

## **Tuning collagen and collagen-alginate mechanics through extrusion bioprinting process parameters**

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### **Supplementary Information**

Figure S1. Bioprinting and Mechanical Setup

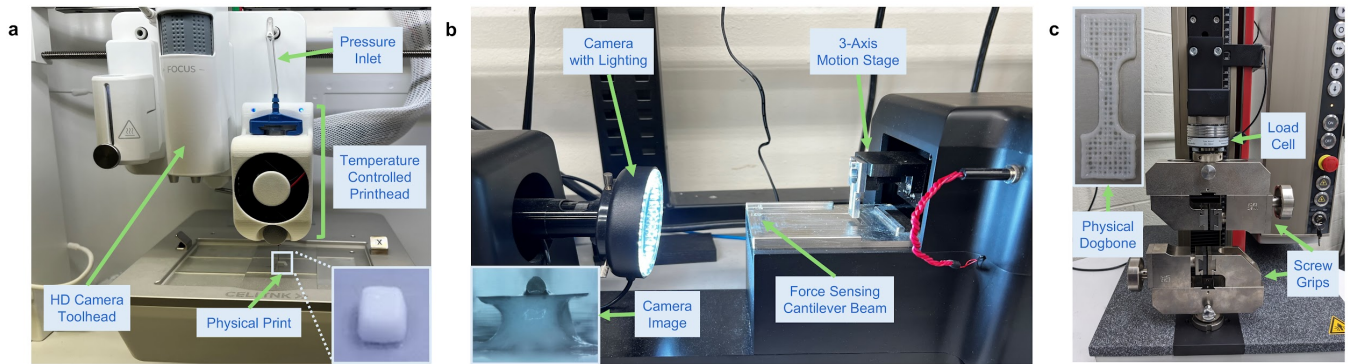
Figure S2. Printability Evaluation Example for More Transparent Composite Blend

