ACCSCIENCE PUBLISHING

International Journal of Bioprinting

RESEARCH ARTICLE

Three-dimensional bioprinting of biomimetic bilayer hydrogels with engineered mechanical and cellular heterogeneity for wound healing

Supplementary File

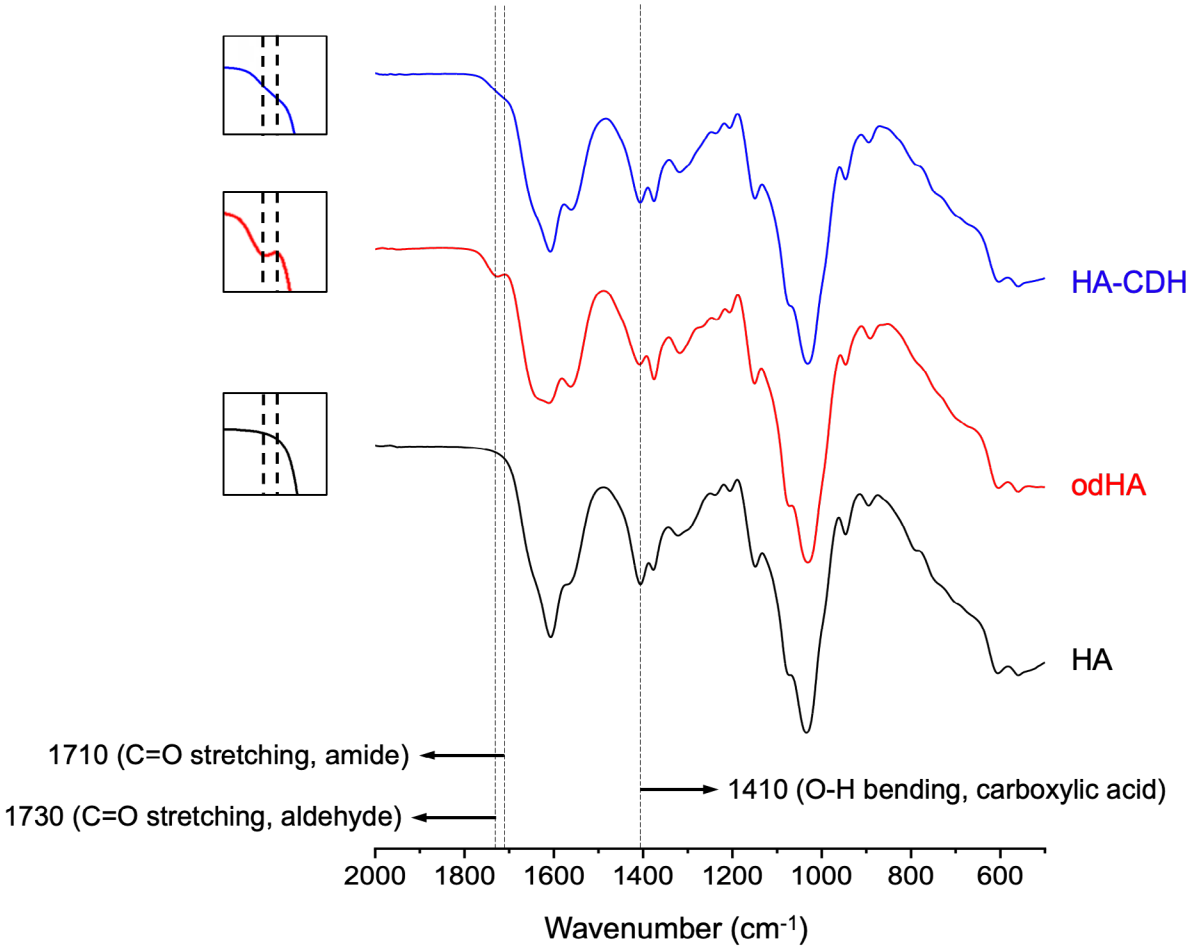

Figure S1. Fourier-transform infrared spectra of hyaluronic acid (HA), carbodihydrazide-conjugated hyaluronic acid (HA-CDH), and oxidized diol-modified hyaluronic acid (odHA)

