SUPPORTING INFORMATION

Incorporation of a Collagen-Binding Chondroitin Sulfate Molecule to a Collagen Type I and II Blend Hydrogel for Cartilage Tissue Engineering

AUTHORS

Claire E. Kilmer[1], Tanaya Walimbe[2], Alyssa Panitch[2,3], Julie C. Liu[1,3,*]

AFFILIATIONS

[1]Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN, 47907, USA

[2]School of Biomedical Engineering, University of California Davis, Davis, CA, 95616, USA

[3]Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN, 47907, USA

* Corresponding author.

E-mail address: julieliu@purdue.edu

Number of Supporting Information Pages: 7

Number of Supporting Information Figures: 5

Number of Supporting Information Tables: 1

Table S1. Primer sequences utilized for quantitative reverse transcription-polymerase chain reaction.

Gene of Interest	Accession number		5' → 3' sequence	Product length (bp)	Primer efficiency	Reference
Collagen Type I	NM 001195668.1	Forward	ATGGATGAGGAAA CTGGCAACT	114	101.9%	Liao, 2010[1]
		Reverse	GCCATCGACAAGA ACAGTGTAAGT			
Collagen Type II	NM 001195671.1	Forward	GCCACCGTGCCCA AGAAGAACT	160	103.1%	Yuan, 2016[2]
		Reverse	ACAGCAGGCGCAG GAAGGTCAT			
Collagen Type X	XM 002714724.3	Forward	GCCAGGACCTCCA GGACTATCA	103	100.0%	Zheng, 2009[3]
		Reverse	CCCAATGTCTCCTT TCGGTCCA			
Aggrecan	XM 008251723.2	Forward	CCTACCAGGACAA GGTCTCG	163	98.1%	Chen, 2016[4]
		Reverse	ACACCTTTCACCA CCACCTC			
SOX9	XM 008271763.2	Forward	GGAAGCTCTGGAG ACTGCTG	135	96.8%	Zhang, 2012[5]
		Reverse	CGTTCTTCACCGA CTTCCTC			
GAPDH	NM 001082253.1	Forward	CGCCTGGAGAAAG CTGCTA	104	96.0%	Morigele, 2013[6]
		Reverse	ACGACCTGGTCCT CGGTGTA			

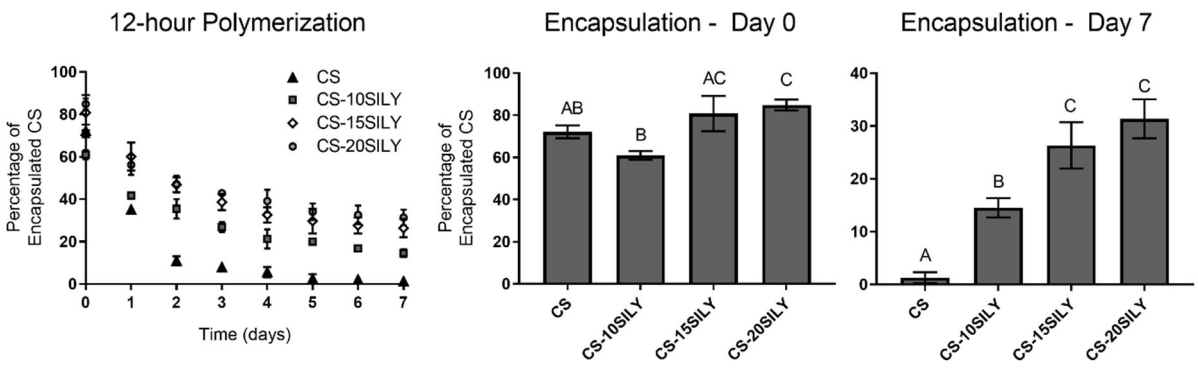

Figure S1. Percentage of encapsulated CS retained in the hydrogel over time after a 12-hour polymerization. CS Encapsulation after a wash or 7-day period are shown. Single factor analysis of variance (ANOVA) and Tukey post hoc tests were performed (n=4). Data that share the same letter do not have statistically significant differences ($p > 0.05$) whereas data that do not share the same letter have statistically significantly differences ($p < 0.05$). Data is represented as the mean ± the standard deviation.

