ACCSCIENCE PUBLISHING

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RESEARCH ARTICLE

Fabrication of biomimetic corneas featuring epithelial, stromal, and endothelial layers via bioprinting

Supplementary file

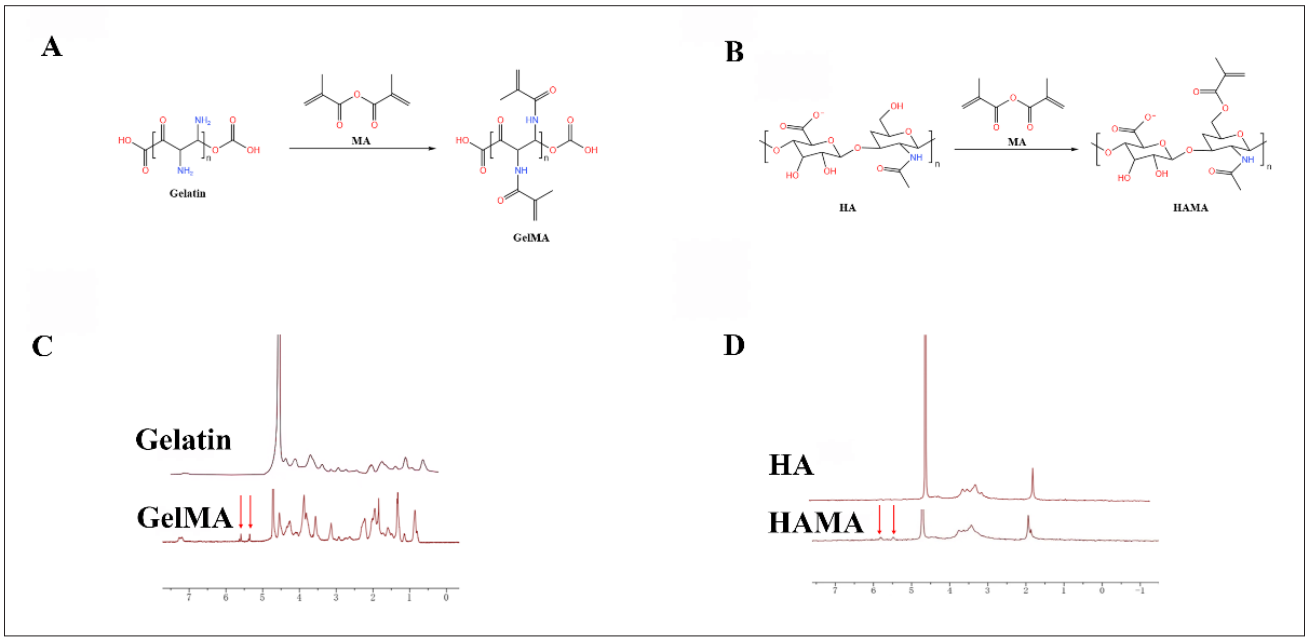

Figure S1. Synthesis and nuclear magnetic resonance (NMR) characterization of gelatin methacryloyl (GelMA) and hyaluronic acid methacryloyl (HAMA). (A) Methacrylation of gelatin using methacrylic anhydride (MA). (B) Methacrylation of hyaluronic acid via MA. (C) ^{1}H NMR spectrum of GelMA (deuterium oxide [D_2O], 400 MHz) showing methacrylate vinyl protons (5.3–6.1 ppm). The degree of methacrylation (DoM) was 73.6 ± 0.3% (mean ± standard deviation [SD], n = 3). (D) ^{1}H NMR spectrum of HAMA (D_2O, 400 MHz) with vinyl proton signals at 5.3–6.1 ppm. The DoM was 13.7 ± 0.1% (mean ± SD, n = 3).