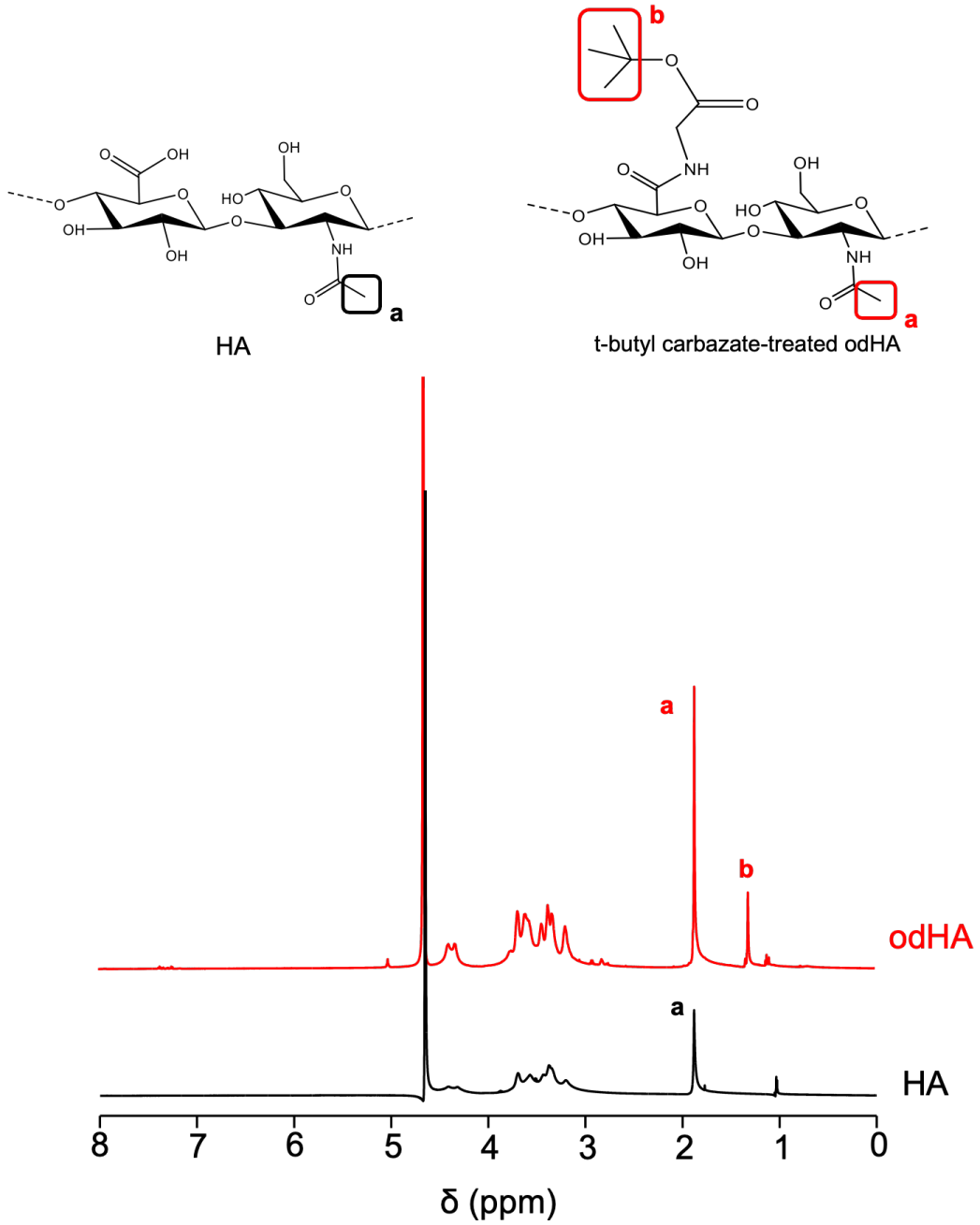

Figure S2. ^{1}H nuclear magnetic resonance spectra of hyaluronic acid (HA) and oxidized diol-modified hyaluronic acid (odHA)

