ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

3D printing of the keloid scar using tunable GelMA-based bioinks for skin fibrosis modeling

Supplementary file

Supplementary Information

1. Swelling ratio

Following printing, the hydrogel constructs were immersed in phosphate-buffered saline at room temperature for 24 h to allow swelling. The swollen hydrogels were then weighed to obtain the wet weight (W_{wet}). Subsequently, the samples were freeze-dried overnight, and the dry weight was measured (W_{dry}). The swelling ratio was calculated as follows:

$$\text{Swelling ratio (\%)} = \frac{Wwet - Wdry}{Wdry} \times 100\% \qquad \text{(SI)}$$

where W_{dry} represents the dry weight of the lyophilized bioinks, and W_{wet} represents the wet weight of the bioinks.

2. Enzymatic degradation assay

The degradation assay was performed by immersing the hydrogel in a medium containing 0.005% collagenase type 2 (cat# LS004177, Worthington, USA). The hydrogel was weighed initially (*Wi*: 0 h) and incubated for 3 days at 37°C. The dry weights were measured at different time points (*Wt*: 24, 48, 64 h). The percentage of degradation was calculated using the following equation:

$$\text{Degradation (\%)} = \frac{Wt - Wi}{Wi} \times 100\% \qquad \text{(SII)}$$

where *Wt* represents the dry weight of the lyophilized bioinks after immersion in collagenase at a specific time point, and *Wi* represents the dry weight of the lyophilized bioinks at time point = 0.

Supplementary Figures and Tables

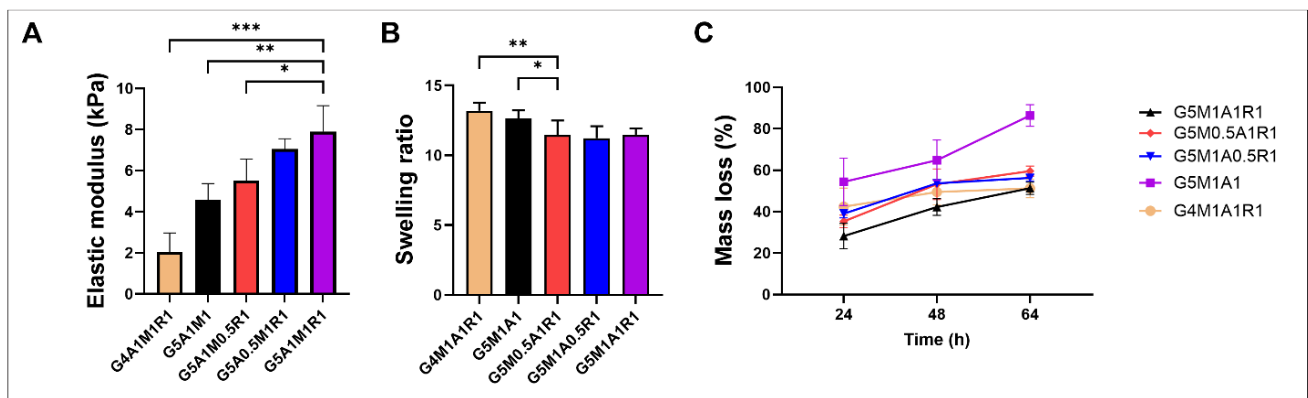

Figure S1. Measurement of elastic modulus (A), swelling ratio (B), and mass loss (C) of printed hydrogel. n = 5 (G4M1A1R1), 3 (G5M1A1), 3 (G5M10.5A1R1), and 4 (G5M1A1R1). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. the other groups.

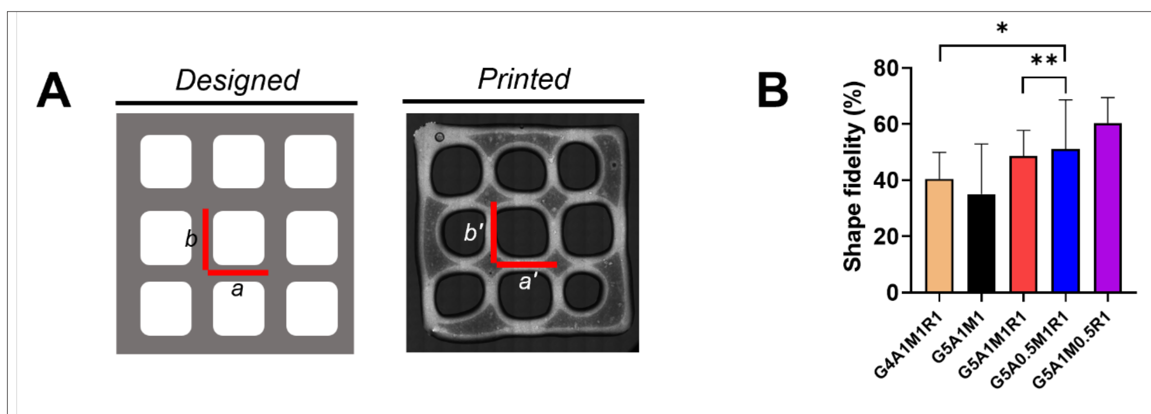

Figure S2. Quantification of shape fidelity was performed by comparing the measured pore size of printed constructs to the intended design specifications. $^*p < 0.05$, $^{**}p < 0.01$ vs. the other groups.

