Supporting Information

***In Situ* Printing of Adhesive Hydrogel Scaffolds for the Treatment of Skeletal Muscle Injuries**

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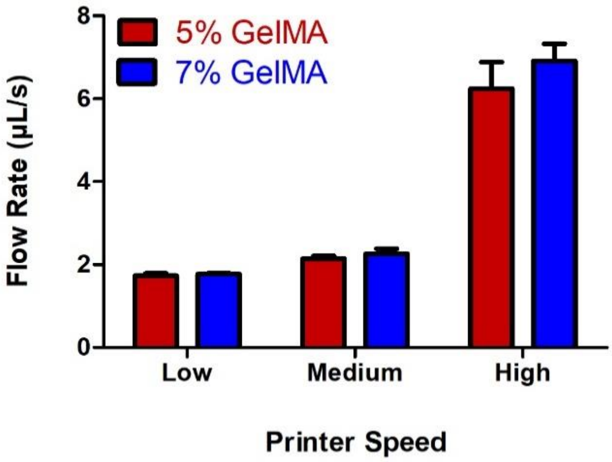

Figure S1: Extrusion rate of handheld bioprinter at low, medium, and high coding inputs.

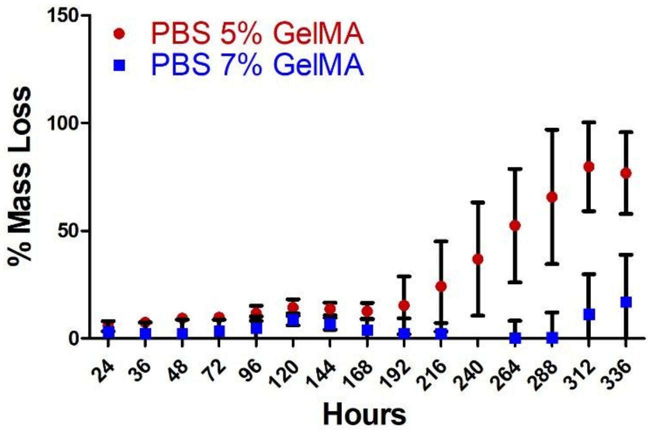

Figure S2: Degradation of 5% and 7% (w/v) GelMA in DPBS over a 14-day period.

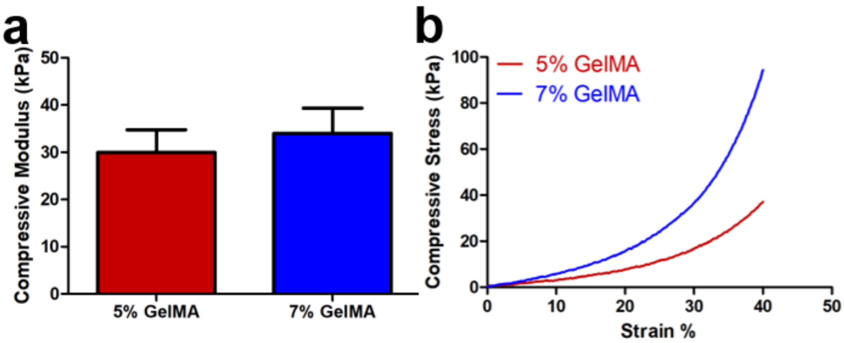

Figure S3: Compressive mechanical properties of GelMA hydrogels with varying gel percentage. (a) The compressive modulus of 5% and 7% (w/v) GelMA hydrogel disks (10mm diameter × 4.5 mm in height). (b) Representative stress-strain curves for 5% and 7% (w/v) GelMA hydrogels.

Effect of temperature on viscosity and elastic modulus of hydrogels

Temperature sweep profiles of 5% and 7% (w/v) GelMA, show that the viscosity is decreasing by increasing the temperature (Figure S4). This shows the necessity of incubation of gel at 4 °C before the printing; where it was observed that the viscosity of both gels at room temperature is nearly zero and to reach to suitable viscosity to print and extrude the gel, the cold incubation is necessary. At room temperature, there was only drops formation rather than fiber deposition.