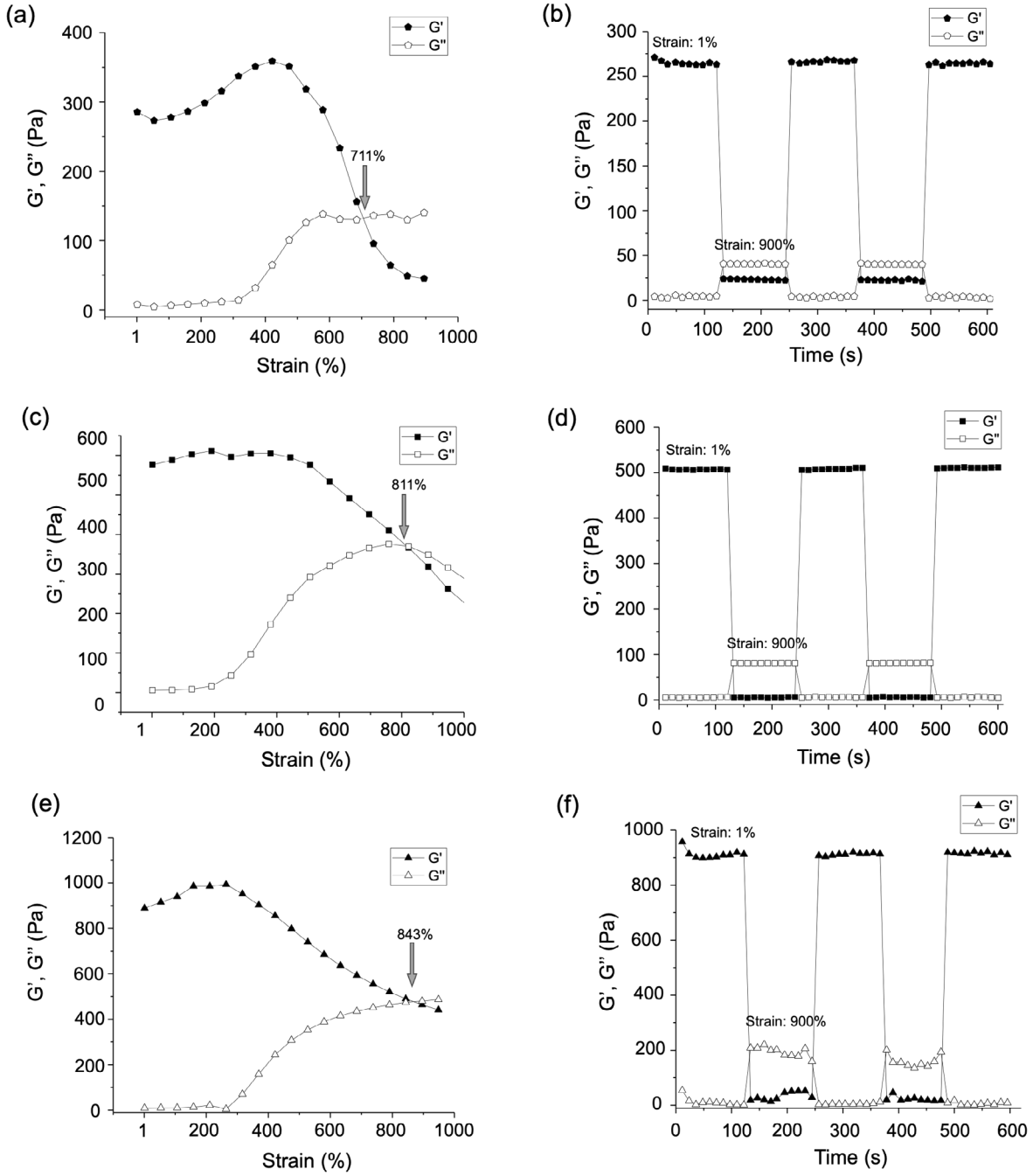

Figure S3. Amplitude sweep tests of (a) HA-CDH 0.5, (c) HA-CDH 1.0, and (e) HA-CDH 1.5 hydrogels. Repeated self-healing behavior of (b) HA-CDH 0.5, (d) HA-CDH 1.0, and (f) HA-CDH 1.5 hydrogels assessed using a rotational viscometer.

