Title: Multi-Material 3D Bioprinting of Human Stem Cells to Engineer Complex Human Corneal Structures with Stroma and Epithelium

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Abstract
Corneal blindness, a leading cause of visual impairment globally, often relies on donor tissues for treatment. Advancing scalable and cost-effective multi-material bioprinting technologies that combine multiple cell types is crucial for creating biomimetic, complex human tissue substitutes and addressing the shortage of transplantable tissues. These developments hold the potential to revolutionize treatments for conditions that currently depend on donor tissues, including corneal blindness.

Here, extrusion-based 3D bioprinting was utilized to combine three clinically relevant hyaluronic acid based bioinks and two human stem cell derived cell types to produce corneal structures consisting of stromal and epithelial layers. Human adipose stem cell -derived corneal stromal keratocytes (hASC-CSKs) were selected for the stromal layer, and our previously developed multi-material 3D bioprinting strategy with cell-laden soft bioink and acellular stiff bioink was utilized to mimic the native corneal stroma organization. Human induced pluripotent stem cell -derived corneal limbal epithelial stem cells (hiPSC-LSCs) were 3D bioprinted in the epithelial layer, where the bioink was customized with ECM components found in native corneal epithelium to match the needs of hiPSC-LSCs.

The 3D bioprinted corneal structures demonstrated excellent cytocompatibility and provided a 3D environment which facilitated cellular interactions and network formation, essential for creating functional tissue substitutes. To the best of our knowledge, corneal structures with stroma and epithelium have not been previously 3D bioprinted using extrusion-based bioprinting technology. Consequently, this study contributes to the advancements in corneal bioprinting, particularly with respect to its translational perspective using clinically relevant cells and bioinks.