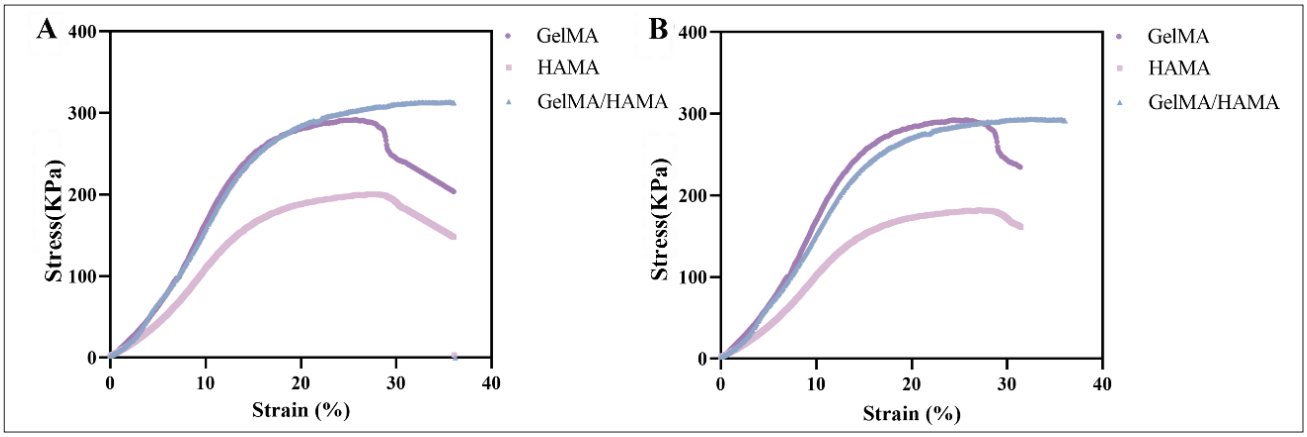

Figure S2. Uniaxial tensile behavior of hydrogels with and without sutures. (A) Engineering stress–strain curves of hydrated gelatin methacryloyl (GelMA), hyaluronic acid methacryloyl (HAMA), and GelMA/HAMA strips (25 × 4 × 1 mm) after phosphate-buffered saline equilibration, tested at 37 °C (10 mm gauge length, 10 mm·min^{-1} extension rate). (B) Tensile response of the same formulations after placement of a single 9-0 nylon stitch (2.0 mm from edge, 1.0 mm bite), showing the constrained mechanical behavior imparted by suturing.

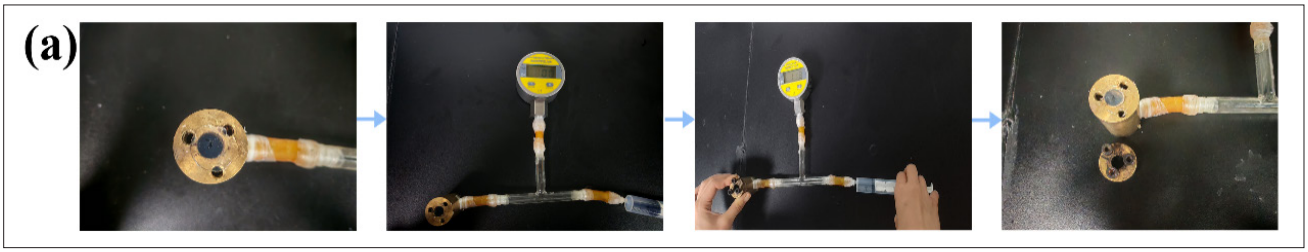

Figure S3. Burst pressure testing of hydrogel discs. A hydrogel disc (12–14 mm diameter, ~0.50 ± 0.02 mm thickness) was mounted in a custom fixture with a 10 mm pressurized aperture and subjected to a controlled pressure ramp (1–2 kPa·s^{-1}) until failure. The mean burst pressure was 11.4 ± 0.31 kPa (mean ± standard deviation, n = 3).

