

RESEARCH ARTICLE

Hyaluronic acid-based self-healing hydrogels with enhanced hydrolytic stability for 3D bioprinting in tissue engineering

Supplementary file

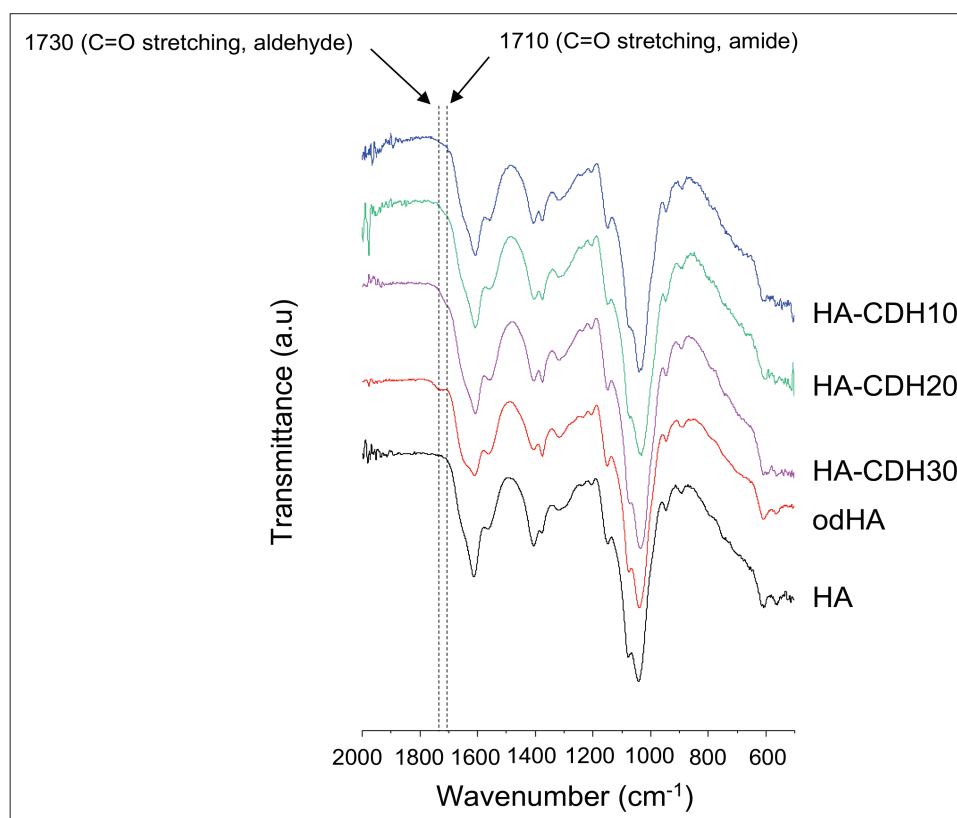


Figure S1. FT-IR spectra of HA, odHA, and HA-CDHs. Abbreviations: FT-IR, Fourier-transform infrared; HA, hyaluronic acid; HA-CDH, carbodihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.

