Two-Photon-Polymerization of Polyacrylamide Cell Scaffolds with Tunable ECM-tethering and Stiffness

Annabelle Sonn[1*], Målin Schmidt[1], Chantal Barwig[1], Christine Selhuber-Unkel[1]
[1]Institute for Molecular Systems Engineering and Advanced Materials (IMSEAM), Heidelberg University, Heidelberg, Germany.
*sonn@stud.uni-heidelberg.de

Introduction

- Mechanical, topographical and chemical cues from the ECM play a vital role in various biological processes in stem cells.[1]
- It was shown that macroscopic polyacrylamide (PAm) hydrogels can be adapted to allow for ECM-tethering and thereby cell adhesion.[2]
- Two-photon-polymerization (2PP) is a powerful tool for the fabrication of hydrogels with features on the micrometer length scale: 2PP utilizes the absorption of two photons with wavelengths in the near-infrared (NIR) range to initiate the polymerization of a resin solely within the laser voxel.[3]
- In this work, we optimize PAm resins with tunable ECM-tethering and thereby cell adhesive and non-adhesive properties. We show their excellent printability and tunable stiffness. Furthermore, we demonstrate the spatial control of the cell adhesion in multimaterial hydrogel structures.

Materials & Methods

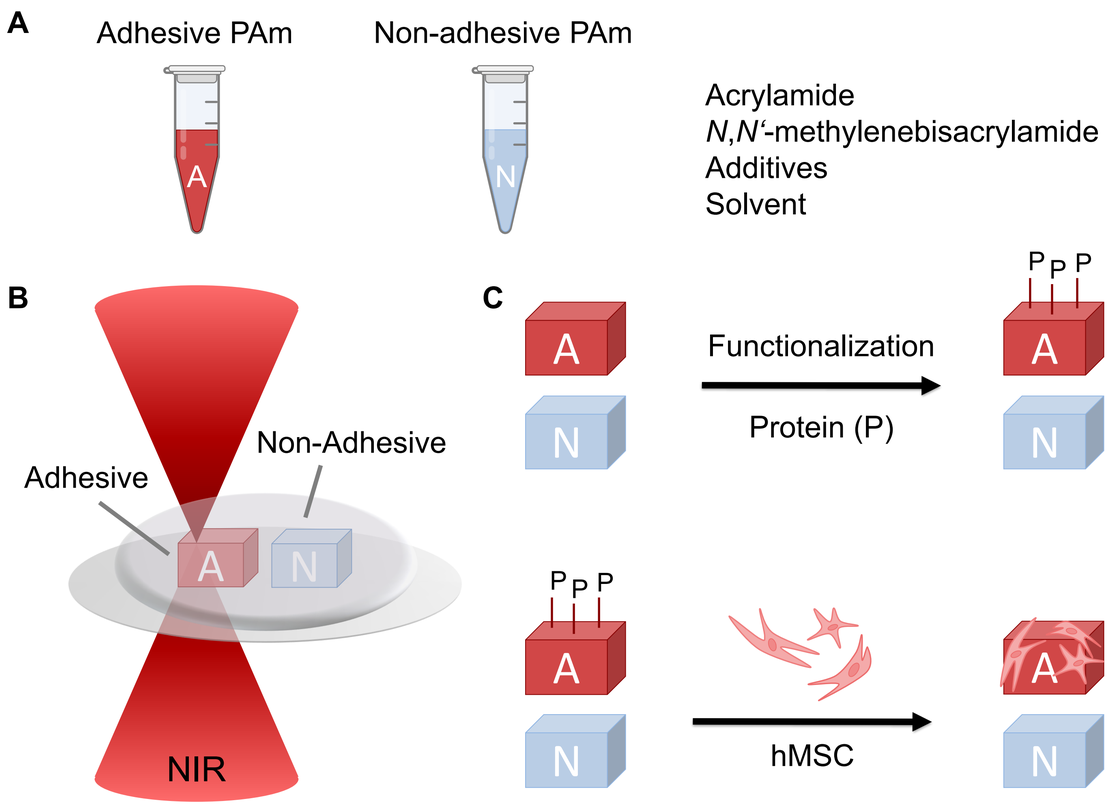


Fig. 1 A) Material composition. **B)** Subsequent multimaterial printing by 2PP. **C)** Post-printing functionalization to enable cell adhesion on adhesive material.

