Injectable biodegradable polysaccharides-based hydrogels for stem cell delivery and cartilage regeneration

Xiaojie Lin,[a] Ruofan Liu,[a] Yang Zhou,[a] Jacob Beitzel,[a] Aya Noguchi,[b] Masayuki Kyomoto [b] and Miqin Zhang [*a]

[a] Department of Materials Science and Engineering, University of Washington, Seattle, WA 98195, USA

[b] Medical R&D Center, Corporate R&D Group, KYOCERA Corporation, 800 Ichimiyake, Yasu, Shiga 520-2362, Japan

***Corresponding author**

[Miqin Zhang]

[mzhang@uw.edu]

Table S1. Primer sequences of the reference and target genes.

Gene	Forward	Reverse
COL2A1	GTTCACGTACACTGCCCTGA	TCCACACCGAATTCCTGCTC
ACAN	AGTCACACCTGAGCAGCATC	TCTGCGTTTGTAGGTGGTGG
SOX9	AGGAAGTCGGTGAAGAACGG	AAGTCGATAGGGGGCTGTCT
GAPDH	TTGGTATCGTGGAAGGACTCA	TGTCATCATATTTGGCAGGTTT

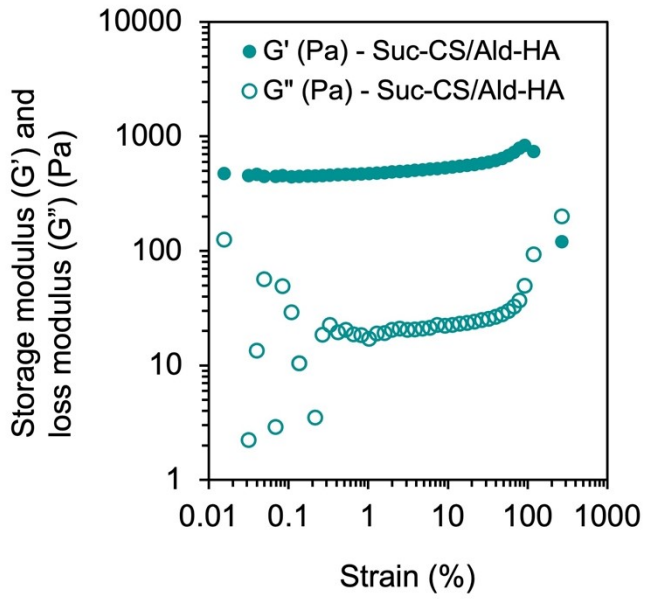

Fig. S1 Rheological analysis of Suc-CS/Ald-HA hydrogels. The storage (G') and loss (G") modulus were recorded under increasing strain (0.01–5000%) at temperature of 37 °C and frequency of 1 Hz. The strain sweep showed that strain at 1% was within the linear viscoelastic regime (LVR), where hydrogel's G' and G" remain constant. Thus, shear strain at 1% was used for time-sweep testing shown in Figure 2.