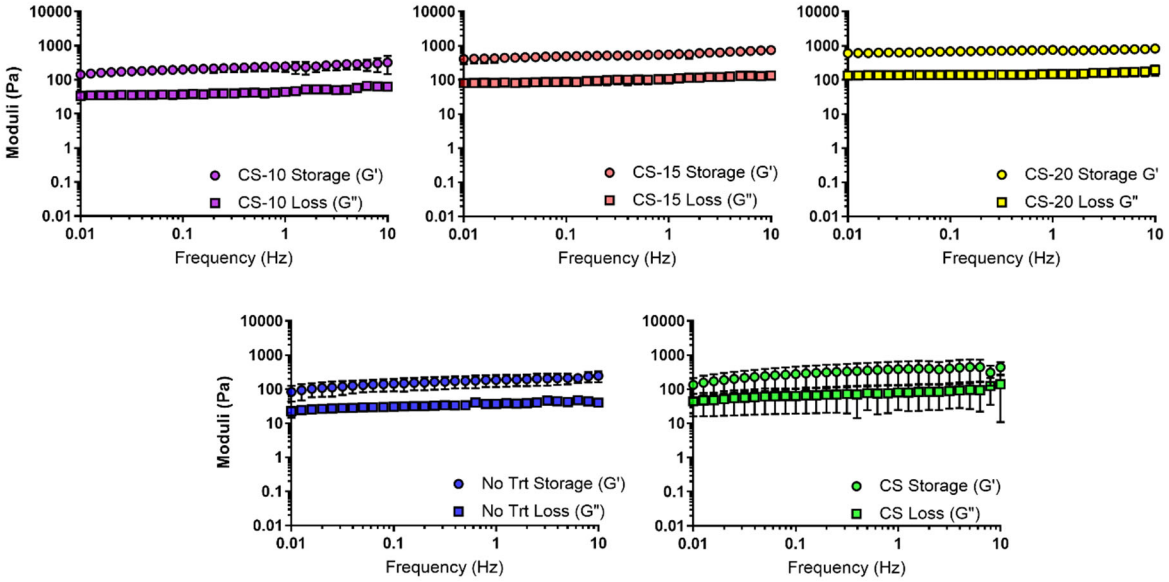

Figure S2. Storage and loss moduli of hydrogels with the addition of CS or CS-SILY molecules. Frequency sweeps from 0.01 to 10 Hz were performed. Data (n=3) is represented as the mean ± the standard deviation.

