3D Bioprinting of corneal stromal models using keratocyte-loaded hydrogels

Rohde M.[1], Duarte Campos D.F.[2], Vogt M.[3], Panfil C.[4], Johnen S.[1], Walter P.[1], Fuest M.[1]

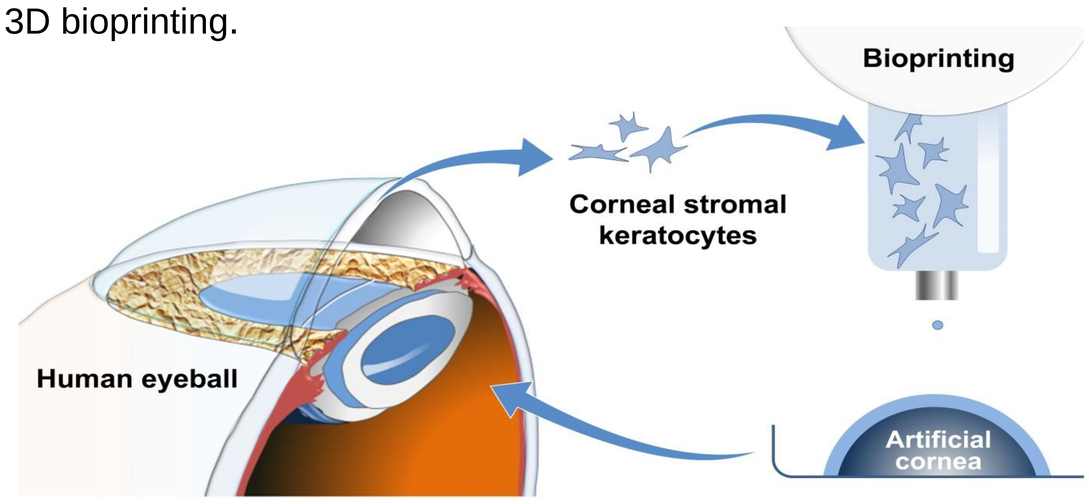
[1]Uniklinik RWTH Aachen, Klinik für Augenheilkunde, Aachen, Germany, [2]Uniklinik RWTH Aachen, Department of Dental Materials and Biomaterials Research, Aachen, Germany, [3]Interdisciplinary Center for Clinical Research IZKF, RWTH Aachen University Hospital, Aachen, Germany, [4]Aachen Center for Technology Transfer in Ophthamology (ACTO), RWTH Aachen University, Aachen, Germany

(Conflicts of interest: none)

Klinik für Augenheilkunde

Background

Corneal transplantation remains the integral treatment for advanced injuries and opacities of the cornea. However, it is limited by graft rejection and especially a global donor shortage. These issues have sparked a search for alternative therapies, among them corneal cell therapy and tissue engineering. Here we present our results for constructing artificial corneal stromal substitutes loaded with human corneal stromal keratocytes (CSK) by 3D bioprinting.



Purpose and Method

CSK were isolated from 27 donor corneas (13 male, 6 female), mean age 69.3 ± 14.9 years (range 34-88), which were unfit for transplantation. The CSK were expanded *in vitro* and passaged up to passage 4. For 3D printing via drop-on-demand technique (DoD) CSK were loaded into a hydrogel consisting of 0.5%agarose/0.2%collagen type I at a concentration of 10^6 cells/ml. The custom-made 3D bio-printer consisted of a 3-axis robotic platform (ISEL, Eichenzell, Germany) and was mounted with an electromagnetic micro-valve with a 300 µm nozzle diameter (Fritz Gyger, Gwatt, Switzerland).

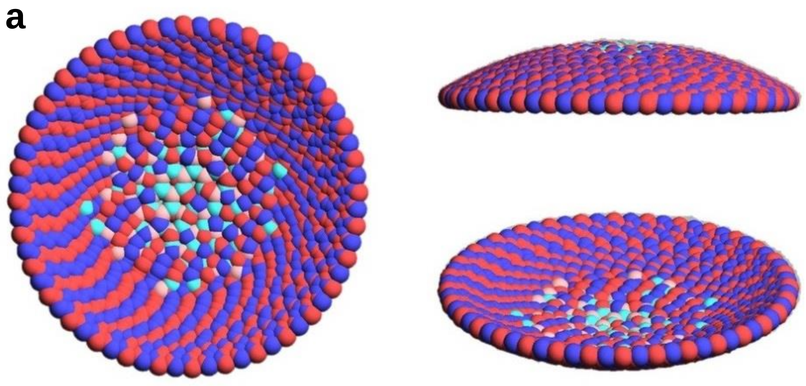


Figure 1. 3D corneal model. ***a.*** 3D corneal model sliced by using a customized slicing software for DoD printing. The software made it possible to distribute the A and B drops (blue and red) in an equal spacing. The remaining gaps were filled with filling drops (green and beige).

b. Bioprinted dome-shaped artificial corneas composed of a 0.5% agarose/ 0.2% collagen type I mixture. The bioprinted 3D model shows high transparency without distorsion of the text.

