Supplementary Materials

S1. Hydroxyproline assay

To quantify the concentration, hydroxyproline was measured to quantify the COL and ColME concentration instead of dry weight. The formation of chromophore from hydroxyproline was measured by adding Ehrlich's reagent[1]. In brief, 200 µL of collagen solution and 200 µL of 2N NaOH were combined in a glass container capable of enduring hydrolytic reaction (120°C, 17 psi for 90 minutes), followed by neutralization with 200 µL of 2N HCl. After cooling the samples, diluted the samples with 2.5 mM citric acid (Sigma-Aldrich, USA) with 5 mM Na_2HPO_4 (Sigma-Aldrich, USA) at pH 6.0. The standard curve was prepared by trans-4-hydroxy-L-proline (Sigma, USA). The standard chemicals and samples were transferred into 96-well plate for 50 µL/well and added with 75 µL of chloramine-T reagent (1.0% w/v of chloramine-T in RO water then diluted with equal volume of citrate phosphate buffer) (Sigma, USA) for 15 minutes at room temperature to form pyrrole. During this period, Ehrlich reagent was freshly prepared from 560 mg of 4-(Dimethylamino)benzaldehyde (DMAB; Sigma, USA) in 1200 µL 70% $HClO_4$ (Sigma-Aldrich, USA) and 6.8 mL of RO water. Subsequently, 75 µL of the Ehrlich reagent was added to each well and incubated at 70°C for 20 minutes. Measurement of chromophore was conducted by a microplate reader (BioTek Synergy H1, USA) at the absorbance of 560 nm. Finally, COL/ColME concentration was calculated by the average composition of hydroxyproline in collagen type I, approximately 13-14% by mass in bovine tendon, so we assumed 13.5%[2].

S2. Designed model with a grid infill pattern

Preliminary investigations of the relationship between nozzle speed, temperature, extrusion pressure, and concentration were based on the STL file (From Solidwork) of a grid structure (16.0 mm in length with 2.0 mm of spacing distance set in Allevi 1's 3D Systems). Then, the characterization of filaments and spaced areas was analyzed through microscope images.

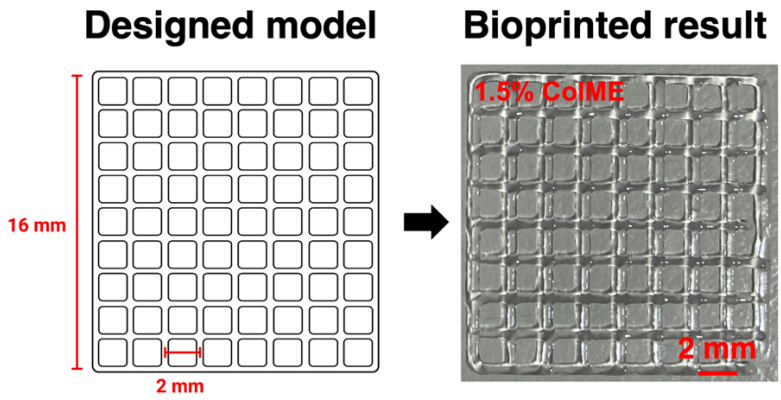

Figure S1. Illustration of a grid infill pattern yielding a monolayered bioprinted structure.

S3. FTIR analysis of COL and ColME

For the preparation prior to FTIR analysis, unmodified collagen and modified collagen were lyophilized prior to the measurement. In the preparation of solid palette for FT-IR measurement, 2 mg of lyophilized samples were first mixed potassium bromide (KBr; Millipore, Germany) powder with 1/50 in weight ratio. Next, the powder was gridded into fine particles followed by palette making by manual hydraulic press machine. Finally, solid sample palettes were transfer onto the holder and scanned by an FTIR spectrophotometer.