Figure S3. Compression Properties Measured for Pure Collagen Samples

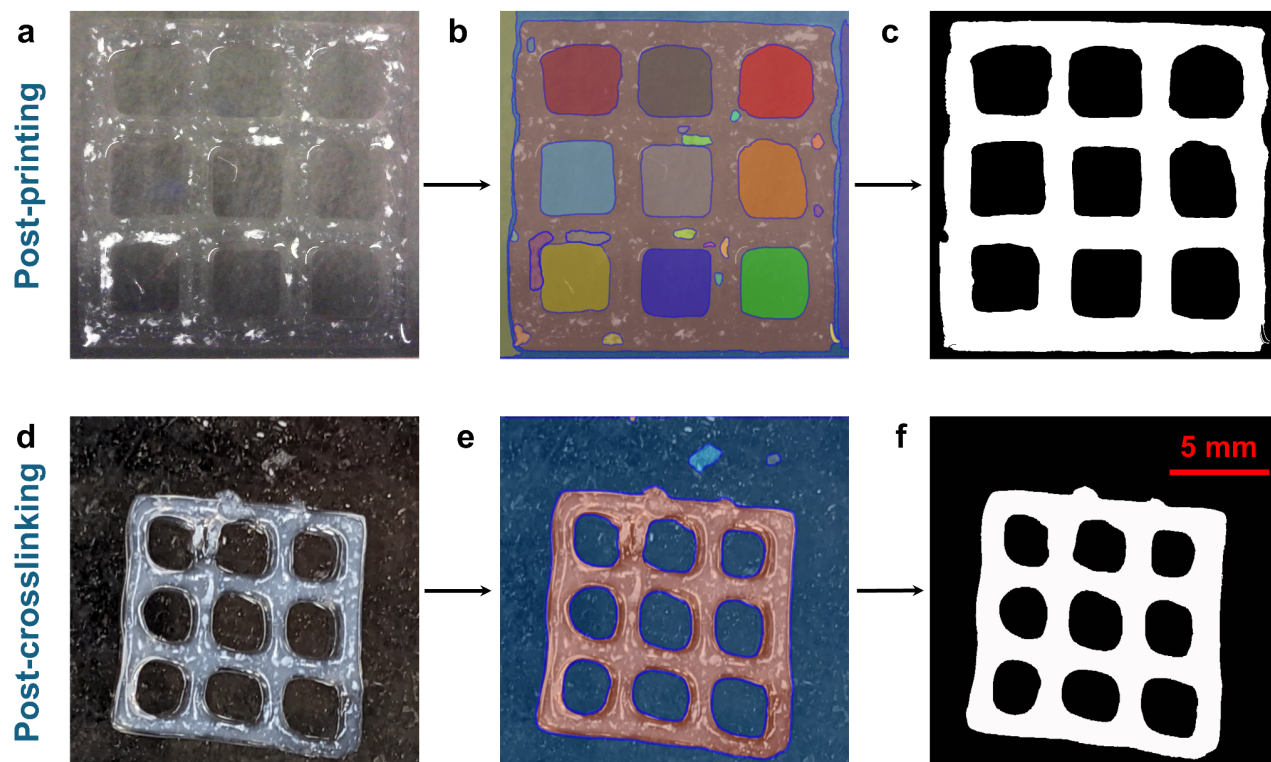
Table S1. Summary of ANOVA Results

Table S2. Summary of ANOVA Results for Pure Collagen Compression Samples

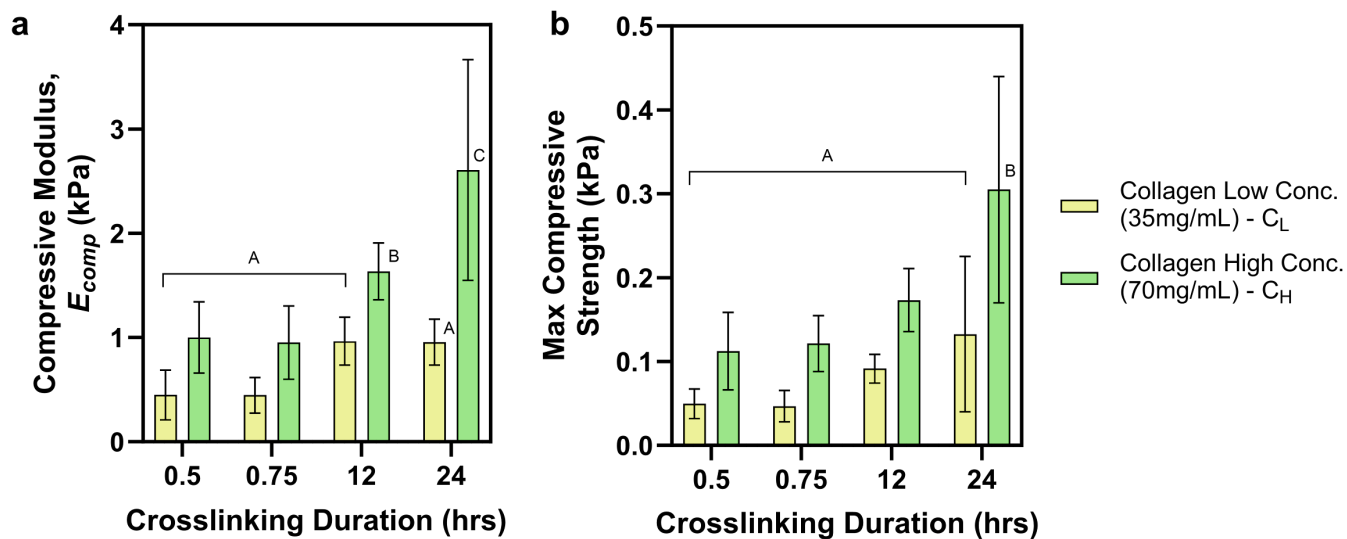
Figure S4. SEM Surface Morphology



**Figure S1. Bioprinting and Mechanical Setup.** (a) CELLINK BioX 3D-printer with a temperature-controlled pneumatic printhead. The inset shows an example of a final pure collagen print. (b) CellScale MicroTester LT compression device with a 3-axis motion stage and force sensing cantilever beam. Inset displays camera view of collagen sample under the compression plate. (c) zwickiLine tensile testing machine using screw grips with inset showing a sample collagen dogbone.



