivation platforms significantly improve the supply of tissue models in vitro. However, a key challenge is the sufficient distribution of oxygen and nutrients within a 3D construct. Here we demonstrate the use of collagen hollow filaments as a functional unit for the generation of a vascular structure in tissue engineering applications.



Fiber Properties

The hollow filaments with a diameter < 1 mm were fabricated by direct extrusion of a collagen fiber suspension through a core-shell nozzle. The filaments were cross-linked, freeze-dried and mechanically characterized. Analyses were undertaken for the swelling behavior of the tubes and the permeability of the tube wall for nutrients and oxygen.

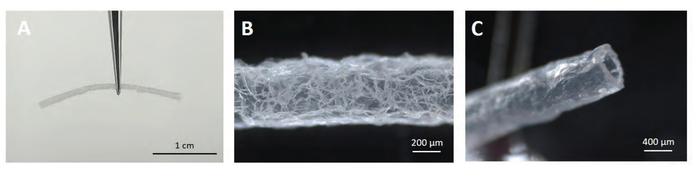


Figure 1. Appearance of collagen hollow filaments.
A) Photograph of a wet hollow fiber. B) Stereomicroscopic image of a dry hollow tube. C) Stereomicroscopic image of a wet, fully rehydrated collagen hollow filament.

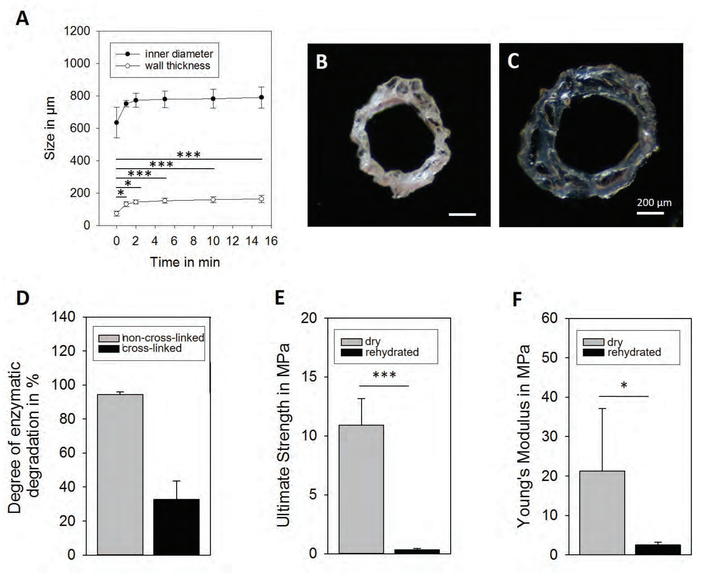


Figure 2. Properties of collagen hollow filaments.
A) Absolute values of the inner diameter and wall thickness of the collagen hollow fibers during rehydration with PBS. Light microscopy images of the hollow tubes before (B) and after (C) rehydration. D) Percentage of degraded collagen after enzymatic digestion with collagenase. Ultimate strength (E) and Young's Modulus (F) of collagen hollow tubes.