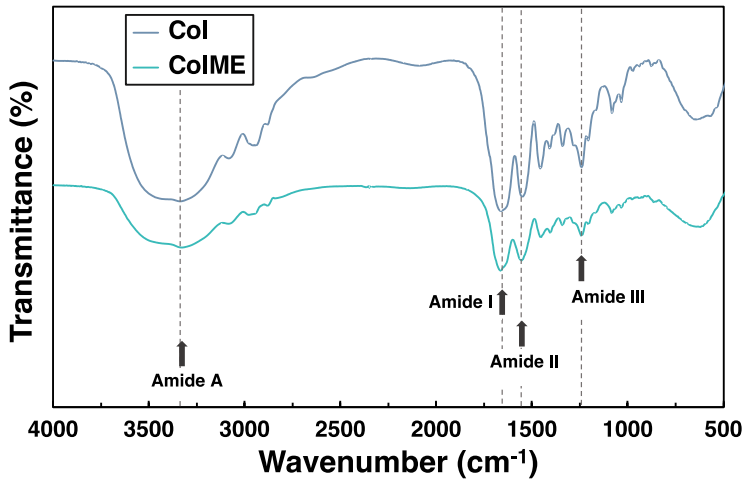

Figure S2. FTIR spectra of COL and ColME. Amide A peak at 3370 cm^{-1}, amide I peak at 1654 cm^{-1}, amide II peak at 1544 cm^{-1} and amide III peak at 1241 cm^{-1}.

S4. Minimum extrusion pressure for bioprinting

The concentrations below 1.0% ColME couldn't form clear grid structures with 2.0 mm spacing, resulting in filament fusion 20°C in our investigation. Therefore, the minimum extrusion pressure of ColME at 1.2%, 1.5%, 1.8%, and 2.0% was recorded using a 25G nozzle, with the minimum extrusion pressure was 23.2, 25.5, 26.5, and 29.2 psi, respectively. Consequently, higher minimum extrusion pressure was found with higher concentrations.

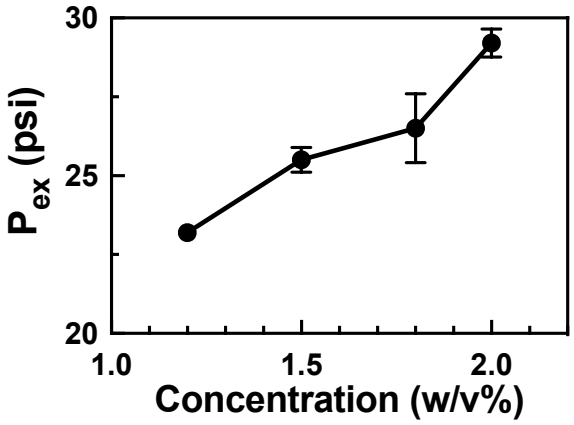

Figure S3. The relationship of minimum extrusion pressure (P_{ex}) to concentrations of ColME from 1.2 to 2.0% at 20°C through 25G.

