Bioengineering Human Corneal Endothelium using Printable Collagen IV

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Corneal endothelium transplantation accounts for 40% of corneal grafts[1]. However, an acute global shortage of donor corneal tissue continues to prevent access to treatment. Bioengineering a corneal endothelium to replace donor cornea is an alternative to solve this global challenge. Collagen IV (Col-IV) is the main contributor to the matrix network of Descemet's membrane. In this study, we have developed a printable Col-IV ink to bioengineer the endothelium through photo-crosslinking. Various printing parameters including UV vs blue light activation were tested to print a 2 layered Col-IV lattice by Edu3D printer (TRICEP, UOW), and the pore size was examined under light microscope. Pore size=1 indicated the optimal printing condition. Transparency of Col-IV membrane was examined by colour Quest XE spectrometer. B4G12 cells and primary human corneal endothelial cells were seeded and cultured on the printed membrane. Cell count and immunostaining were conducted to examine morphology, Ki67, ZO-1 and Na^+K^+ATPase. A mock clinical test was also conducted to demonstrate the compatibility of the bioengineered endothelium with current surgical techniques. All tests were repeated at least twice.

The results showed that the Col-IV ink required longer activation time with UV to be fully crosslink (2.69min) than blue light (400-500nm) which was only 41.73 seconds. However, the storage modulus for hydrogel formed by UV was 3 times higher than blue light. The printability test showed that Col-IV lattice can reach a pore size of 0.93 when printed at room temperature with a printing speed of 150mm/min, extrusion rate of 0.8mm/min and through a 25GA needle tip. The transparency of the Col-IV membrane was at 90.43%. Corneal endothelial cells can reach full confluency on the membrane which showed classic hexagonal shapes and strong expression of Ki67, ZO-1, and Na^+K^+ATPase (Figure. 1). The mock clinical test showed that the bioengineered endothelium can be successfully transplanted into the artificial eye and porcine eyes without obvious loss of endothelial cells.

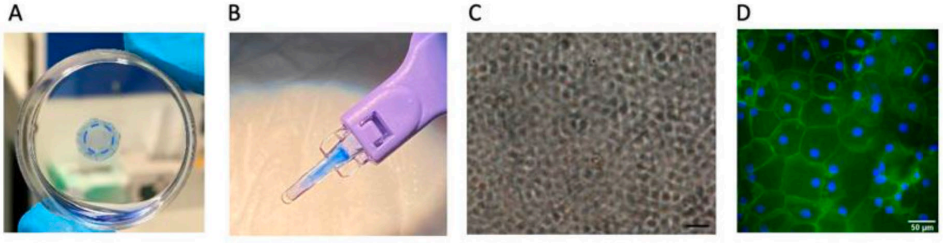

Figure. 1: *Col-IV based bioengineered corneal endothelium. A: the membrane stained with vision blue (a dye commonly used to stain corneal endothelium layer). B: the membrane can be aspirated into the Stryker injector. C-D: corneal endothelial cells maintained hexagonal morphology and expressed Na+K+-ATPase shown as the green borders in D.*

Our study demonstrated the successful development of a printable Col-IV ink to bioengineer human corneal endothelium. As Col-IV is the main matrix protein in Descemet's membrane, this new bioengineered corneal endothelium has high potential to replace donor endothelium. The printable Col-IV ink required short light activation which can also have a wide range of potential applications such as a carrier for primary endothelial cell culture and for cell printing.

[1]Gain, P., et al. *JAMA Ophthalmol* **2016,** 134, 167-173.