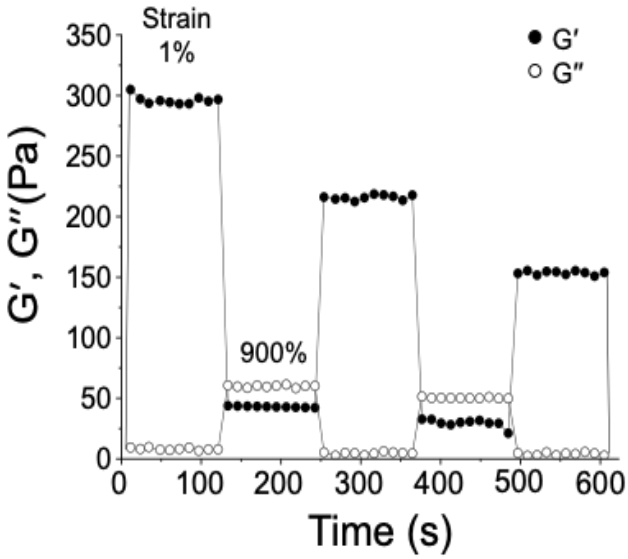

Figure S4. Repeated self-healing behavior of HA-CDH/odHA hydrogels (without adipic acid dihydrazide) assessed using a rotational viscometer

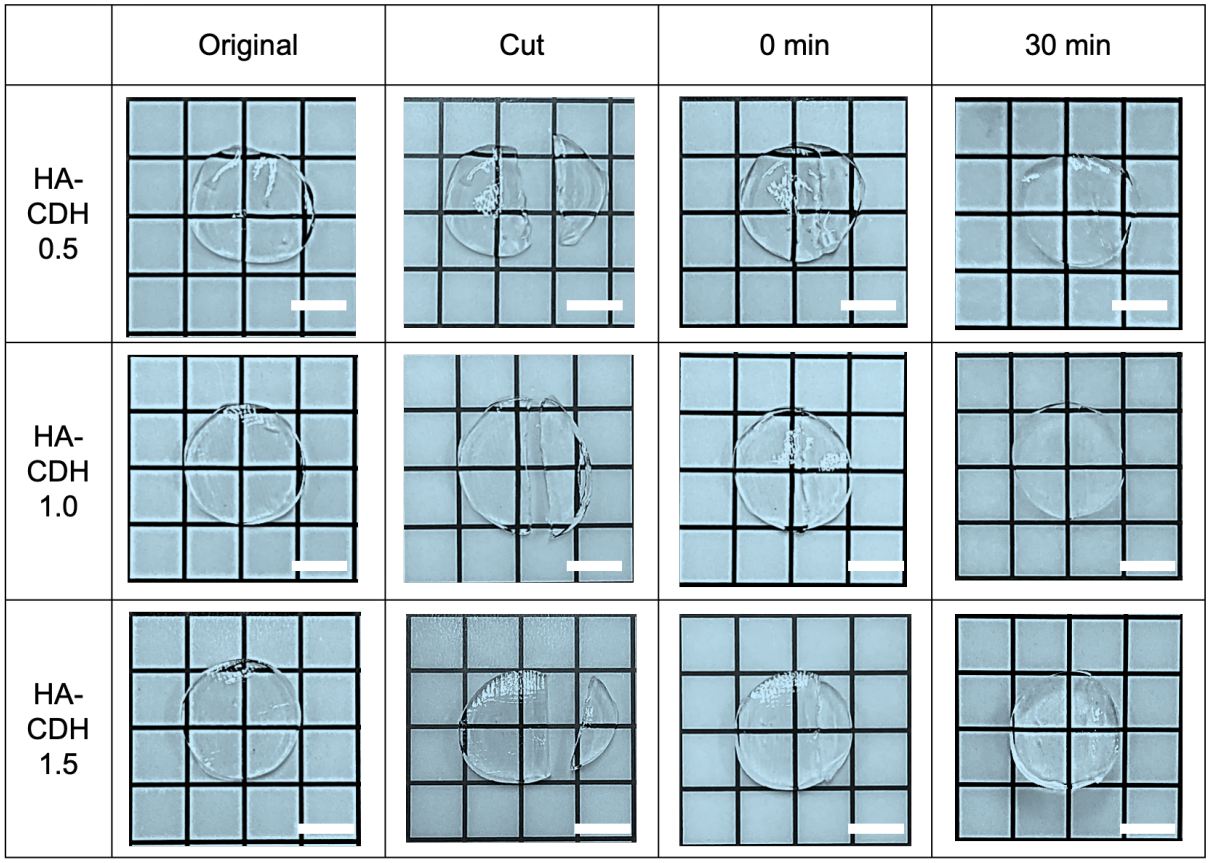

Figure S5. Self-healing behavior of HA-CDH 0.5, HA-CDH 1.0, and HA-CDH 1.5 hydrogels. The hydrogel disks were cut into two pieces and then reattached, forming fully integrated gel disks within 30 min at room temperature.