S5. Characterization of digestion products using SDS-PAGE

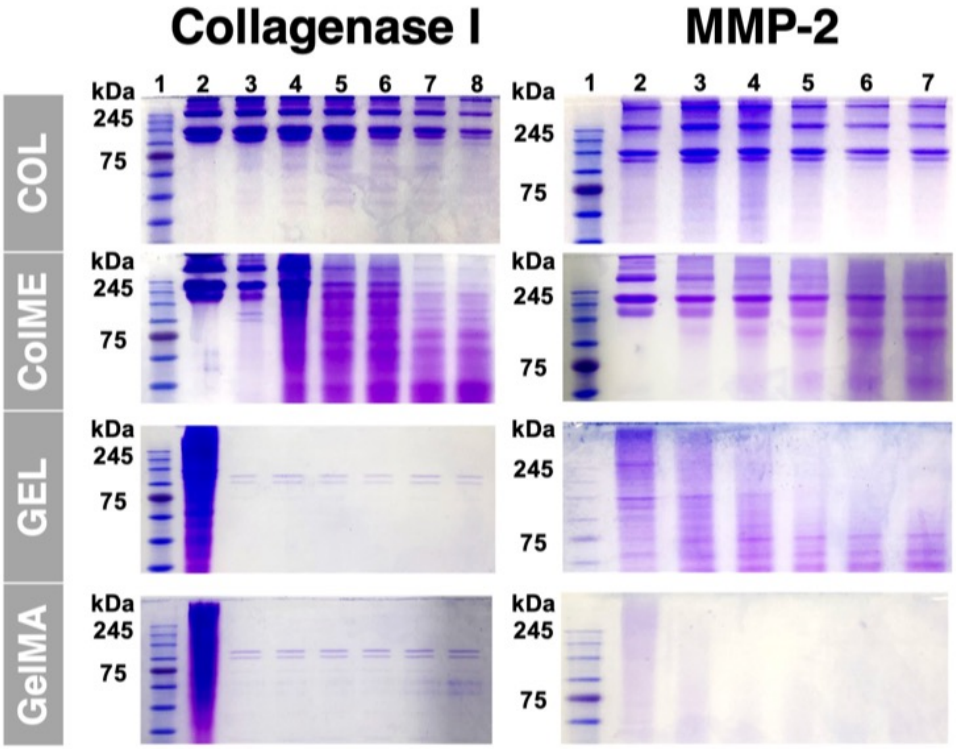

Figure S4. The enzymatic degradation of collagen (COL), ColME, gelatin (Gel) and GelMA obtained by SDS-PAGE. In collagenase I profiles: Lane 1: protein standard; Lane 2 to 8: samples degraded for 0 to 60 minutes. In MMP-2 profiles: Lane 1: protein standard; Lane 2 to 7: samples degraded for 0, 1, 2, 4, 8, and 12 hours.

S6. Formation of ColME and GelMA hydrogel

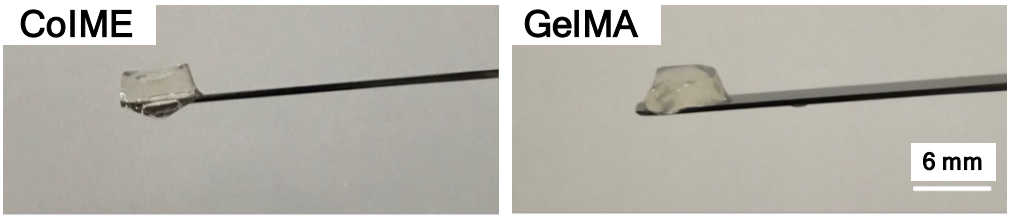

Figure S5. Hydrogel formation of 0.8% ColME and 3.2% GelMA with the addition of 0.6 mg/mL of LAP, then subsequently exposed to UV irradiation.

S7. Cell attachment, viability, and proliferation in GelMA and ColME hydrogel

L929 fibroblasts were suspended in a 8 mg/mL ColME precursor solution or 32 mg/mL GelMA precursor solution, both containing 0.6 mg/mL LAP. The cell density was adjusted to 1×10^6 cells/mL. This mixture was then exposed to UV irradiation at 365 nm with an intensity of 100 mW/cm² for 60 seconds. Following irradiation, the cell-laden ColME hydrogel was cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, USA), supplemented with 10% fetal bovine serum (Biological Industries, Israel), 1% antibiotic-antimycotic solution (Hyclone, USA), and 0.04% 2-mercaptoethanol. Cell viability within the ColME and GelMA hydrogels was evaluated after one and five days of culture using the LIVE/DEAD cell viability assay (Invitrogen, USA). The morphology of L929 cells was captured by optical microscope (Nikon Eclipse TS 100, Nikon, Japan) from day 1 to day 5.