**Figure S2. Printability Evaluation Example for More Transparent Composite Blend.** (a) Representative  $C_{1A_{10}}$  printed sample before crosslinking is quite transparent, causing the SAM model to create (b) a labeled object with edges poorly detected due to optical effects. (c) Binary hydrogel lattice has rougher edges due to small boundary regions improperly excluded but aggregates the main region decently well. (d) The same  $C_{1A_{10}}$  sample after 24 h crosslinking, which has become more opaque and the grid region better labeled in (e) as a single unit. (f) Extracted lattice with binary mask demonstrates significant sample shrinkage after long crosslinking periods. Scale bar = 5 mm.



**Figure S3. Compression Properties Measured for Pure Collagen.** (a) Compressive modulus and (b) max compressive strength evaluated across for pure collagen at 35 mg/mL and 70 mg/mL. Stronger mechanical behaviors are seen with higher concentration and longer crosslinking durations, suggesting that total time of crosslinking may need to be more than manufacturer's recommended minimum time of 30 min. Different letters (A or B) by the top of each bar indicate pairwise differences, where there are significant differences between any two conditions receiving different letters ( $p < 0.05$ ).



**Table S1. Summary of ANOVA Results.** Two-way ANOVA and three-way ANOVA were performed for compression and tension data shown in the main manuscript, respectively, due to the number of independent variables for comparison.