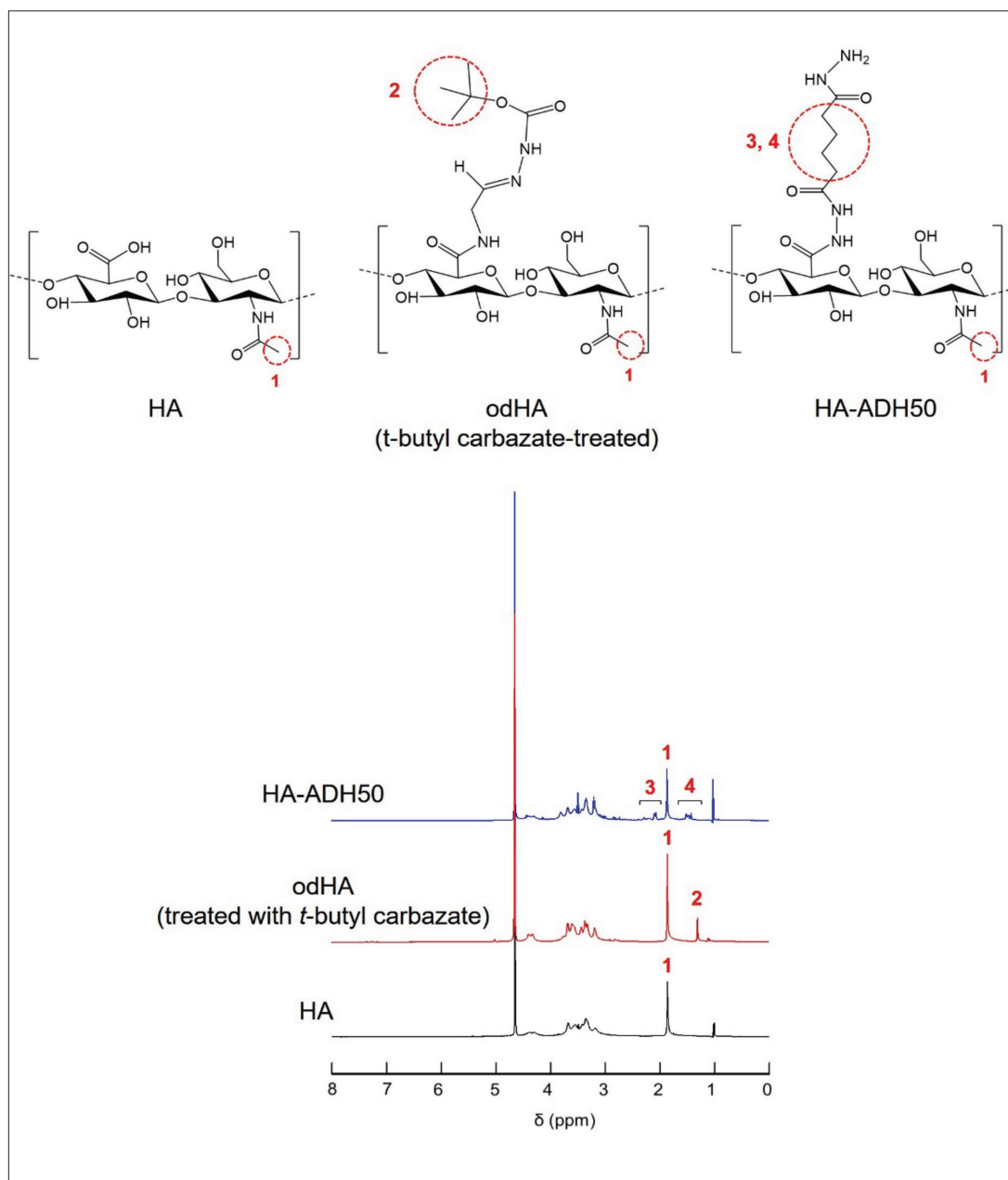


Figure S2. ¹H NMR spectra of HA, odHA (treated with *t*-butyl carbazate), and HA-ADH50. Abbreviations: HA, hyaluronic acid; HA-ADH, adipic acid dihydrazide-modified hyaluronic acid; NMR, nuclear magnetic resonance; odHA, oxidized diol-modified hyaluronic acid.