For DNA quantification, the hydrogels were enzymatically digested using papain (Sigma, USA) digestion at 60 °C for 24 hours. DNA was then isolated and quantified using the Quant-iT™ PicoGreen™ dsDNA assay kit (Invitrogen, USA), following the manufacturer's instructions. Fluorescence intensity for DNA quantification was measured using a microplate reader (Synergy H1, BioTek, USA) at an excitation wavelength of 480 nm and emission detection at 520 nm.

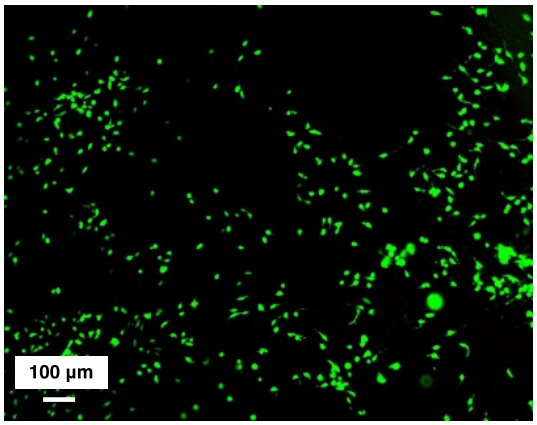

Figure S6. Live/dead staining of L929 cells embedded in ColME hydrogel after one day of culture. Most of the cells were alive.