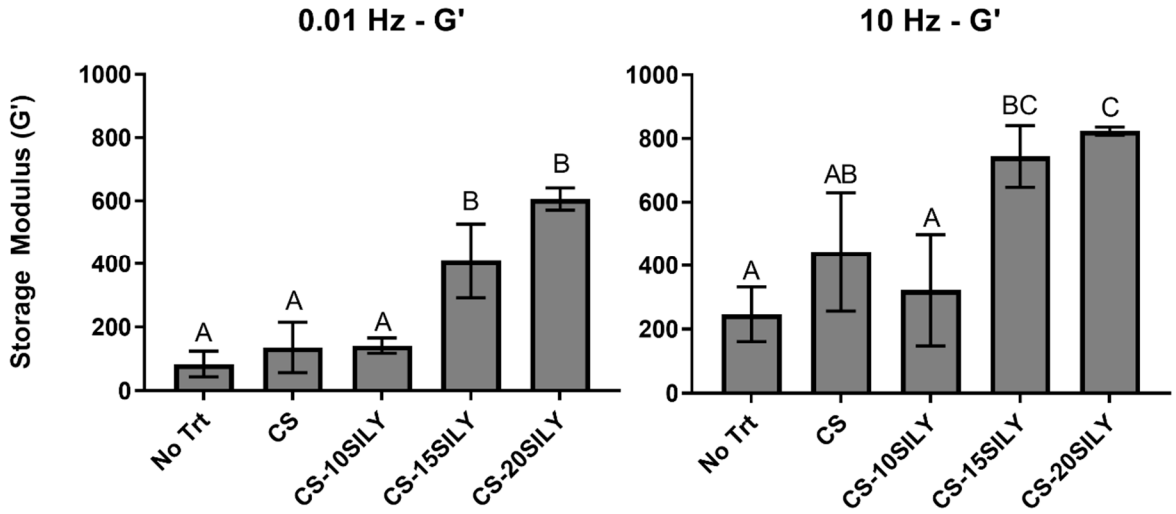

Figure S3. The storage modulus (G') of hydrogels with the addition of CS or CS-SILY molecules. Frequency sweeps from 0.01 to 10 Hz were performed. Single factor analysis of variance (ANOVA) and Tukey post hoc tests were performed (n=3) at 0.01 and 10 Hz. Data that share the same letter do not have statistically significant differences ($p > 0.05$) whereas data that do not share the same letter have statistically significantly differences ($p < 0.05$). Data is represented as the mean ± the standard deviation.