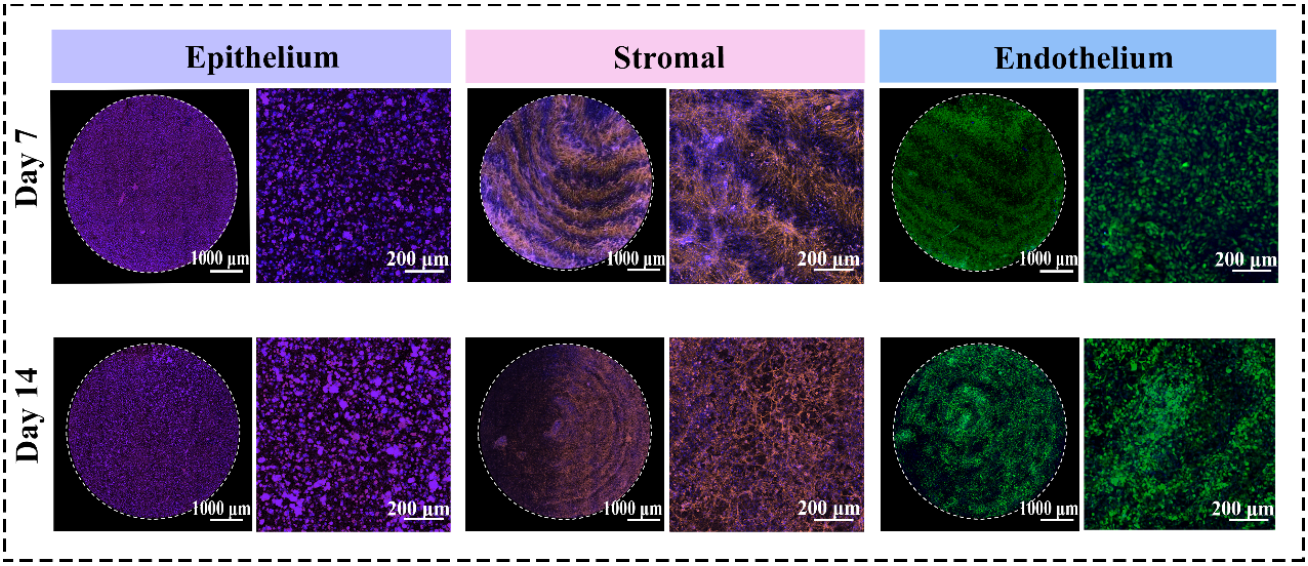

Figure S4. Layer-resolved confocal imaging of the bioprinted trilayer corneal construct. Whole-mount overviews and zoomed views of the epithelial, stromal, and endothelial layers at day 7 and day 14. The epithelium maintains dense and continuous coverage; the stromal layer shows progressively organized extracellular matrix; and the endothelium forms a confluent, polygonal monolayer. Scale bars: whole-mount: 1,000 μm, zoom: 200 μm.