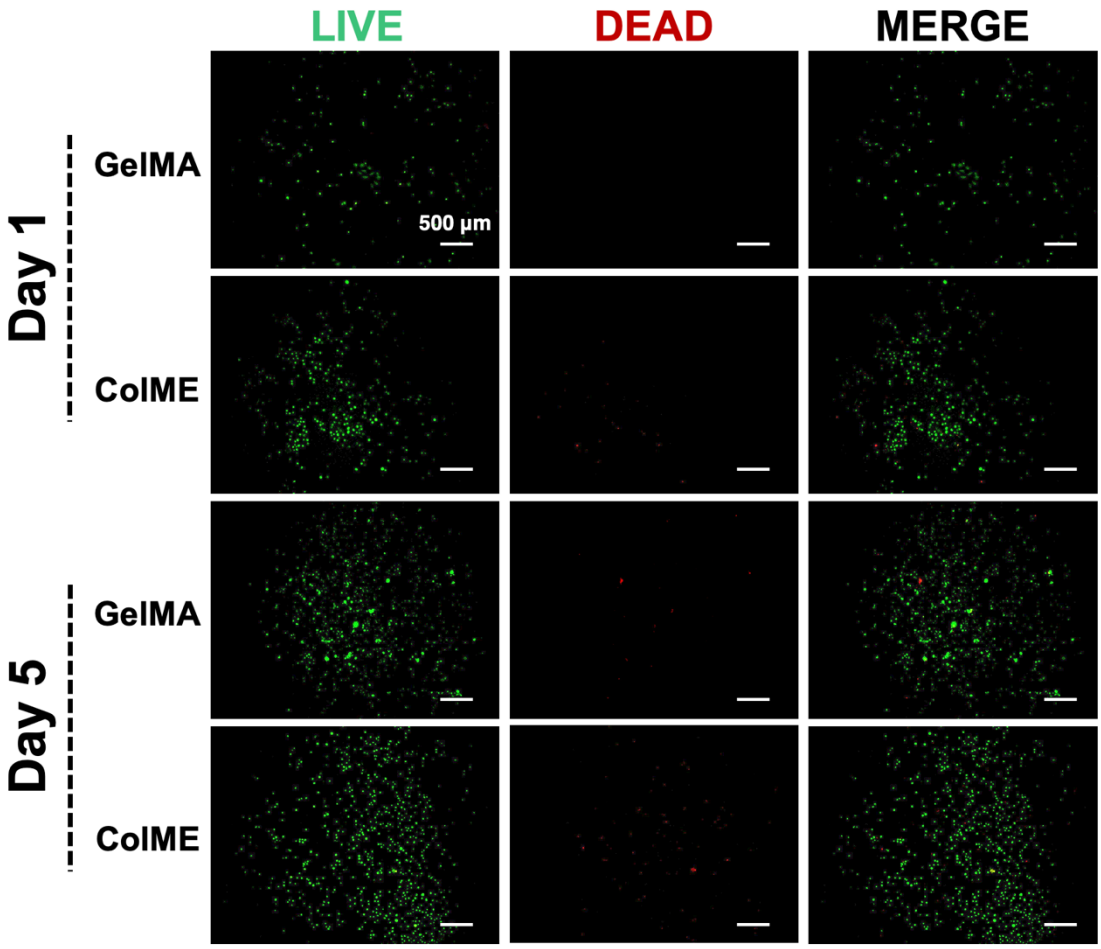

Figure S7. Live/dead staining of L929 cells embedded in GelMA and ColME hydrogel after one day and five days of culture. Live cells were in green, and dead cells were in red.