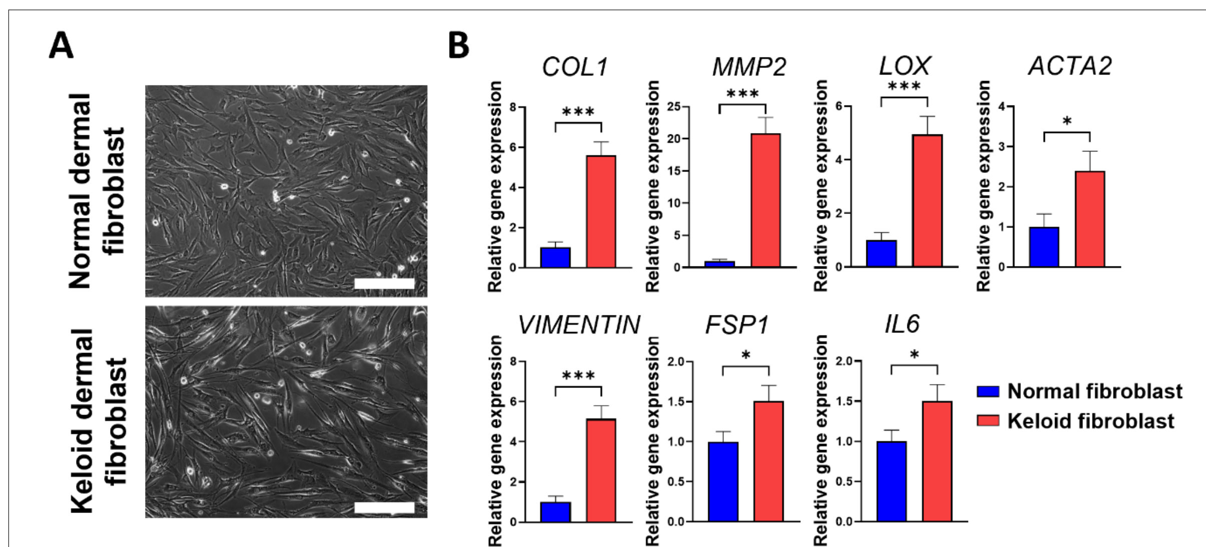

Figure S3. Comparative fibrotic gene expression between normal and keloid dermal fibroblasts. (A) Representative phase-contrast images of normal dermal fibroblasts (purchased from Daewoong Pharmaceutical, Korea) and patient-derived keloid dermal fibroblasts cultured on tissue culture plastic (TCP). Scale bar: 275 μm; magnification: 10×. (B) Quantitative gene expression analysis of fibrosis-related markers (*COL1, MMP2, LOX, ACTA2, VIMENTIN, FSP1, and IL6*) in keloid dermal fibroblasts compared to normal dermal fibroblasts on TCP. Data are presented as mean ± SD ($n = 3$). $^*p < 0.05$, $^{***}p < 0.001$.