The 3D corneal model *(Figure 1)* was designed with AutoCAD (Autodesk, San Rafael, CA, USA). Constructs were evaluated for optical properties by coherence tomography and bio-mechanical strength by compression testing at 20% strain *(Figure 2).* CSK viability and phenotype were tested at 1 and 7 days using FDA/PI staining and two-photon laser scanning microscope imaging (FV1000MPE, Olympus, Tokyo, Japan), as well as immunohistochemistry *(Figures 3-5).*

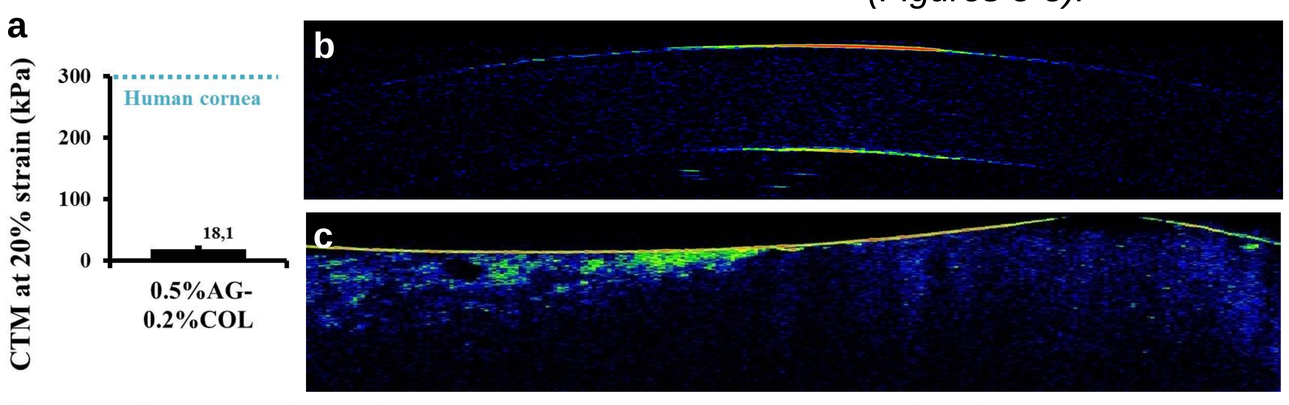


Figure 2. Evaluation of optical properties. ***a.*** Compressive tangent modulus of a bioink composed of 0.5% agarose/0.2% collagen measured by unconfined compression testing at 20% strain in comparison to the stiffness of native corneas. ***b.*** Optical coherence tomography images of native rabbit corneas in comparison with ***c.*** Optical coherence tomography images of CSK-loaded hydrogel mixture

Results

Printed corneal models had a diameter of 20 mm, a height of 4 mm and a thickness of 0.3 mm. Coherence tomography showed that the density of cell-free constructs and CSK-loaded bioprinted models was qualitatively similar to that measured for rabbit corneas *(Figure 2).* The biomechanical strength at 20% strain was found to be 18.1 ± 3.5 kPa, which only represents about 6% of the reported stiffness of a human cornea *(Figure 2).*

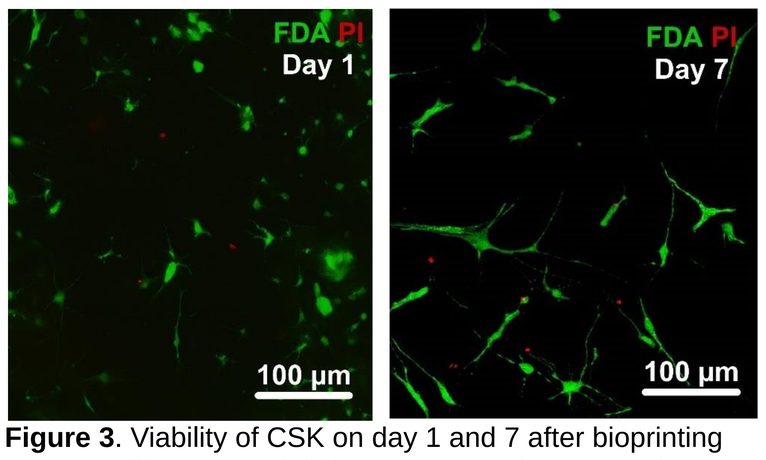
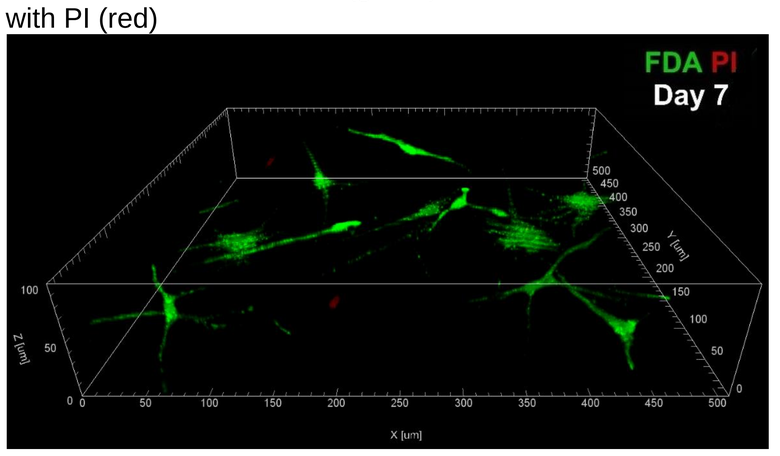