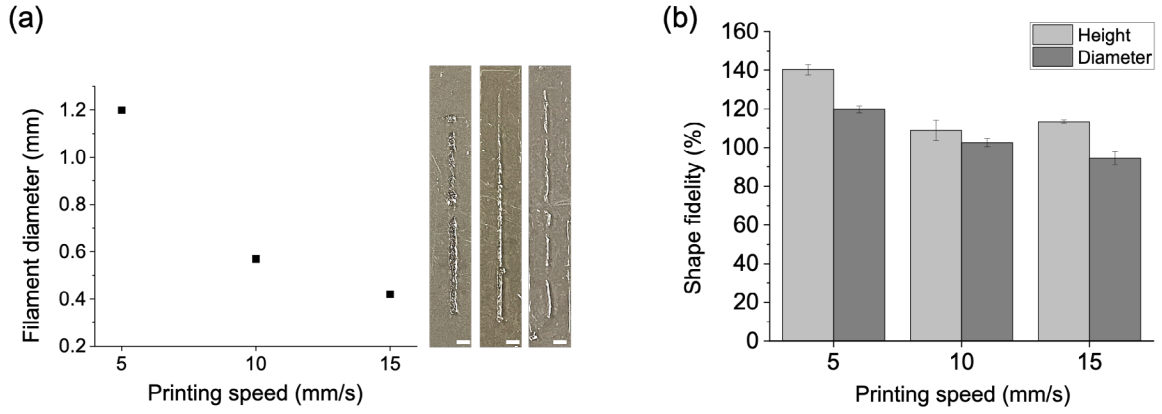

Figure S6. Printability and shape fidelity of HA-CDH 1.5 hydrogels. (a) Changes in filament diameters and images of the filaments of HA-CDH 1.5 hydrogels printed at various printing speeds (scale bars: 2 mm). (b) Shape fidelity of HA-CDH 1.5 hydrogels at various printing speeds (n = 4).

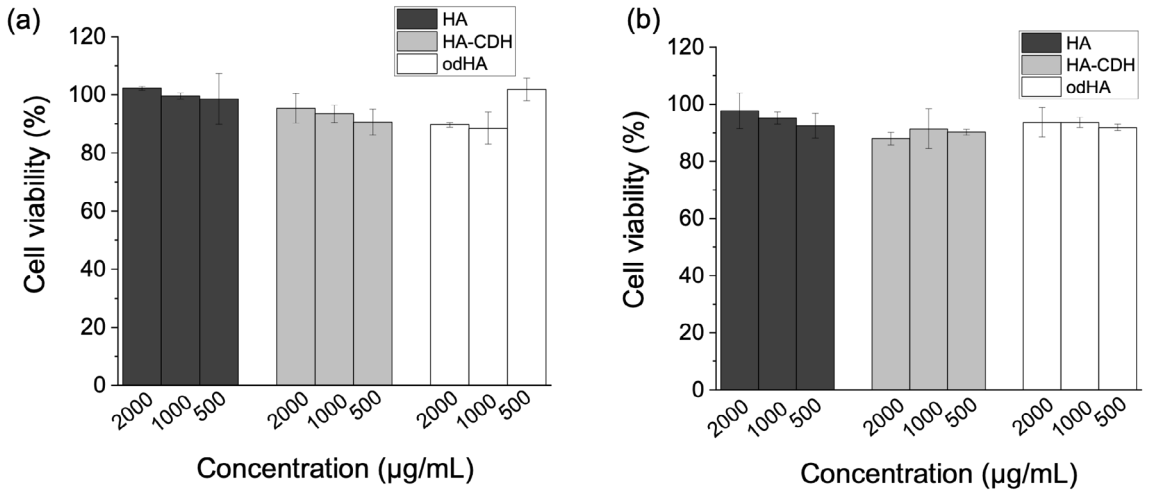

Figure S7. Relative viability of (a) HEKn and (b) HDFn cells treated with hyaluronic acid (HA), carbodihydrazide-conjugated hyaluronic acid (HA-CDH), and oxidized diol-modified hyaluronic acid (odHA) solutions at various concentrations (EZ-Cytox, cell seeding density = 5.0×10^3 cells/well, n = 4).