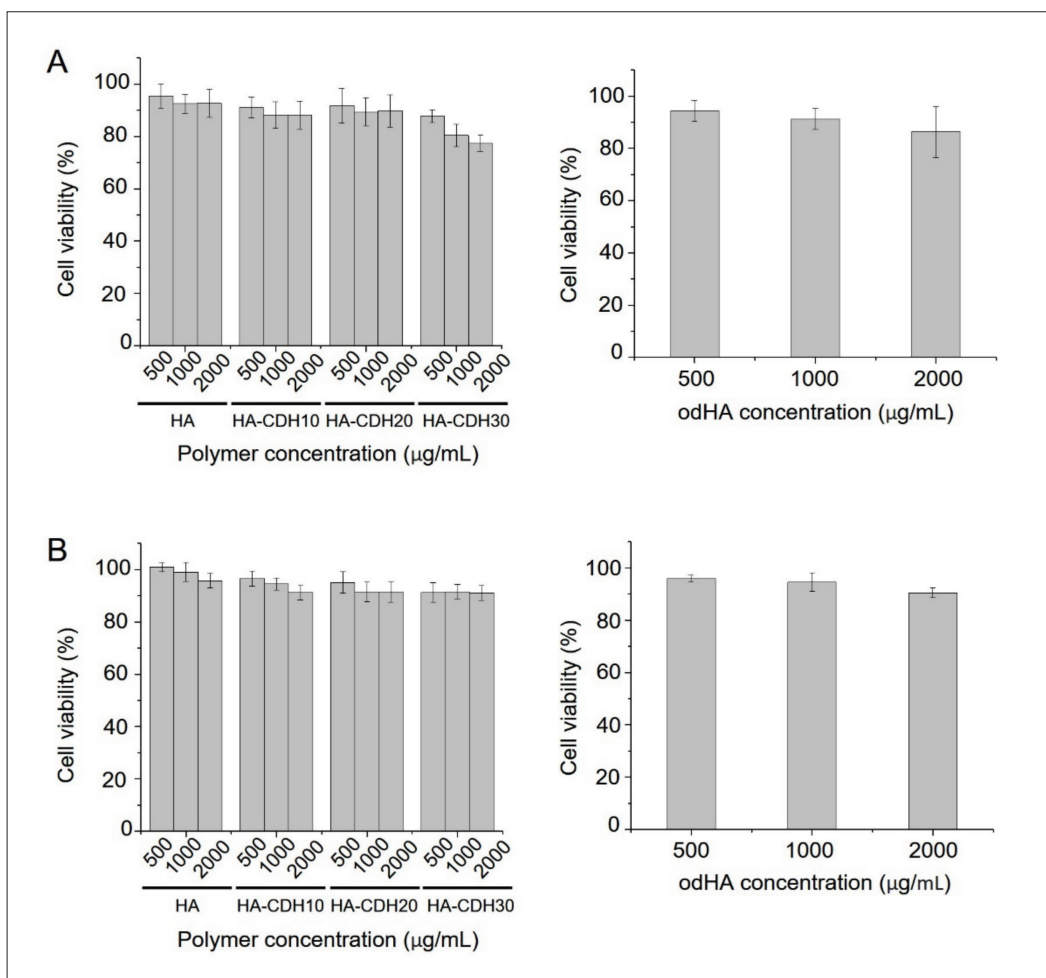


Figure S3. Relative viability of ATDC5 (A) and MC3T3-E1 cells (B) treated with various concentrations of HA, HA-CDH, and odHA. Abbreviations: HA, hyaluronic acid; HA-CDH, carbodihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.

