

RESEARCH ARTICLE

3D printing collagen type IV for corneal endothelium transplantation

Supplementary file

(A) Printability test

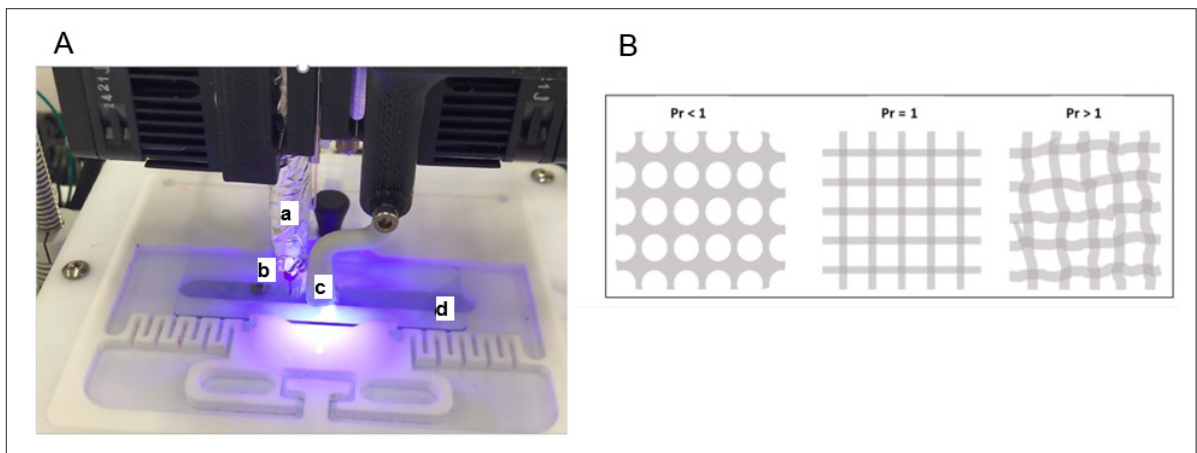


Figure S1. Methods of characterizing printable collagen type IV (Col-IV) ink. (A) Set-up of the Edu3D printer for pore testing: (A, a) Col-IV ink is loaded on the foiled wrapped syringe; (A, b) printing a two-layered lattice structure; (A, c) light activation; (A, d) glass slide. (B) Representation of structures classified based on printability (Pr) values, where Pr = 1 is the optimum printability.

(B) Compression test

A compression test was conducted to evaluate Young's modulus for the photo-crosslinked Col-IV scaffold. The EZ-L Table-Top Universal Testing Instrument (Shimadzu, Japan) equipped with a 2 N load cell was used for this test. Col-IV hydrogel discs (5×5 mm) were prepared using a homemade syringe mold (Figure S2A, Supporting Information). The Col-IV scaffold was removed from the mold and placed in the center of the bottom clamp. The scaffold was then compressed at a rate of 1 mm/min to 65% of its height. A stress vs. strain graph was generated, and Young's modulus was calculated as the slope of its linear region (Figure S2B, Supporting Information). The test was repeated five times.