Figure 3. Viability of CSK on day 1 and 7 after bioprinting using a 0.5%agarose/0.2%collagen type I hydrogel; Live cells are stained with FDA (green) and dead cells stained with PI (red)



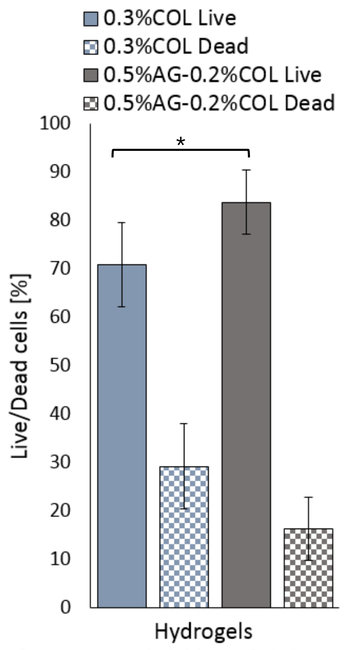


Figure 4. Viability of CSK 7 days after bioprinting in comparison with casted CSK-loaded collagen type I samples; *p<0.05 two sample t-test

After bioprinting, CSK remained viable up to 7 days in culture *(Figure 3)* with a viability of 83 ± 6.61 %. Casted CSK-loaded 0.3%collagen type I samples were used as controls and showed a significantly lower viability (p = 0.02) than CSK in the printed hydrogel mixture *(Figure 4).* Regarding cell shape, CSK showed a rounded shape one day after printing, but grew into their typical dendritic form by day 7, also expressing the typical CSK markers lumican and keratocan *(Figure 5).*

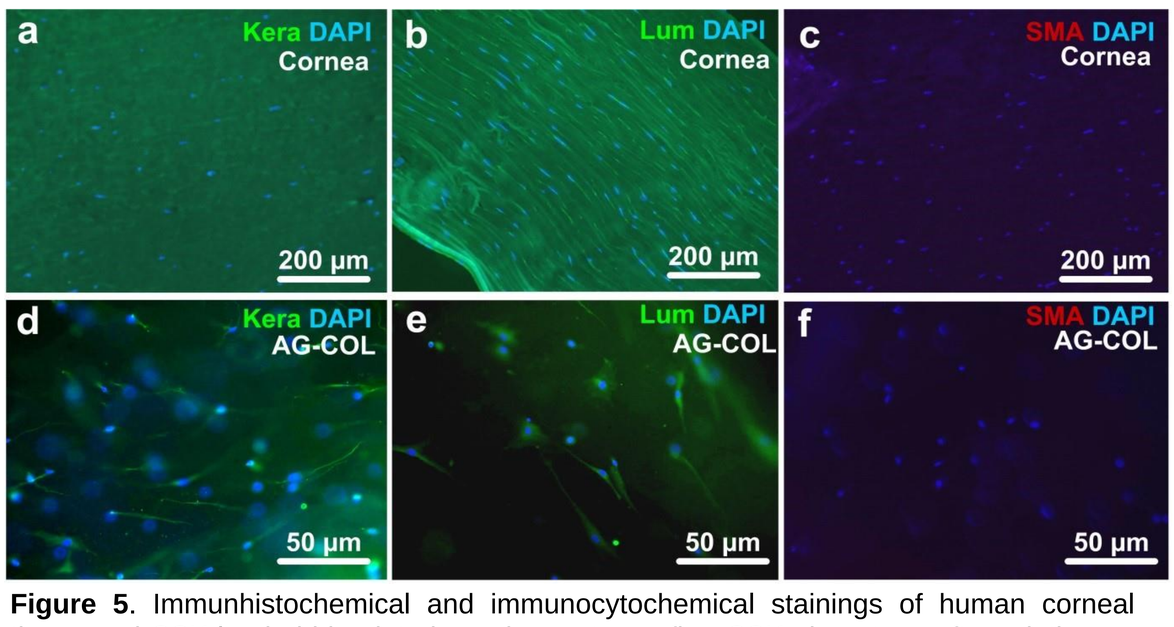


Figure 5. Immunhistochemical and immunocytochemical stainings of human corneal tissue and CSK-loaded bioprinted specimens to confirm CSK phenotype after printing. ***a.*** keratocan, ***b.*** lumican and ***c.*** smooth muscle actin (SMA) stainings of human corneal tissue slides as postive controls. ***d.*** keratocan, ***e.*** lumican and ***f.*** SMA stainings of CSK-loaded bioink blends 7 days post print. Printed CSK show a positive expression of CSK-markers keratocan and lumican and no expression of SMA, which is usually found in other cell types such as fibroblasts.

Conclusion

3D bioprinting is a suitable technique in corneal tissue engineering. CSK survived the DoD printing process and maintained a typical dendritic phenotype expressing CSK markers for 7 days. However, mechanical properties and transparency need to be improved in the future.