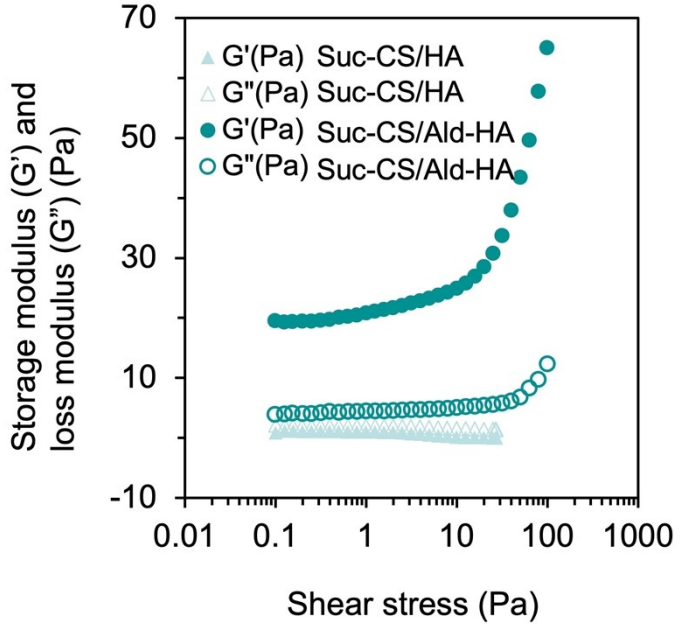

Fig. S2 Rheological analysis of Suc-CS/Ald-HA and Suc-CS/HA. The storage (G') and loss modulus (G") were recorded under increasing shear stress to indirectly confirm the Schiff base covalent reaction between the amino of Suc-CS and aldehyde groups of Ald-HA (temperature: 37 °C, frequency: 1 Hz). Each polymer was dissolved into DPBS at 20 mg/mL concentration. The storage modulus was larger than loss modulus (G' > G") for Suc-CS/Ald-HA, confirming the hydrogel formation, while the storage modulus was smaller than loss modulus (G' < G") for Suc-CS/HA, indicating no occurrence of Schiff base reaction between Suc-CS and HA.

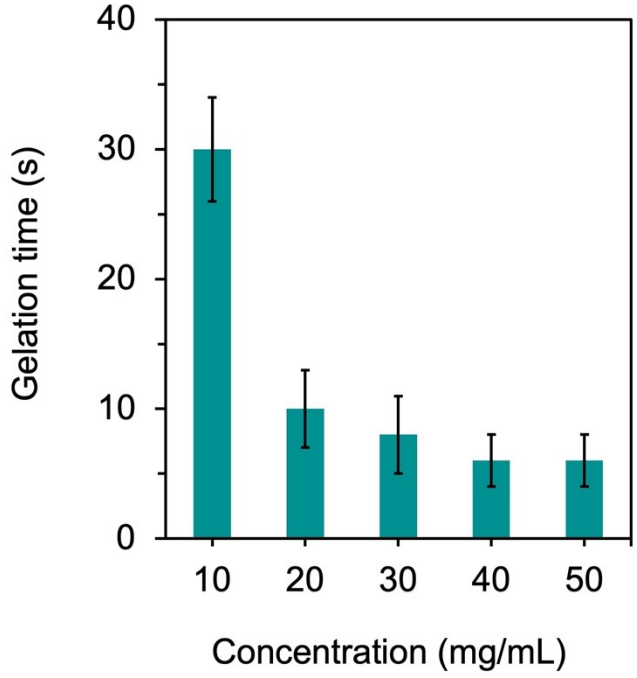

Fig. S3 The gelation times of Suc-CS/Ald-HA hydrogels with different concentrations (10, 20, 30, 40, and 50 mg/mL), acquired using the inverting tube method. The gelation occurred within 1 min upon mixing two polymer solutions at the same concentrations (1/1, v/v) at 37 °C. Suc-CS and Ald-HA were dissolved separately in DPBS at different concentrations (10, 20, 30, 40, and 50 mg/mL). Suc-CS/Ald-HA hydrogels were prepared by mixing two polymer solutions of the same concentration (1/1, v/v) at room temperature using a double barrel syringe (Duploject syringe, Baxter) with a 21 G needle (Becton Dickinson).