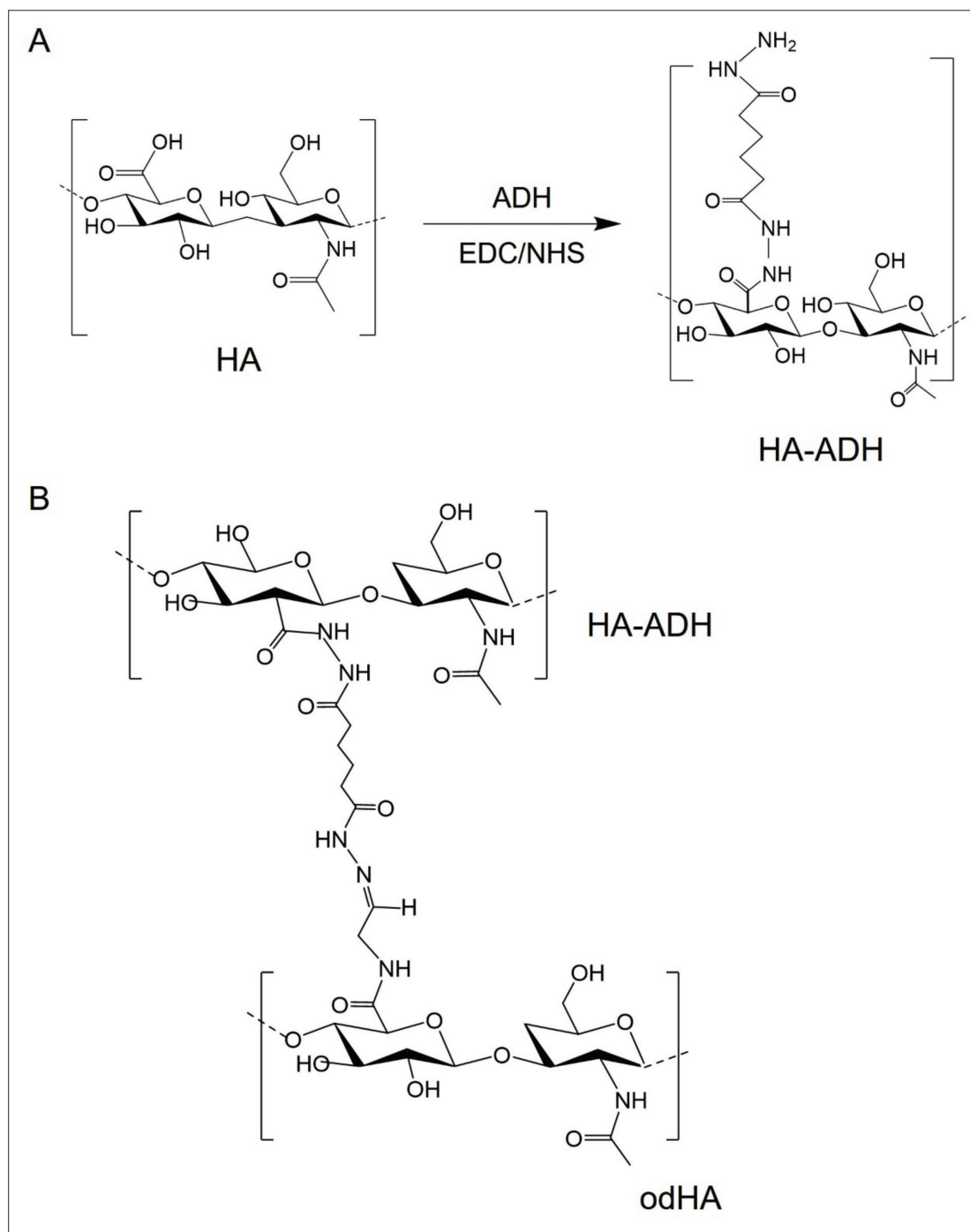


Figure S4. Synthetic scheme of HA-ADH (A) and chemical structure of HA-ADH/odHA hydrogels (B). Abbreviations: ADH, adipic acid dihydrazide; EDC, 1-ethyl-3-(dimethylaminopropyl) carbodiimide; HA, hyaluronic acid; HA-ADH, adipic acid dihydrazide-modified hyaluronic acid; NHS, *N*-hydroxysulfosuccinimide sodium salt; odHA, oxidized diol-modified hyaluronic acid.

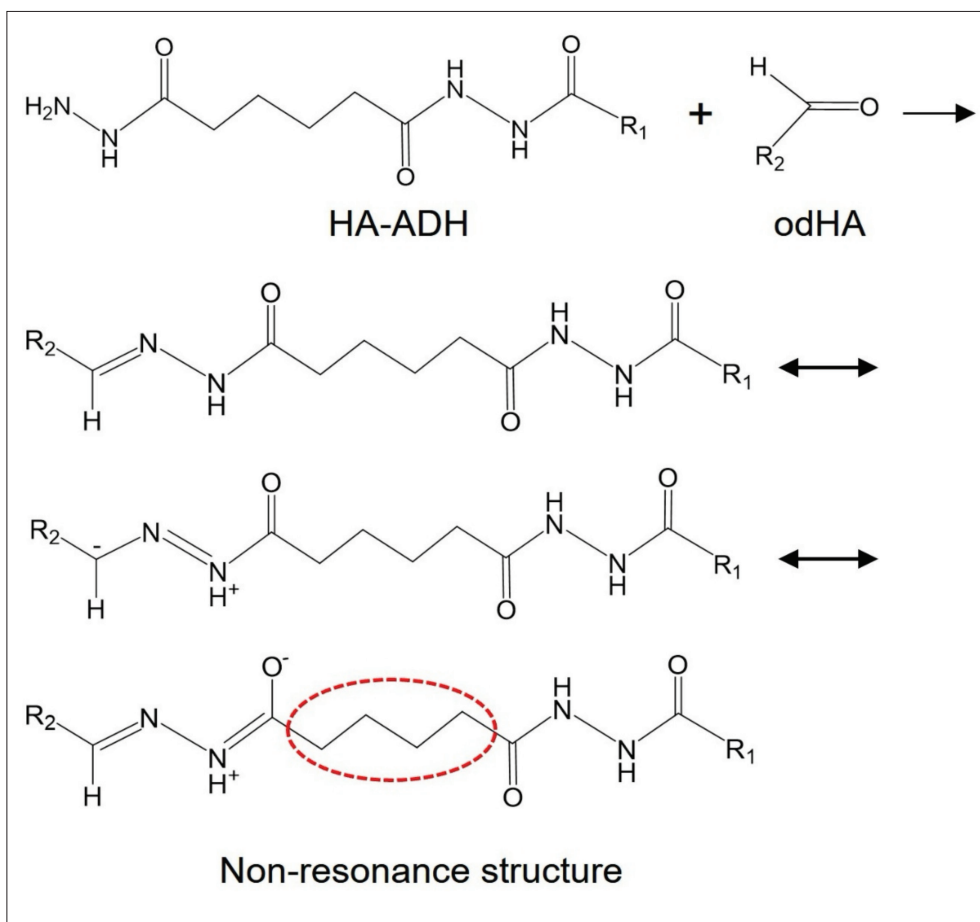


Figure S5. Non-resonance structure of the hydrazone bond formed between HA-ADH and odHA. Abbreviations: HA-ADH, adipic acid dihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.