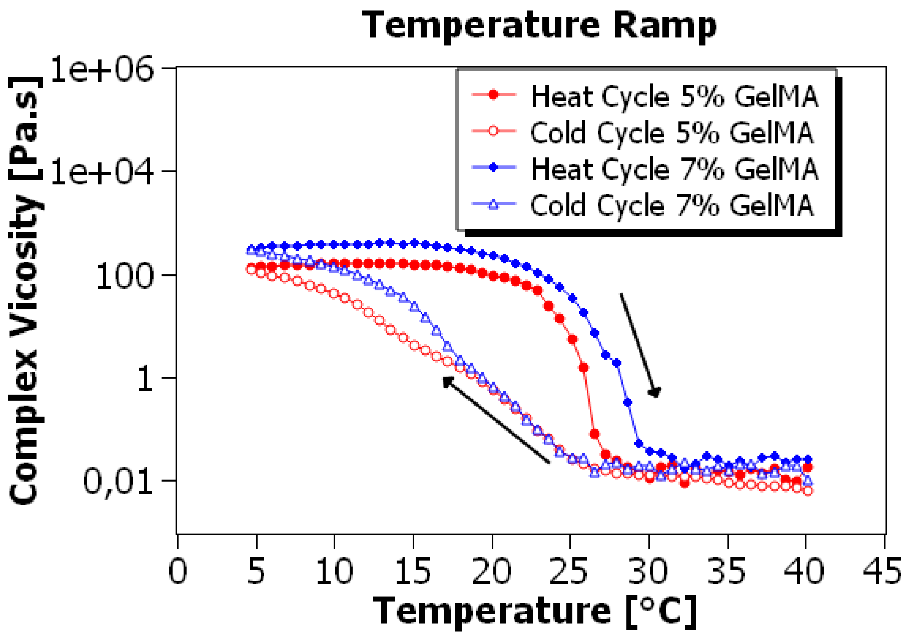

Figure S4: Temperature sweep to measure complex viscosity of non-cross-linked 5% and 7% (w/v) GelMA hydrogels solution in oscillatory mode from 4 to 37 °C, at a constant frequency and strain of 1 Hz and 1%, respectively.

Comparing storage modulus (G') between 5% and 7% (w/v) un-cross-linked GelMA showed that when the polymer concentration is higher, stiffer constructs are achieved that corresponds to a higher cross-linking density (Figure S5 a and b). Moreover, the gel point of two concentrations happens in less than 37 °C, and at this point, the almost zero G' is due to the cleavage of hydrogen bonds in the range from 30 at 37 °C. Upon cooling, G' of the hydrogels attempted to recover and increased rapidly but, they just crossed over the G'' indicating the typical behavior of a gel-like structure.

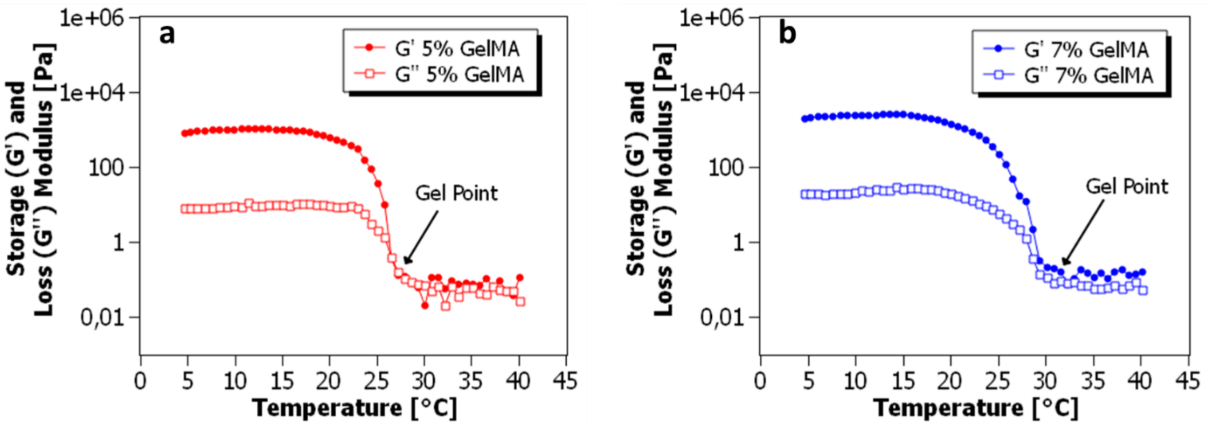

Figure S5: Storage modulus (G′) and loss modulus (G″) and their temperature dependency for different concentration of non-cross-linked (a) 5% and (b) 7% (w/v) GelMA hydrogels solution in oscillatory mode from 4 to 37 °C, at constant frequency and strain of 1 Hz and 1%, respectively.