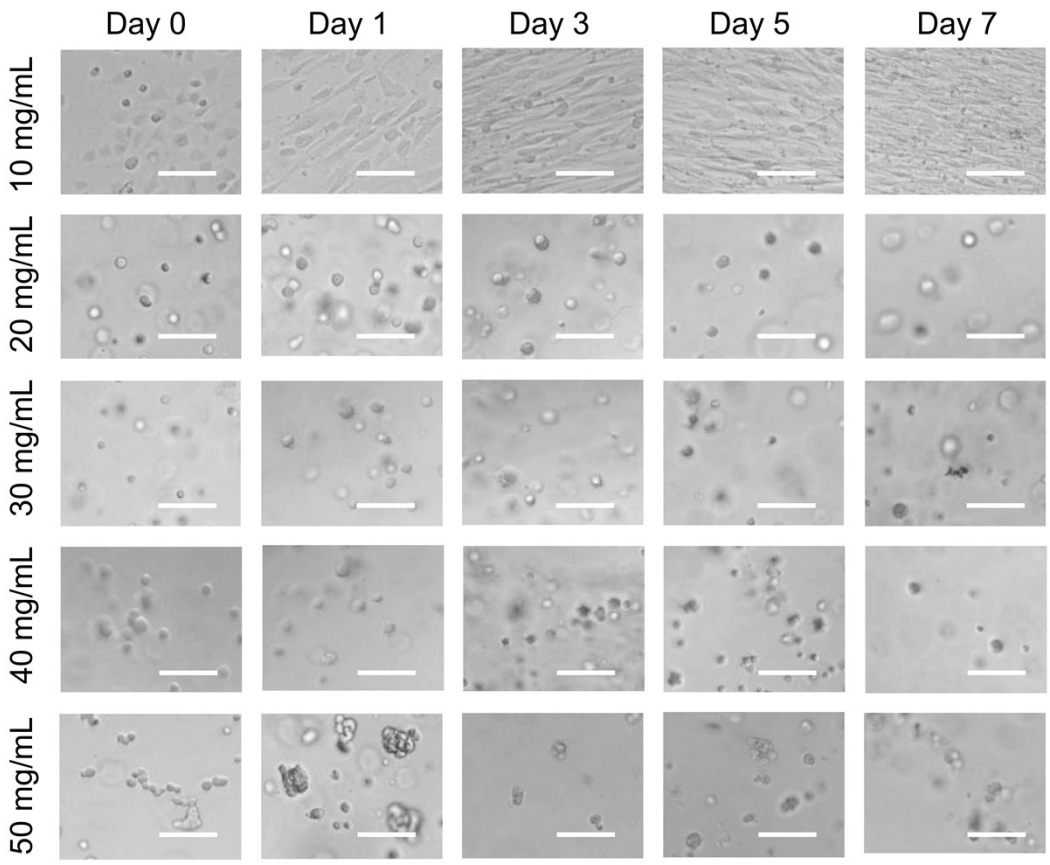

Fig. S4 hADSC morphology in hydrogels. The morphology of hADSCs encapsulated in Suc-CS/Ald-HA hydrogels prepared at different concentrations (10, 20, 30, 40, and 50 mg/mL) on day 0, 1, 3, 5, and 7. Scale bars represent 100 μm. In the hydrogel with a concentration of 10 mg/mL, on day 0, a portion of hADSCs adhered to the bottom of the cell culture dish while the remaining cells exhibited a spherical morphology; from day 1, all the cells were able to attach and grow on 2D surfaces. In the hydrogels with concentrations of 20, 30, 40, and 50 mg/mL, hADSCs showed a spherical shape for 7 days, suggesting the potential of the hydrogels to serve as a 3D scaffold supporting cell growth.