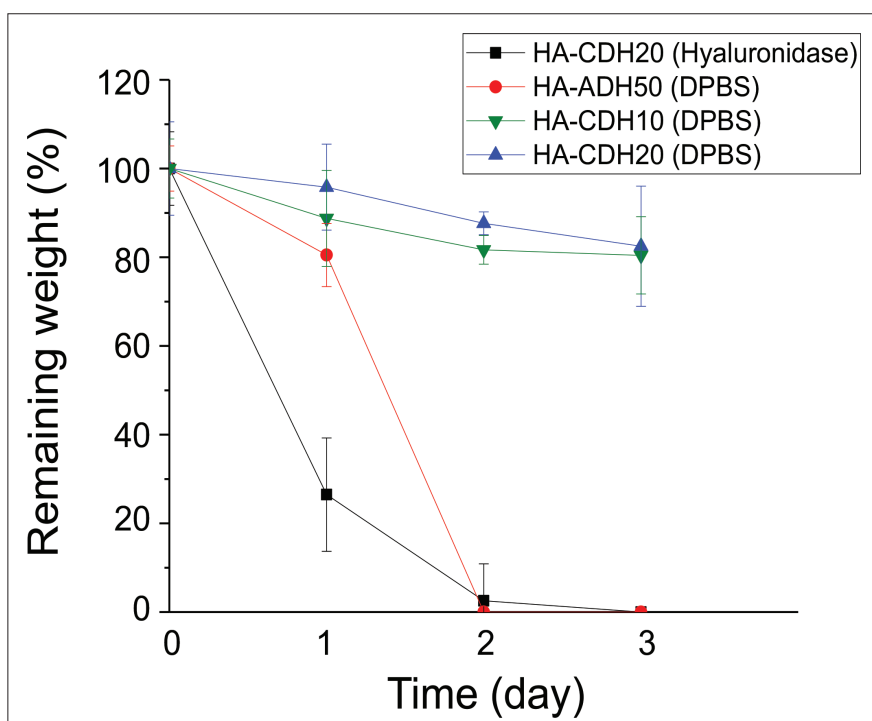


Figure S6. Changes in the remaining weights of HA-CDH/odHA/ADH and HA-ADH/odHA/ADH hydrogels incubated in DPBS at 37°C over time. The HA-CDH20/odHA/ADH hydrogel was also incubated in the presence of hyaluronidase ([HA-CDH] = 1.5 wt.%, [HA-ADH] = 1.5 wt.%, [odHA] = 1 wt.%, [ADH] = 0.1 wt.%, [hyaluronidase] = 1 µg/mL). Abbreviations: ADH, adipic acid dihydrazide; HA-ADH, adipic acid dihydrazide-modified hyaluronic acid; HA-CDH, carbodihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.