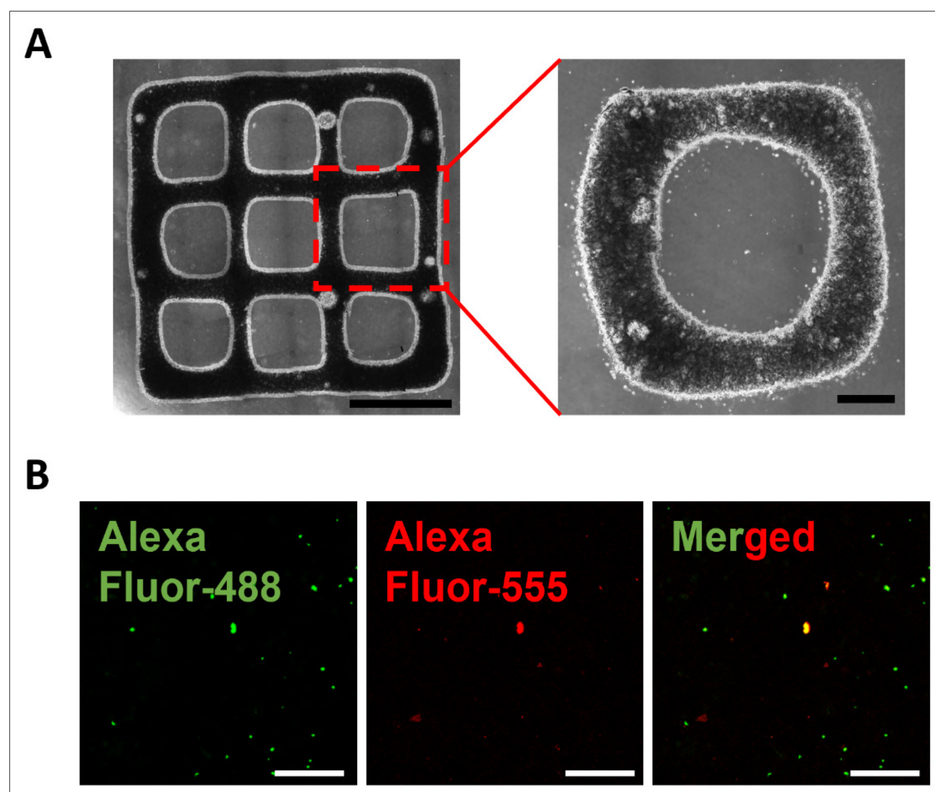

Figure S4. a single square-shaped bioprinted hydrogel construct, adapted from one unit of a previously used 3×3 square grid design, and utilized for subsequent immunofluorescence staining and imaging. Scale bars: 5 mm (3 × 3 grid) and 1 mm (single square). (B) Representative image of the negative control for immunofluorescence staining, confirming antibody specificity and minimal background signal. Scale bar: 200 μm; magnification:10×.

Table S1. Bioink compositions

Groups	Concentration (wt%)			
	GelMa (G)	Alginate (A)	Methylcellulose (M)	RDS (R)
G4A1M1R1	4	1	1	1
G5A1M1	5	1	1	0
G5A1M1R1	5	1	1	1
G5A0.5M1R1	5	0.5	1	1
G5A1M0.5R1	5	1	0.5	1

Table S2. Pressure and speed for the printing process of each bioink

Groups	Pressure (kPa)	Speed (mm/s)
G4A1M1R1	5	80
G5A1M1	3	250
G5A1M1R1	5	150
G5A0.5M1R1	6	300
G5A1M0.5R1	5	150

Table S3. List of primer used in this study

Gene name	Sequence (5′-3′)
COL1	Forward: CAAGACAG TGATTGAATACAAAACCA Reverse: ACGTCGAAGCCGAATTCCT
FSP1	Forward: TCTTTCTTGGTTTGATCCTGACT Reverse: AGTTCTGACTTGTTGAGCTTGA
ACTA2	Forward: AAGCACAGAGCAAAAGAGGAAT Reverse: ATGTCGTCCCAGTTGGTGAT
VIMENTIN	Forward: AATCCAAGTTTGCTGACCTCTCTGA Reverse: ACTGCACCTGTCTCCGGTACTC
MMP2	Forward: GATACCCCTTTGACGGTAAGGA Reverse: CCTTCTCCCAAGGTCCATAGC
LOX	Forward: TTCCAGTACGGTCTCCCAGA Reverse: TGGCCAGACAGTTTTCCTCC
IL6	Forward: ACTCACCTCTTCAGAACGAATTG Reverse: CCATCTTTGGAAGGTTCAGGTTG
GAPDH	Forward: CACTCCACCTTTGACGC Reverse: GGTCCAGGGGTCTTACTCC

Table S4. List of primary and secondary antibodies used for immunofluorescence

Cat#	Manufacturer	Antibody	Dilution factor
ab5694	Abcam	α-SMA	1:100
sc-100784	Santa Cruz Biotechnology	MTS-1	1:100
A34055	Invitrogen	Alexa Fluor™ 555 Phalloidin	1:400
A11001	Invitrogen	Goat anti-Mouse IgG (H+L), Alexa Fluor™ 488	1:200
A31573	Invitrogen	Donkey anti-Rabbit IgG (H+L, Alexa Fluor™ 647	1:200

Table S5. Mechanical properties of bioink formulations

Sample	Toughness (kJ/m^3)	Stress at break (kPa)	Strain at break (m/m)
G4A1M1R1	0.41 ± 0.27	2.93 ± 1.72	0.42 ± 0.24
G5A1M1	5.96 ± 1.04	39.52 ± 8.53	0.70 ± 0.00
G5A1M1R1	7.35 ± 1.88	50.89 ± 12.36	0.67 ± 0.02
G5A0.5M1R1	9.09 ± 1.38	59.31 ± 8.96	0.70 ± 0.00
G5A1M0.5R1	2.36 ± 0.67	14.20 ± 5.72	0.57 ± 0.01

Table S6. Shape fidelity of each bioink construct

Sample	Printed area (mm^2)	Designed area (mm^2)	Shape fidelity (%)
G4A1M1R1	8.21 ± 1.91	20.25 ± 0	40.52 ± 9.41
G5A1M1	7.11 ± 3.61	20.25 ± 0	35.10 ± 17.82
G5A1M1R1	9.86 ± 1.85	20.25 ± 0	48.68 ± 9.12
G5A0.5M1R1	10.37 ± 3.54	20.25 ± 0	51.19 ± 17.46