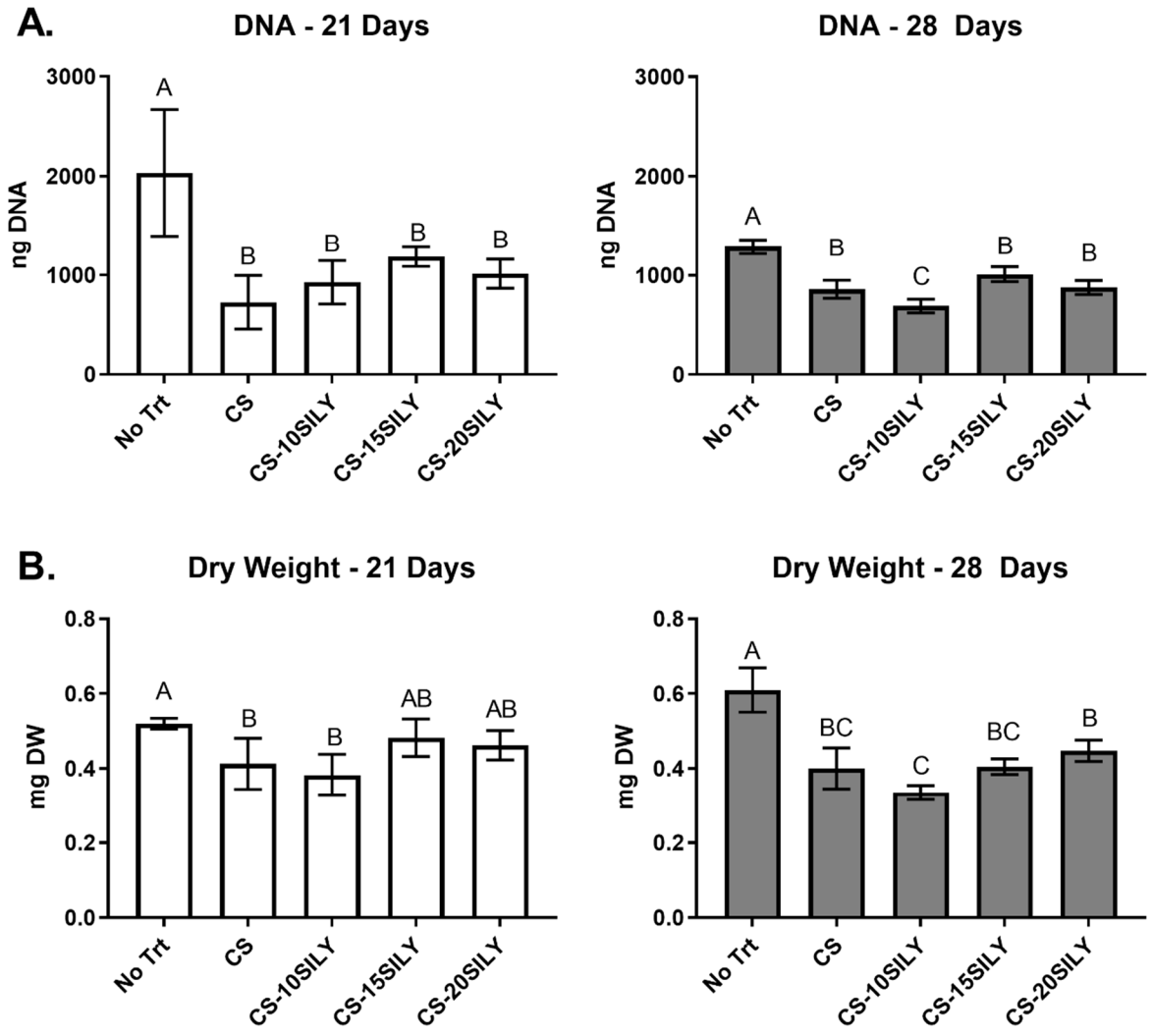

Figure S4. The mass of (A) DNA or (B) dry weight (DW) of the cell-hydrogel constructs with or with added CS or CS-SILY molecules after a 21-day or 28-day culture period. Values are expressed as mean ± the standard deviation (n = 4). An ANOVA and Tukey's post hoc test were performed. Data that share the same letter do not have statistically significant differences ($p > 0.05$) whereas data that do not share the same letter have statistically significantly differences ($p < 0.05$).