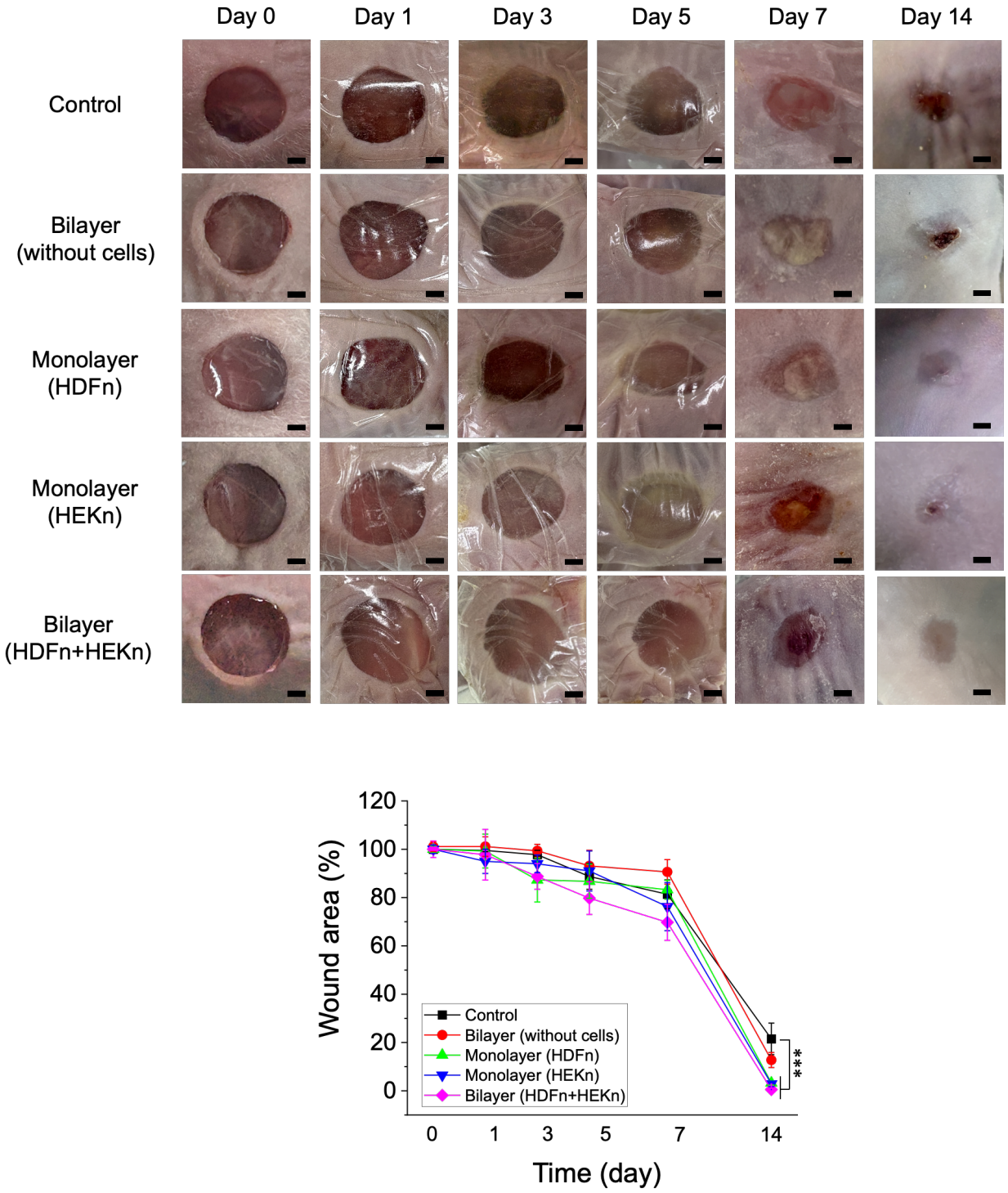

Figure S8. *In vivo* wound-healing outcomes after treatment with various hydrogel patches. Representative images of wound sites on days 0, 1, 3, 5, 7, and 14 (scale bars: 2 mm) and corresponding wound-area measurements over time (***$p < 0.001$, $n = 4$).