Characterization

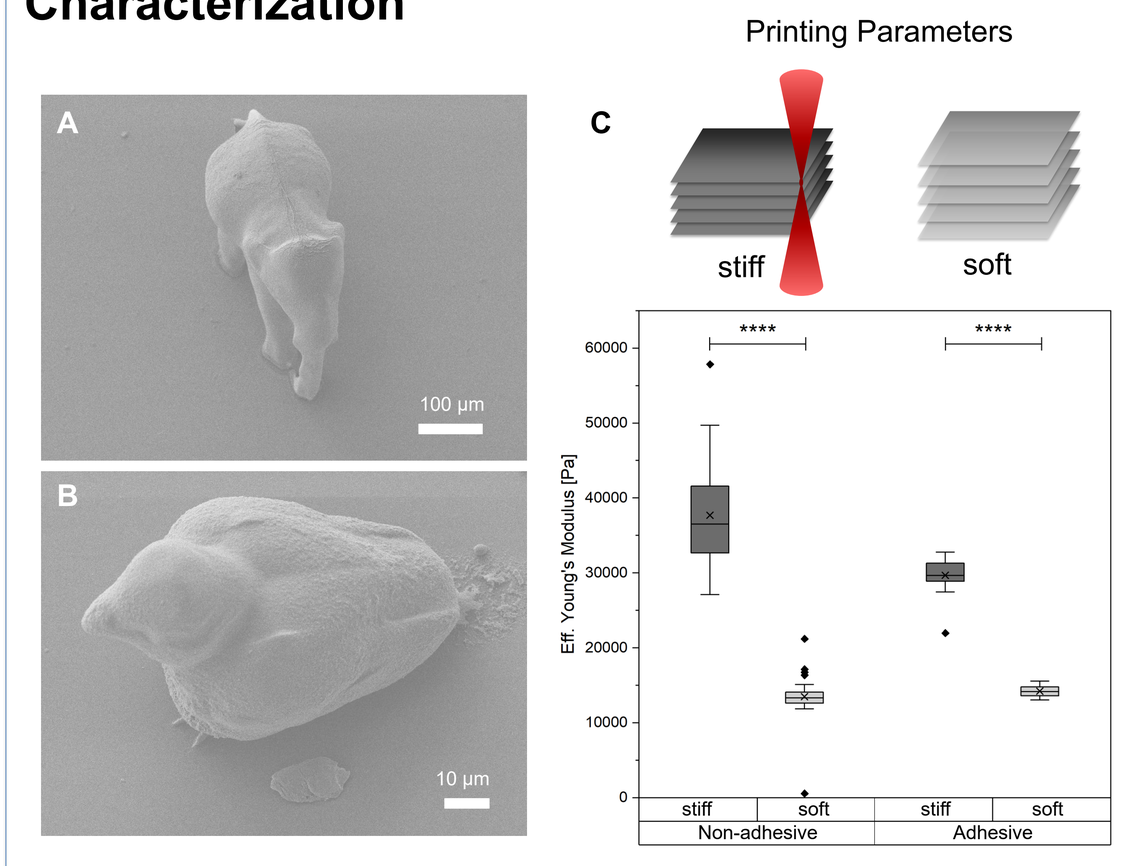


Fig. 2 A-B) Scanning electron microscopy of structures printed with different objectives. **C)** Stiffness measurements for the resins printed with different parameters.