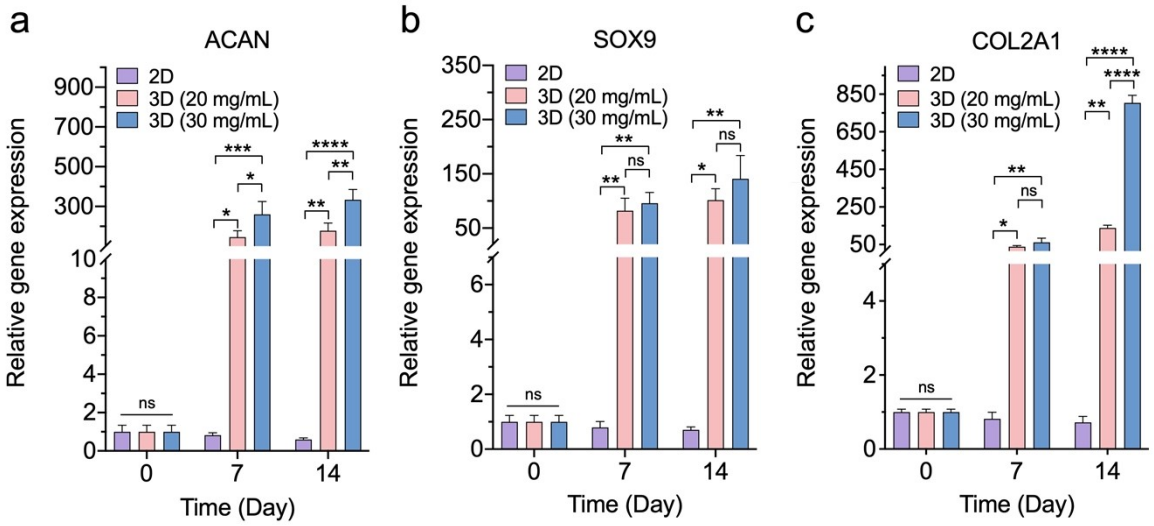

Fig. S5 Expression of genes associated with chondrogenic differentiation. Relative expression of (a) ACAN, (b) SOX9, and (c) COL2A1 in hADSCs cultured on 2D TCPS, in 3D hydrogels at 20 mg/mL, and in 3D hydrogels at 30 mg/mL in normal stem cell culture medium for a 14-day culture period. Gene expression was first normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (reference gene) and then normalized to the expression associated with 2D TCPS culture on day 0 (set as 1-time). One-way ANOVA followed by Tukey's multiple comparisons test was used to determine whether the differences were statistically significant (mean ± SD, n = 3; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$; ns, nonsignificant). hADSCs encapsulated within the 3D hydrogels exhibited elevated expression levels of ACAN, SOX9, and COL2A1 in comparison to the cells cultured on the 2D surface, even when the cells were maintained in a normal stem cell culture medium rather than a chondrogenic differentiation medium. This result further indicates that our hydrogel can stimulate chondrogenic differentiation.

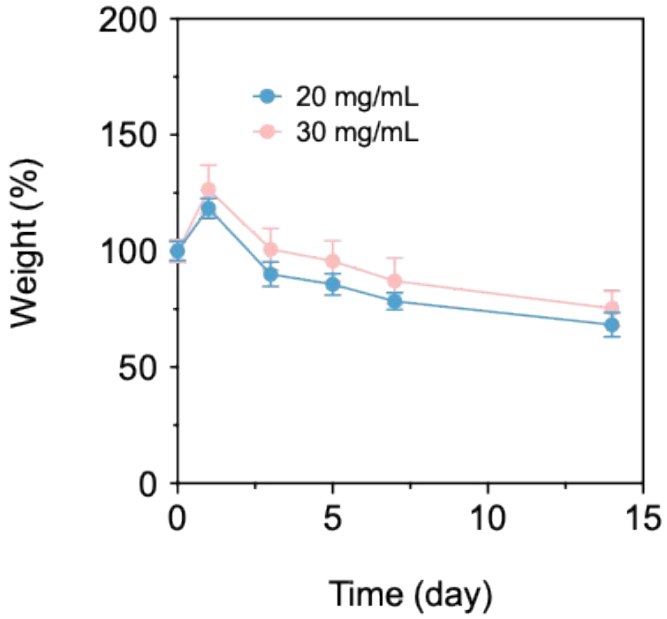

Fig. S6 Hydrogel degradation behaviors. Degradation of Suc-CS/Ald-HA hydrogels at different concentrations (20 and 30 mg/mL) incubated in human mesenchymal stem cell medium and maintained at 37 °C for 14 days. At different time points, the hydrogels were collected and promptly weighed with a microbalance. The value of $W_t/W_0 \times 100\%$ was used to calculate the weight remaining ratio, where W_t and W_0 are the weights of the hydrogels at different time points and at the initial state, respectively.