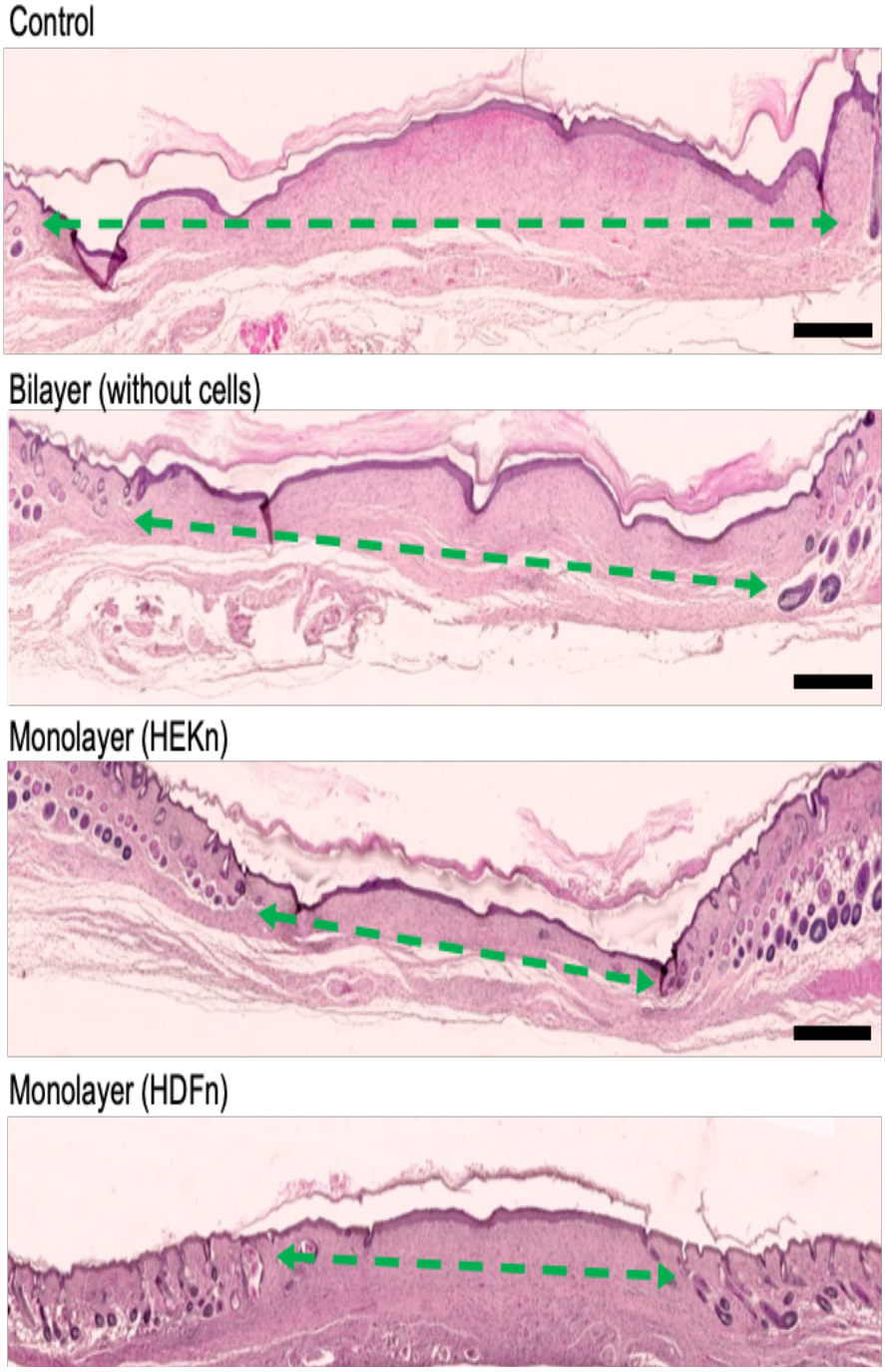

Figure S9. Representative hematoxylin and eosin-stained images of skin tissue sections used for quantification of dermal gap. Scale bars: 500 μm; magnification: 20×.