Cell Scaffolds

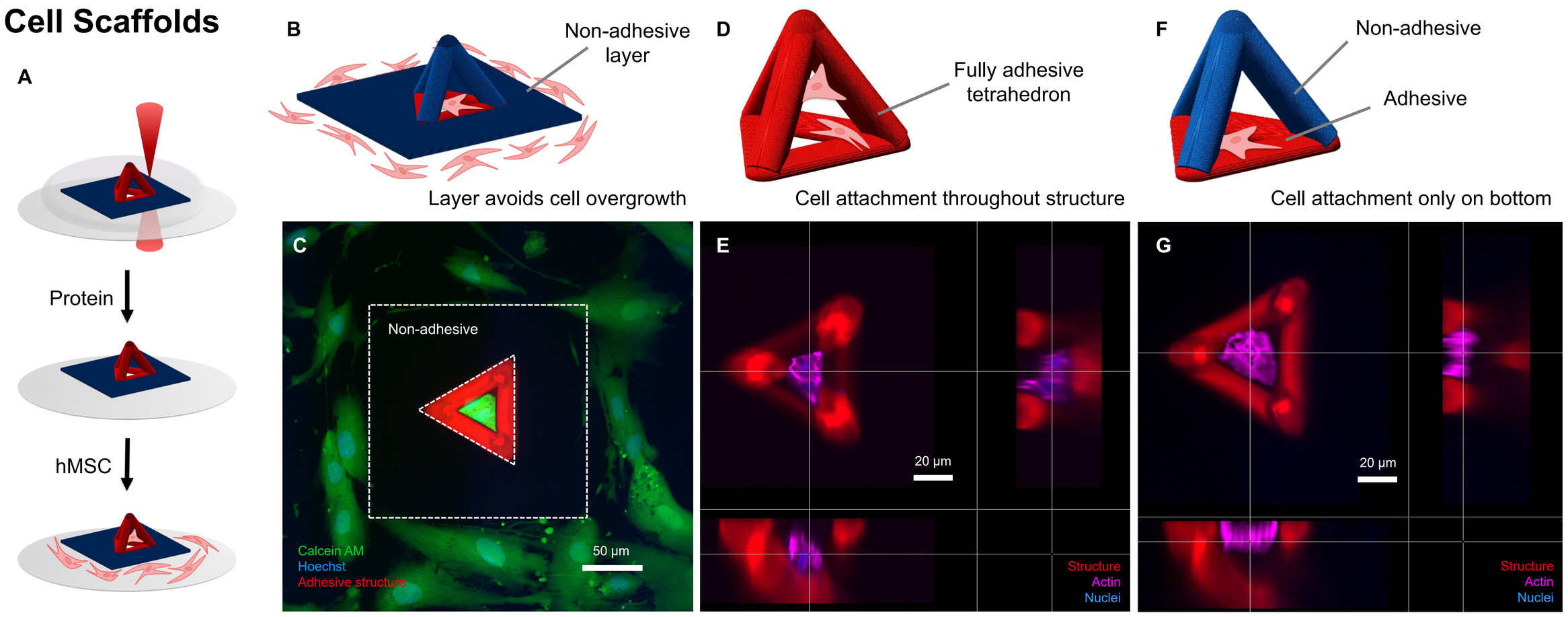


Fig. 3 A) Fabrication workflow of scaffolds for hMSC. **B)** Non-adhesive layer avoids cell overgrowth **C)** Z-projection of a confocal image of hMSC seeded onto the multimaterial scaffold. **D-E)** Scheme and cross section of z-stack of a fully adhesive structure. **F-G)** Scheme and confocal z-stack of a partially adhesive structure.

Conclusion

- We successfully show the implementation of the material for 2PP and optimized the printing and functionalization workflow.
- The resins show good printability with various print sets. Young's moduli are tunable between 10-20 kPa and 30-40 kPa.
- Cell studies with hMSC reveal that cell adhesion can be spatially controlled in multimaterial structures made of adhesive and non-adhesive resins.



Acknowledgements
We acknowledge the funding received by the collaborative research training group „Perpharmance" (BW6_07, Ministerium für Wissenschaft und Kunst (MWK), Baden-Württemberg). The authors thank the funding INST 35/1643-1 FUGG for the supply of the Nanoscribe Photonic Professional GT2. We thank Florine Sessler and the Biomechanics Core Facility for providing the Pavone Nanoindenter. We acknowledge Dr. Irene Wacker, Ernest Ronald Curticean and Prof. Dr. Rasmus R. Schröder for providing the SEM protocol and devices. We thank Sophia Schmidt, Mario Feinauer, Charlotte Weiß, Federico Colombo and Sebastian Weber for their support.

References
[1] Murphy W.L., McDevitt T.C., Engler A.J., Materials as stem cell regulators. *Nature Materials,* 2014,13(6), 547–557.
[2] Labouesse C., Tan B., Agley C. *et al.*, StemBond hydrogels control the mechanical microenvironment for pluripotent stem cells. *Nat. Commun.,* 2021, 12, 6132.
[3] O'Halloran S., Pandit A., Heise A., Kellett A., Two-Photon Polymerization: Fundamentals, Materials, and Chemical Modification Strategies. *Adv. Sci.*,2023, 10, 2204072.

UNIVERSITÄT HEIDELBERG ZUKUNFT SEIT 1386
3D MATTER MADE TO ORDER
DFG Deutsche Forschungsgemeinschaft
Excellence Strategy, EXC 2082/1 – 390761711
Carl Zeiss Stiftung
HELMHOLTZ RESEARCH FOR GRAND CHALLENGES
Excellence Networks, Phase 2 & 3
HEiKA Heidelberg Karlsruhe Strategic Partnership
PERPHARMANCE