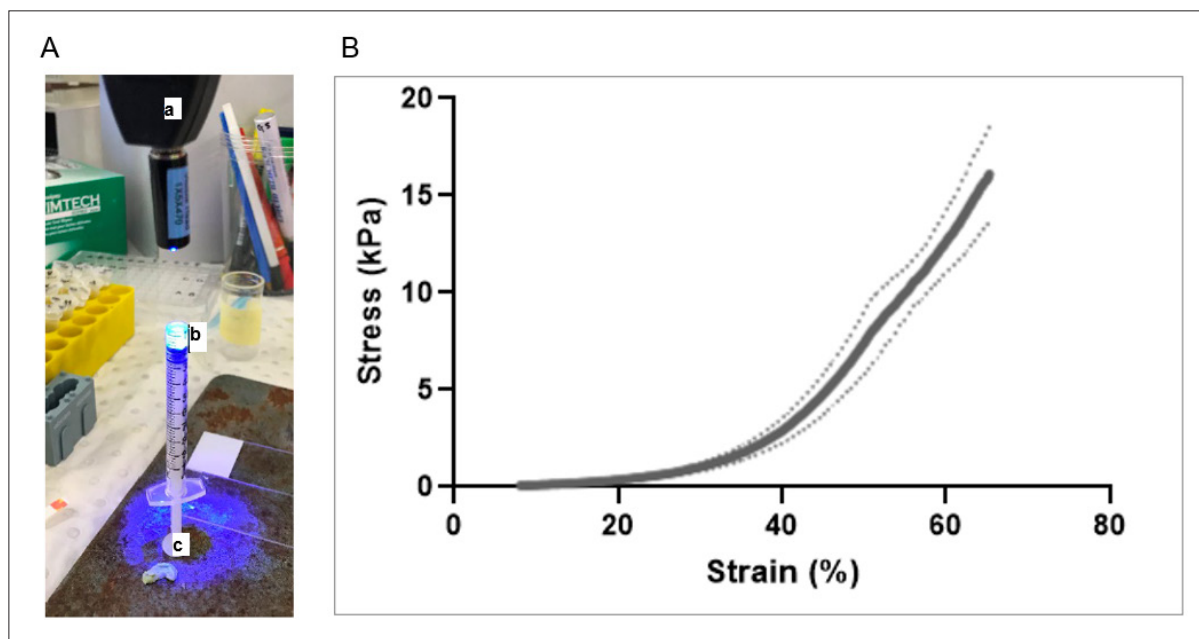


Figure S2. Mechanical testing of collagen type IV (Col-IV) hydrogel. (A) A mold created from a syringe is used to create thick Col-IV crosslinked hydrogels of varying thicknesses through adjustments of the syringe height; (A, a) a light source, (A, b) Col-IV solution filling the height set in the mold, and (A, c) the syringe secured at the base. (B) Stress vs. strain graph of Col-IV hydrogel.