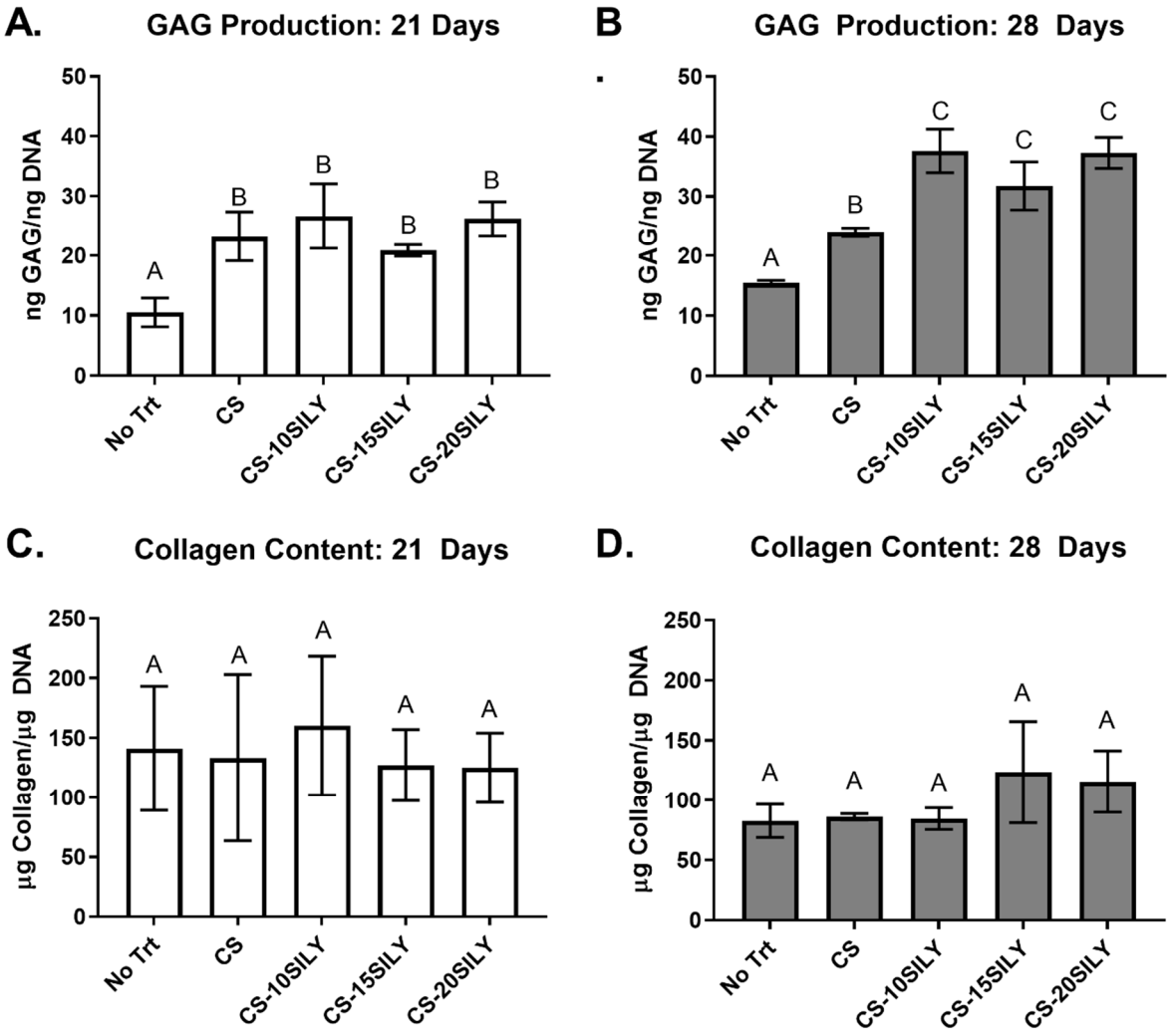

Figure S5. The addition of CS-SILY molecules increases normalized sulfated GAG production after 28 days in culture and has no change on normalized collagen content in scaffolds. GAG/DNA ratio of the cell-hydrogel constructs (n = 4) with or with added CS or CS-SILY molecules after a (A) 21-day or (B) 28-day culture period. Total collagen/DNA ratio of the cell-hydrogel constructs (n = 3) with or with added CS or CS-SILY molecules after a (C) 21-day or (D) 28-day culture period. Values are expressed as mean ± standard deviation. ANOVA and Tukey's post hoc tests were performed. Data that share the same letter do not have statistically significant differences ($p > 0.05$) whereas data that do not share the same letter have statistically significantly differences ($p < 0.05$).

REFERENCES

(1) Liao, J.; Guo, X.; Grande-Allen, K. J.; Kasper, F. K.; Mikos, A. G. Bioactive Polymer/Extracellular Matrix Scaffolds Fabricated with a Flow Perfusion Bioreactor for Cartilage Tissue Engineering. *Biomaterials* **2010**, *31* (34), 8911-8920. DOI: 10.1016/j.biomaterials.2010.07.110.

(2) Yuan, L.; Li, B.; Yang, J.; Ni, Y.; Teng, Y.; Guo, L.; Fan, H.; Fan, Y.; Zhang, X. Effects of Composition and Mechanical Property of Injectable Collagen I/II Composite Hydrogels on Chondrocyte Behaviors. *Tissue Eng. Part A* **2016**, *22* (11-12), 899-906. DOI: 10.1089/ten.tea.2015.0513.

(3) Zheng, L.; Sun, J.; Chen, X.; Wang, G.; Jiang, B.; Fan, H.; Zhang, X. In Vivo Cartilage Engineering with Collagen Hydrogel and Allogenous Chondrocytes after Diffusion Chamber Implantation in Immunocompetent Host. *Tissue Eng. Part A* **2009**, *15* (8), 2145-2153. DOI: 10.1089/ten.tea.2008.0268.

(4) Chen, C.-H.; Kuo, C.-Y.; Wang, Y.-J.; Chen, J.-P. Dual Function of Glucosamine in Gelatin/Hyaluronic Acid Cryogel to Modulate Scaffold Mechanical Properties and to Maintain Chondrogenic Phenotype for Cartilage Tissue Engineering. *Int. J. Mol. Sci.* **2016**, *17* (11), 1957. DOI: 10.3390/ijms17111957.

(5) Zhang, L.; Yuan, T.; Guo, L.; Zhang, X. An In Vitro Study of Collagen Hydrogel to Induce the Chondrogenic Differentiation of Mesenchymal Stem Cells. *J. Biomed. Mater. Res. A* **2012**, *100A* (10), 2717-2725. DOI: 10.1002/jbm.a.34194.

(6) Morigele, M.; Shao, Z.; Zhang, Z.; Kaige, M.; Zhang, Y.; Qiang, W.; Yang, S. TGF-β1 Induces a Nucleus Pulposus-Like Phenotype in Notch 1 Knockdown Rabbit Bone Marrow Mesenchymal Stem Cells. *Cell Biol. Int.* **2013**, *37* (8), 820-825. DOI: 10.1002/cbin.10109.