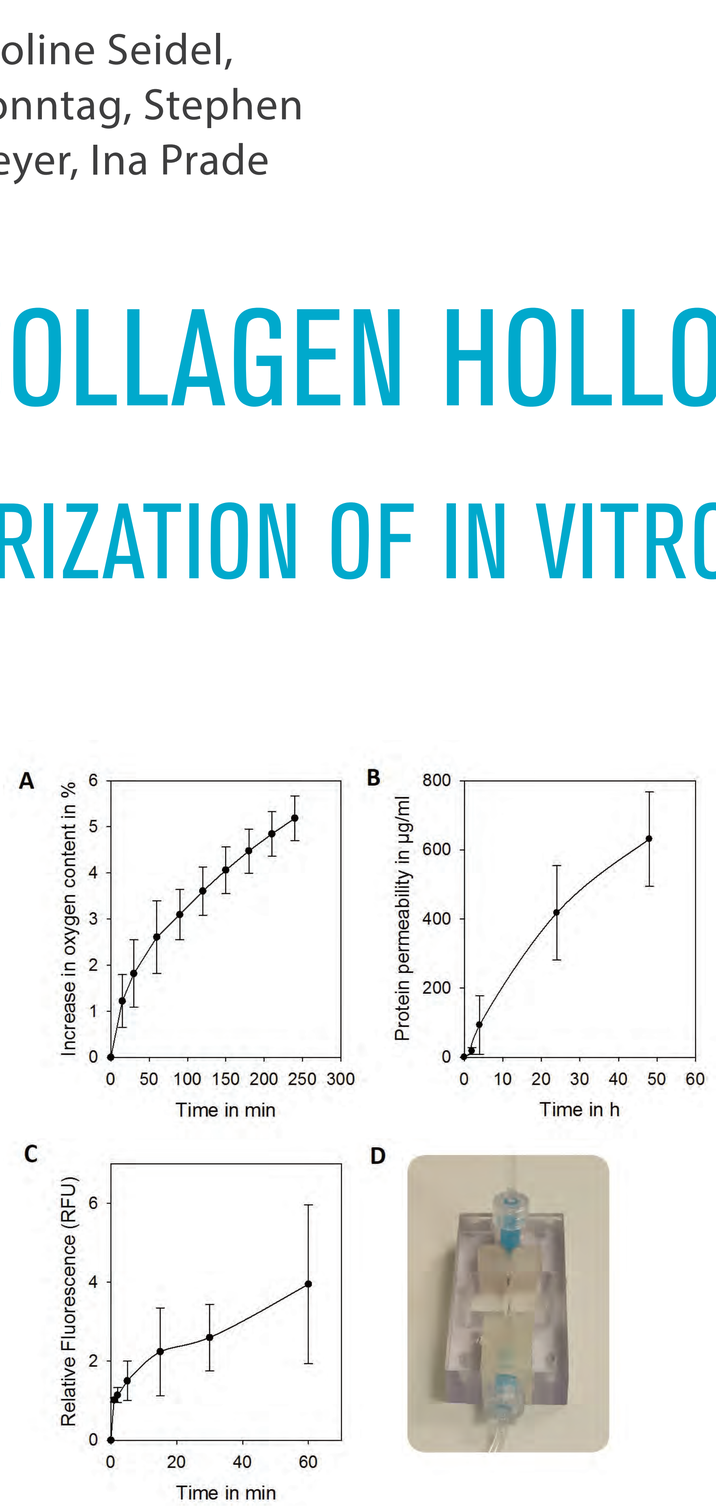


Figure 3. Permeability for oxygen (A) and proteins (B). Dextran permeability (C) through collagen hollow filaments was analyzed using a perfusion chamber (D).

Cell Response

Human endothelial cells (ECs) were seeded on the inner surface of the tubes and cultured under perfused conditions in a microfluidic circulation system.

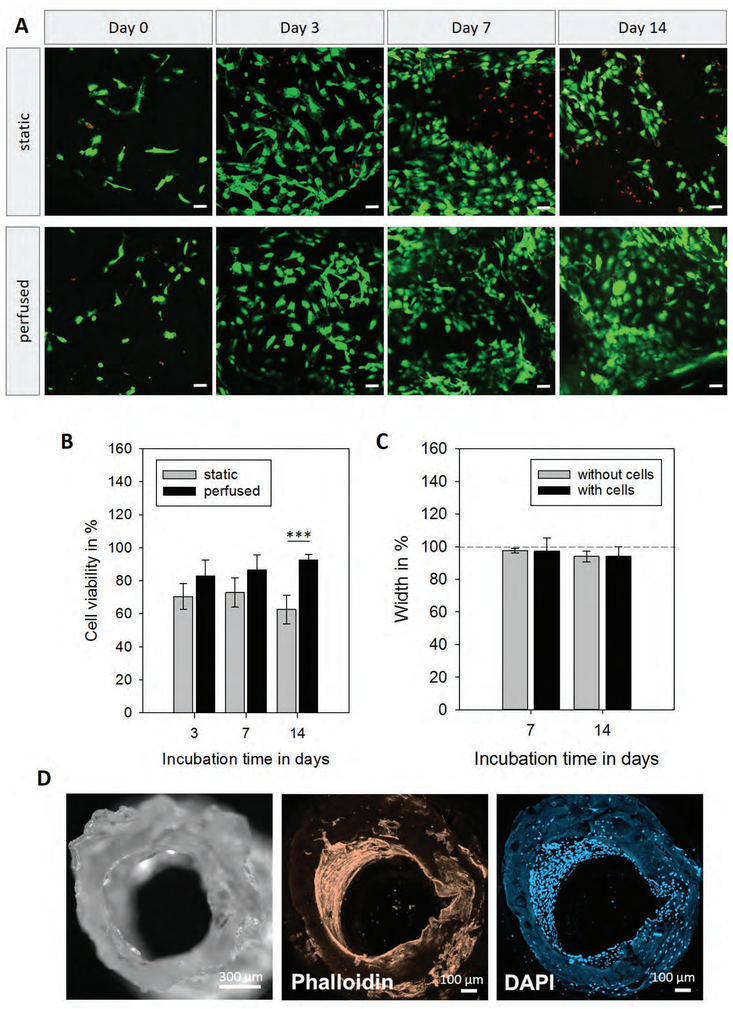


Figure 4. Cell cultivation within the collagen hollow filaments.
A) Live/dead staining of cell-laden collagen hollow fibers during cultivation under static or perfused conditions. B) Cell viability of HUVECs cultured up to 14 days in the collagen tubes. C) Changes in the width of collagen fibers in the presence or absence of cells. D) Cross-section of collagen hollow filament.