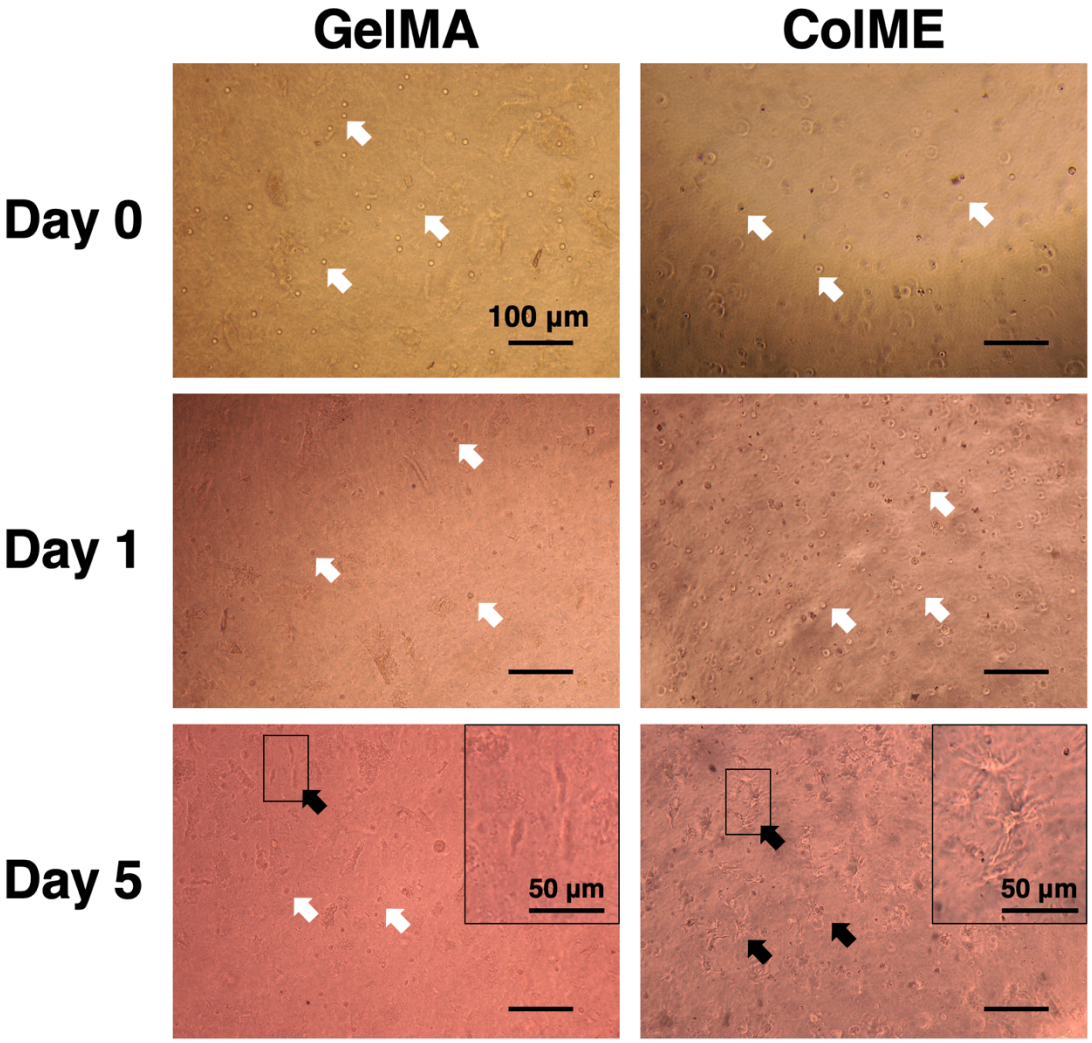

Figure S8. Morphology of L929 cells in GelMA and ColME hydrogel observed from an optical microscope. Spread spindle-shaped cells were observed (black arrows), while more rounded cells (white arrows) were observed in GelMA hydrogel.

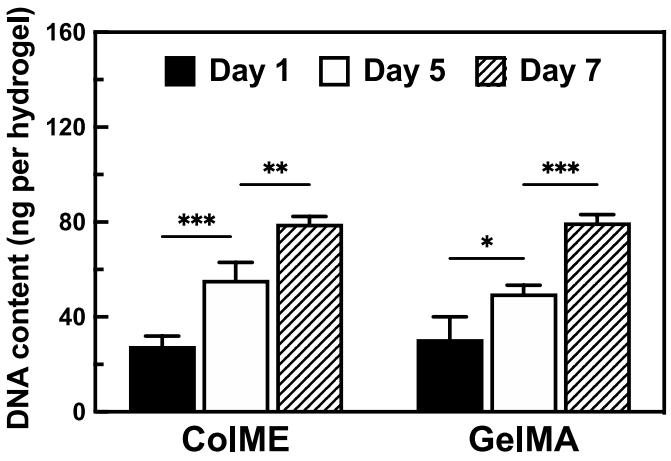

Figure S9. The DNA content of L929 cells in ColME and GelMA hydrogel after one, five, and seven days of incubation. Sample number = 4.

S8. Microscopic images of printed grids with corresponding Pr values

Each concentration of ColME had the broadest printable nozzle speeds at 30.0, 30.7, 32.2, and 35.2 psi for concentrations of 1.2, 1.5, 1.8, and 2.0%, respectively. When the printability is close to 1, a more squared-spaced area can be obtained, indicating great shape fidelity for the bioprinting parameter. As shown in Figure S12, the printability gradually increases and more square-spaced areas were found with increasing nozzle speeds.