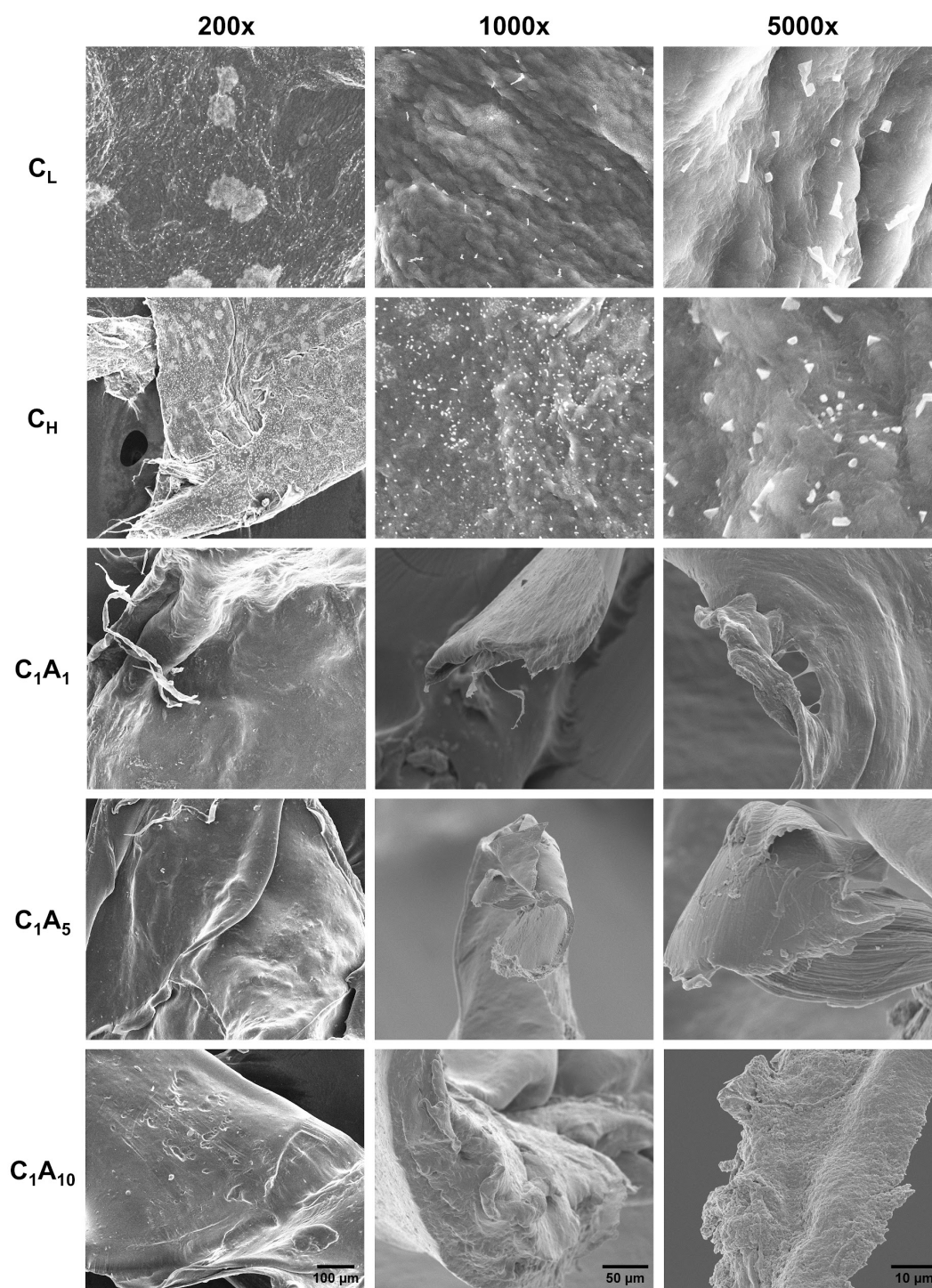
	Source	SS	df	F	p
<b>Compression</b>	Between				
	Composition	950.292	4	19.129	7.31E-09 **
	Crosslinking Duration	15.703	1	1.264	0.268
	Composition × Crosslinking Duration	392.737	4	7.905	8.57E-05 **
	Error	496.791	40		
	Between				
	Composition	5.427	4	15.196	1.23E-07 **
	Crosslinking Duration	0.296	1	3.315	0.076
	Composition × Crosslinking Duration	2.794	4	7.822	9.35E-05 **
	Error	3.571	40		
<b>Tension</b>	Between				
	Composition	2.070	2	36.125	2.68E-10 **
	Crosslinking Duration	0.117	1	4.089	0.049 *
	Infill Density	0.000	1	0.003	0.953
	Composition × Crosslinking Duration	0.256	2	4.475	0.017 *
	Composition × Infill Density	0.121	2	2.117	0.131
	Crosslinking Duration × Infill Density	0.480	1	16.771	1.61E-04 **
	Composition × Crosslinking Duration × Infill Density	0.445	2	7.775	1.19E-03 *
	Error	1.375	48		
	Between				
	Composition	1.619	2	19.889	5.11E-07 **
	Crosslinking Duration	0.009	1	0.232	0.633
	Infill Density	0.076	1	1.876	0.177
	Composition × Crosslinking Duration	0.146	2	1.789	0.178
	Composition × Infill Density	0.204	2	2.510	0.092
	Crosslinking Duration × Infill Density	0.924	1	22.709	1.78E-05 **
	Composition × Crosslinking Duration × Infill Density	0.330	2	4.060	0.023 *
	Error	1.953	48		

Note: \*  $p < 0.05$ ; \*\*  $p < 0.001$ .

**Table S2. Summary of ANOVA Results for Pure Collagen Samples.** Two-way ANOVA was performed for collagen-only samples shown in supplementary compression data to identify the effects of variables (composition and crosslinking duration) and for their interaction effects for two data sets (compressive modulus and max compressive strength).

	Source	<i>SS</i>	<i>df</i>	<i>F</i>	<i>p</i>
<b>Compression (Pure Collagen)</b>	Between				
	Compressive Modulus				
	Composition	7.122	1	35.168	1.00E-06 **
	Crosslinking Duration	8.025	3	13.210	9.00E-06 **
	Composition × Crosslinking Duration	2.212	3	3.642	0.023 *
	Error	6.480	32		
	Between				
	Max Compressive Strength				
	Composition	0.096	1	23.678	2.90E-05 **
	Crosslinking Duration	0.124	3	10.207	7.20E-05 **
	Composition × Crosslinking Duration	0.019	3	1.561	0.218
	Error	0.130	32		

Note: \*  $p < 0.05$ ; \*\*  $p < 0.001$ .



**Figure S4. SEM Surface Morphology.** Comparison of microstructures for collagen and collagen-alginate printability grids captured at different magnifications. Scale bars are the same for each image in the respective magnification column.