ECs growing on the inner surface showed typical features of a well-formed endothelium including VE-cadherin expression, cellular response to flow and secretion of extracellular matrix proteins. Cell growth was analyzed over a period of 21 days.

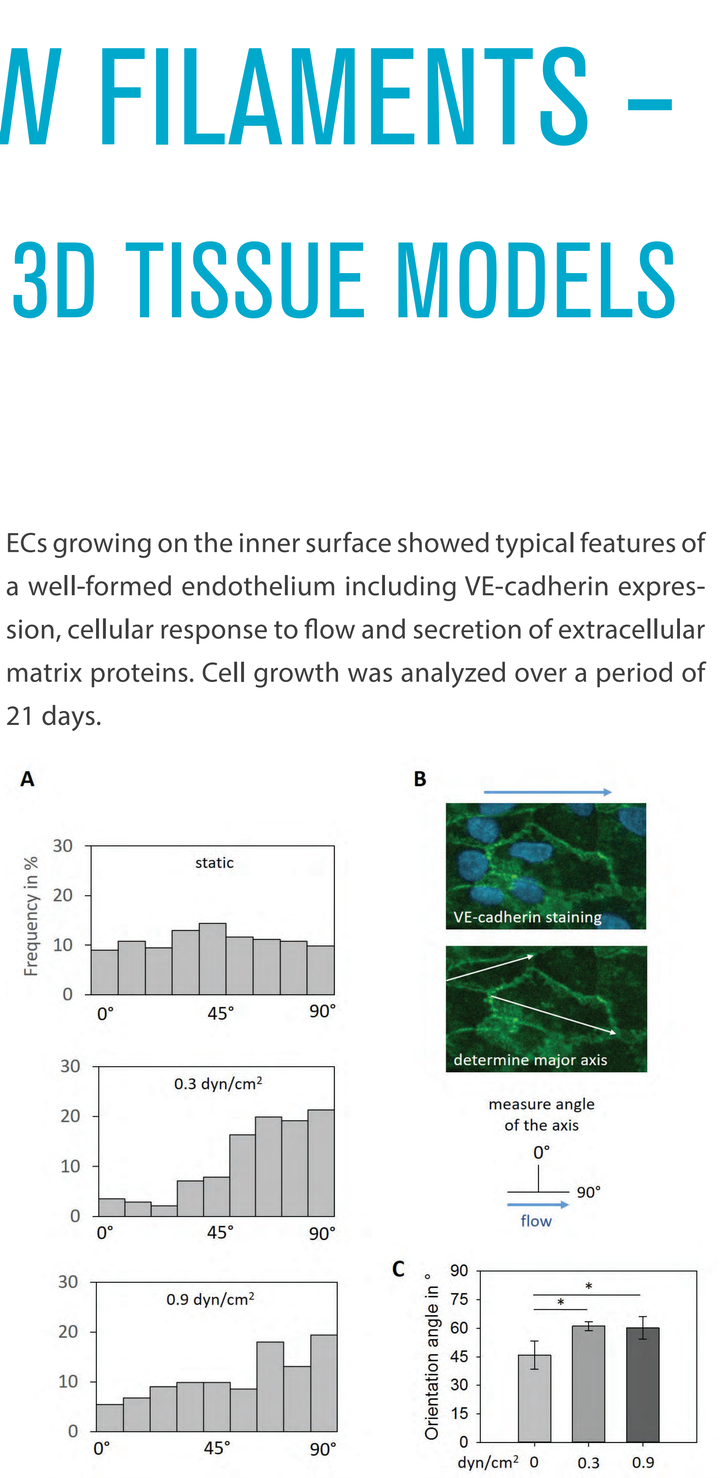


Figure 5. Analysis of the endothelial layer.
A) to C) Cell orientation in perfused collagen hollow filaments. D) to G) Confocal images of VE-cadherin, Laminin, Collagen type IV and fibronectin staining and DAPI nuclear marker in HUVECs cultured for 2 days under perfused conditions in the collagen hollow filaments.

Conclusion

Collagen hollow filaments support the formation of a living vascular tissue over a long period. The fibers enable the delivery of nutrients and oxygen to a surrounding compartment. The collagen hollow filaments could be used as a template for the fabrication of prevascularized tissue engineering constructs.

Contact
Franziska Ullm, Telefon: +49 3731 366-226
Email: franziska.ullm@filkfreiberg.de
FILK Freiberg Institute gGmbH
Meißner Ring 1–5, 09599 Freiberg, Germany
www.filkfreiberg.de/en

Partners
Fraunhofer-Institut für Werkstoff- und Strahltechnik IWS
Mikro- und Biosystemtechnik
Winterbergstraße 28, 01277 Dresden
Telefon +49 351 83391
http://www.iws.fraunhofer.de

Funded by
DFG Deutsche Forschungsgemeinschaft
German Research Foundation

Acknowledgement
This study was supported by The Deutsche Forschungsgemeinschaft (DFG) grant numbers ME 3734/6-1. We would like to thank for the support granted.