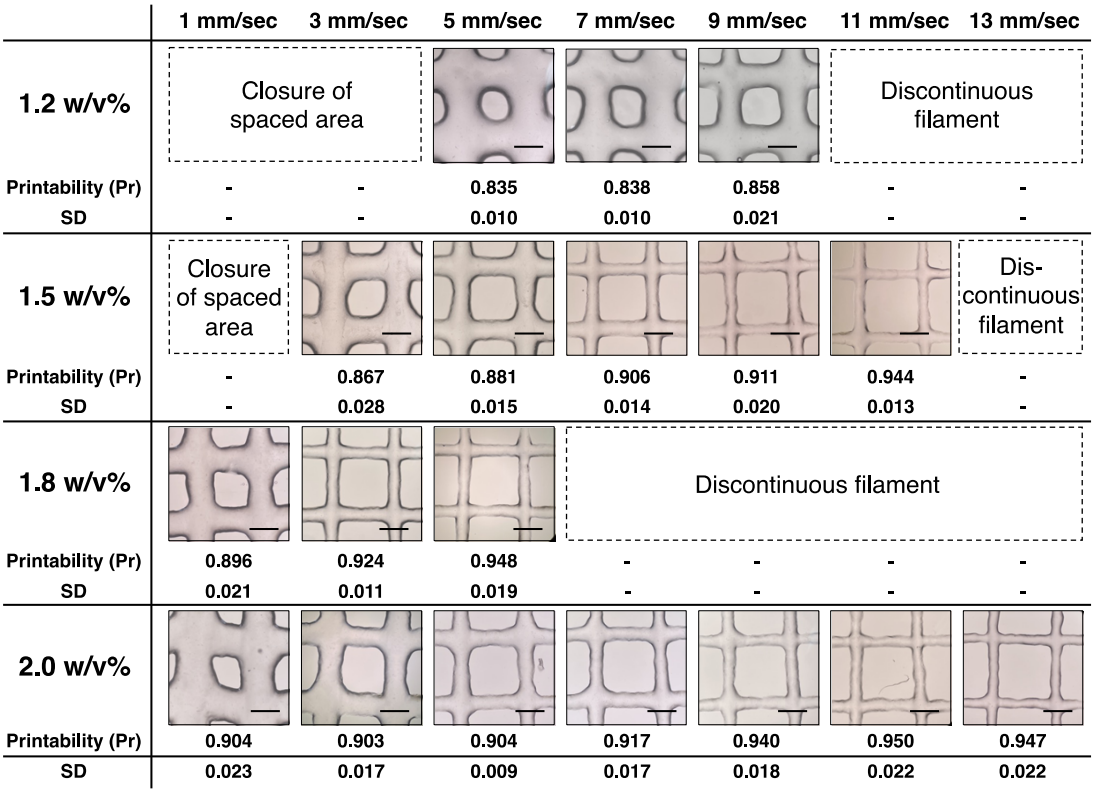

Figure S10. The printability (Pr) and microscopic images depicting spaced areas for each concentration of ColME across nozzle speeds ranging from 1 to 13 mm/s are shown. Scale bar = 1.0 mm.

S9. Variation of minimum extrusion pressure when cells encapsulated in ColME solution

The minimum extrusion pressure at each concentration of ColME increased after the addition of L929 shown in Table S1. According to the Einstein model, the viscosity difference of cell-encapsulated bioink to acellular bioink was 2.5^{V_c} (where ^{V_c} represents the volume fraction of cells in the bioink)[3]. Therefore, stiffer fluid was presented in the solution with cells, resulting in a higher minimum extrusion pressure. The minimum extruding pressure increased by 2.5, 5.2, 5.2, and 5.5 psi at concentrations of 1.2, 1.5, 1.8, and 2.0%, respectively, for the 25G. On the other hand, a lesser extent of increase was observed with the 20G, which was 1.5 and 0.5 psi at concentrations of 1.8 and 2.0%, respectively.

Nozzle size	Bioink type	Minimum extruding pressure (psi) @20°C			
		1.2 w/v%	1.5 w/v%	1.8 w/v%	2.0 w/v%
25G	Acellular	23.2	25.5	26.5	29.2
	With L929	25.7	30.7	31.7	34.7
	With L929 with elevated pressure	26.7	31.7	32.7	35.7
20G	Acellular	-	-	26.2	28.2
	With L929	24.7	27.2	27.7	28.7

Table S1. The recorded minimum extrusion pressure, both acellular and with L929 cells, along with elevated pressure, varied between different nozzle sizes and concentrations at 20°C. It was observed that 1.2% and 1.5% ColME solutions were not printable due to the closure of spaced areas.

References

1. G. K. Reddy and C. S. Enwemeka, *Clinical biochemistry*, 1996, **29**, 225-229.
2. R. E. Neuman and M. A. Logan, *J Biol Chem*, 1950, **184**, 299-306.
3. N. Diamantides, C. Dugopolski, E. Blahut, S. Kennedy and L. J. Bonassar, *Biofabrication*, 2019, **11**, 045016.