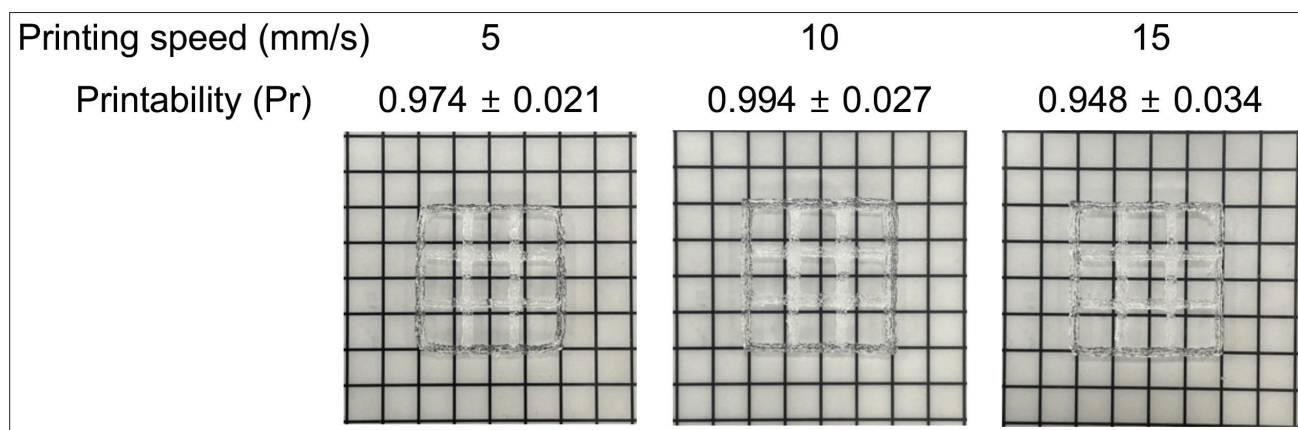


Figure S7. Representative images of lattice structures used to evaluate the printability (Pr) of HA-CDH/odHA/ADH hydrogels fabricated via 3D printing at different printing speeds ([HA-CDH20] = 1.5 wt.%, [odHA] = 1 wt.%, [ADH] = 0.1 wt.%). Abbreviations: ADH, adipic acid dihydrazide; HA-CDH, carbodihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.

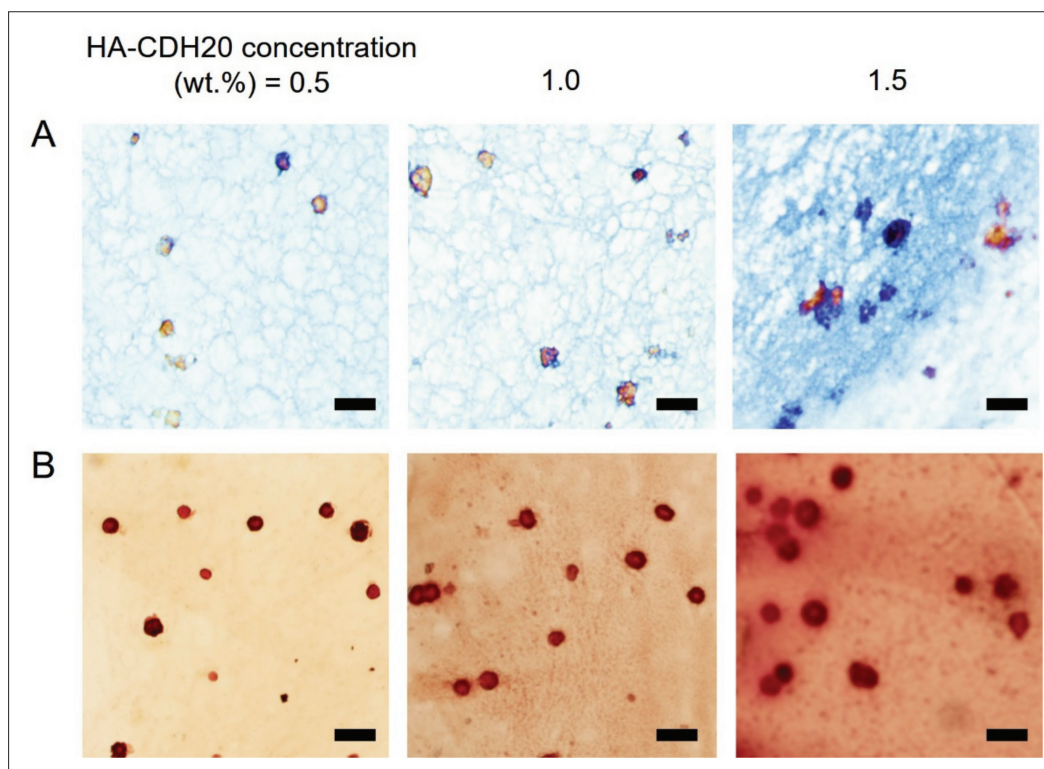


Figure S8. Histological images of HA-CDH20/odHA/ADH hydrogels encapsulating ATDC5 (A) and MC3T3-E1 cells (B). The cell/hydrogel constructs were incubated at 37°C in a 5% CO₂ atmosphere for 14 days. Hydrogel sections were stained with Alcian blue (A) and Alizarin red (B) (scale bar: 10 μm). Abbreviations: ADH, adipic acid dihydrazide; HA-CDH, carbodihydrazide-modified hyaluronic acid; odHA, oxidized diol-modified hyaluronic acid.