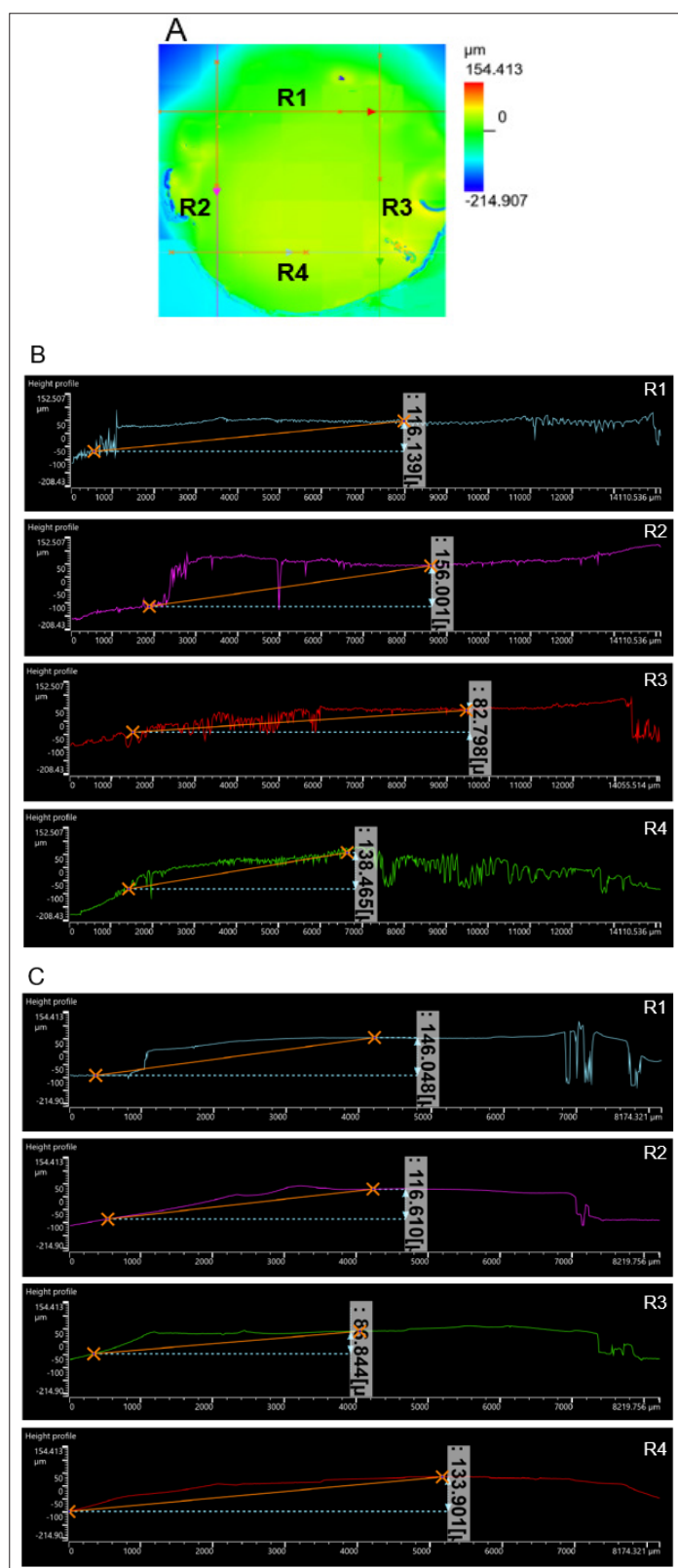


Figure S3. Thickness measurement of membranes. (A) A representative image of the membrane where four different regions were selected for thickness measurement. (B) Bioprinted membrane areas R1–R4 were used to generate height profiles with an average measured membrane thickness of $123.4 \pm 26 \mu\text{m}$. (C) Molded membrane areas R1–R4 were used to generate height profiles with an average measured thickness of $120.9 \pm 26 \mu\text{m}$.

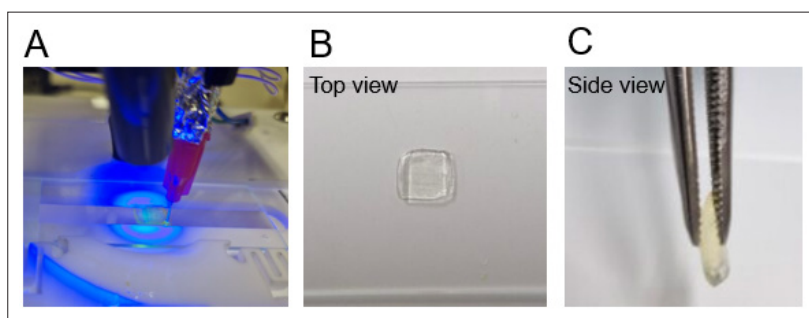


Figure S4. 3D printing of collagen type IV (Col-IV) four-layered square structure. (A) The printing process. (B) Top-view of the printed structure. (C) Side-view of the printed structure.

(C) Summary of antibodies used

Table S1. Primary and secondary antibodies used for immunofluorescent labeling

Antibody	Final concentration/ dilution	Source
Anti-ZO-1	5 µg/mL	33-9100; Invitrogen, USA
Anti-Na ⁺ /K ⁺ -ATPase	4 µg/mL	sc-71638; Santa Cruz Biotechnology, USA
Anti-Ki67	4 µg/mL	ab15580; Abcam, UK
Anti-laminin β1	5.3 µg/mL	ab256380; Abcam, UK
Donkey anti-mouse IgG Alex Fluor 488	1:1000	R37114; Invitrogen, USA
Donkey anti-rabbit IgG Alexa Fluor 594	1:1000	R37119; Invitrogen, USA

Abbreviations: IgG: Immunoglobulin G; UK: United Kingdom; USA: United States of America.