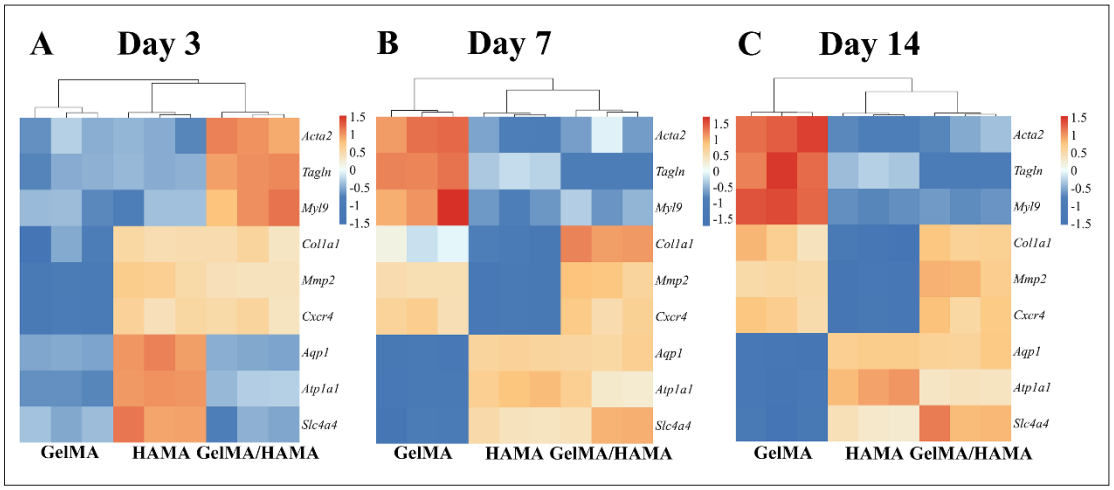

Figure S5. Temporal gene expression profiles of gelatin methacryloyl (GelMA), hyaluronic acid methacryloyl (HAMA), and GelMA/HAMA (GH) matrices. (A–C) Heatmaps show normalized mRNA levels at days 3, 7, and 14. GH sustains downregulation of myofibroblast markers (*Acta2*, *Tagln*, *Myl9*) while upregulating extracellular matrix remodeling genes (*Col1a1*, *Mmp2*), the migration receptor *Cxcr4*, and corneal pump genes (*Aqp1*, *Atp1a1*, *Slc4a4*), supporting its dual role in suppressing fibrosis and promoting functional repair. Data represent *z*-score normalized expression (red: high; blue: low) from three biological replicates. Data are shown as mean ± standard deviation for $n = 3$ biological replicates, with each sample tested in triplicate. Statistical significance was evaluated using one-way analysis of variance with Tukey's honestly significant difference post hoc test. The *p*-values were adjusted for multiple testing using the false discovery rate.

Table S1. Primer sequence

Gene	Primer	Sequence (5' to 3')
Actb		
	Forward primer	GGACACGTACCGTTCTCCG
	Reverse primer	CATTCCCACCATCACACCCT
Klf4		
	Forward primer	GAAGGGAGAAGACACTGCGT
	Reverse primer	GGGGGAAGTCGCTTCATGTG
Pax6		
	Forward primer	CCCGAATTCTGCAGACCCAT
	Reverse primer	AGTCGCCACTCTTGGCTTAC
Col1a1		
	Forward primer	TTCTCCTGGCAAAGACGGAC
	Reverse primer	CGGCCACCATCTTGAGACTT
Col4a4		
	Forward primer	CCCATGGGATATTCCGGAGC
	Reverse primer	CTGTCCCTTCACTCCCACAC
Zeb1		
	Forward primer	CTGCTCCCTGTGCAGTTACA
	Reverse primer	GTGCACTTGAACTTGCGGTT
Foxc1		
	Forward primer	CGCTTCAAGAAGAAGGACGC
	Reverse primer	GGACACGTACCGTTCTCCG

Table S1. Primer sequence

Gene	Primer	Sequence (5' to 3')
Acta2		
	Forward primer	GAACTGTCACCCCAGCGAAC
	Reverse primer	GCCTTTGCAACAGCTTGCTT
Tagln		
	Forward primer	GCAGTTTGGCTGTGACCAAAA
	Reverse primer	TGCTCCTGGGCTTTCTTCATA
Myl9		
	Forward primer	CCTCAGGCTTCATCCACGAG
	Reverse primer	CCTCCTCATCCGTGAATCGG
Mmp2		
	Forward primer	ACAAGTGGTCCGCGTAAAGT
	Reverse primer	AGGCCATGGGTTGGATCTTC
Cxcr4		
	Forward primer	CCGGTACCTCGCTATTGTCC
	Reverse primer	CTATCGGGGTAAAGGCGGTC
Aqp1		
	Forward primer	GTCGACAATTCACTTGGCCG
	Reverse primer	AGCTGCAGAGTGCCAATGAT
Atp1a1		
	Forward primer	CTGGCTGAGAACGGTTTCCT
	Reverse primer	AACGCTGTATGGCAGGTGAA
Slc4a4		
	Forward primer	GCCTGTCAGTCTTCATGGCT
	Reverse primer	TGAGGCCACCCCCATATACA