Effect of shear rate on viscosity of hydrogels

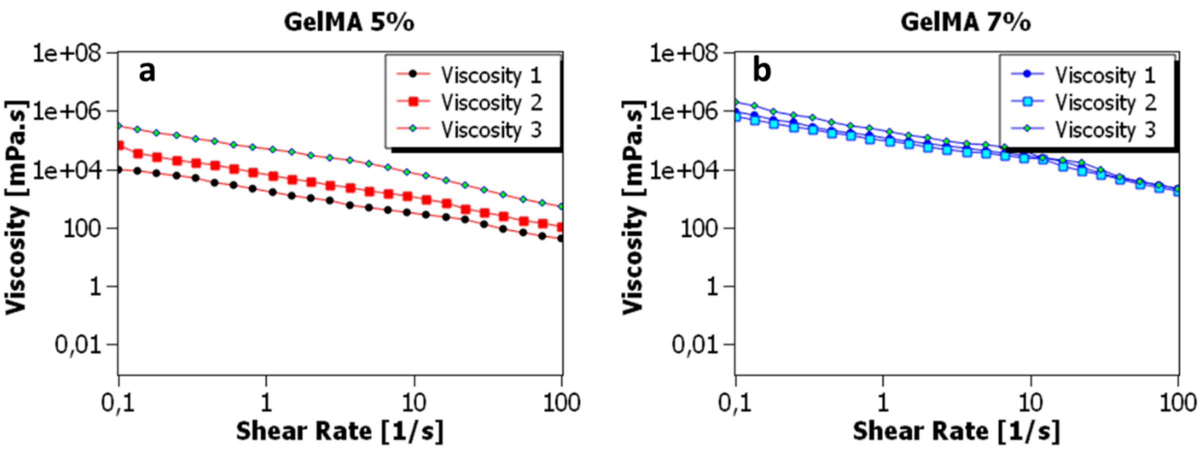

Figure S6: Shear ramp of un-cross-linked GelMA hydrogel at room temperature in a shear rate range of 1 to 100 1/s for (a) 5% and (b) 7% (w/v).

Figure S6 displays a shear-thinning behavior, where viscosity is highly dependent on the shear rate. This measurement in combination with the temperature ramp measurements proved that hydrogel viscosity is not only affected by the thermal sensitivity of GelMA but also by the shear rate. For the higher strain rates, the viscosity decreases. The data also shows the mechanical properties of the hydrogel begin to decrease after applying the shear force. The variation in the viscosity in the 5% (w/v) solution is due to its higher sensitivity to temperature.

The range of the shear rates considered in these measurements was calculated in the 400 μm (22 G) and 600 μm (20 G) nozzle for both of the hydrogel concentrations as following and based on the flow rates reported in Figure S1 used for bioprinting. The following equation was used to calculate the shear rate ($\dot{\gamma}$):

$$\dot{\gamma} = \frac{4Q}{\pi R^3} \quad \text{(S1)}$$

where Q is the volumetric flow rate and R is the radius of the nozzle [1].

Using Equation S1, the maximum shear rate values are reported in Table S1.

Table S1. Estimated shear rate (1/s) for GelMA 5 and 7% (w/v) based on different flow rates.

Lower Rate		**Medium Rate**		**Higher Rate**	
5%	**7%**	**5%**	**7%**	**5%**	**7%**
274.81	83.39	341.03	106.84	993.48	326.01

While the range of the maximum shear stress is beyond the upper limit of the rheological tests, the lowest flow rate was used in all printing which results in a total shear rate range from 0 to 274.8 s-1. According to the values reported above and correlating the results shown in Figure S6, it can be described that the lack of a Newtonian-plateau region is due to the high applied shear rates. The utilized printing speeds allowed for fiber deposition and did not result in high enough flow-induced shear stresses to reduce cell viability (Figure S7 and S8).

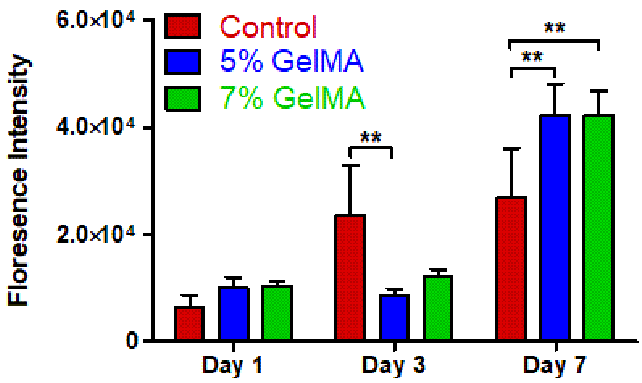

Figure S7: PrestoBlue™ cell viability of 20 μL, 500 μm sheets of 5% and 7% (w/v) GelMA.

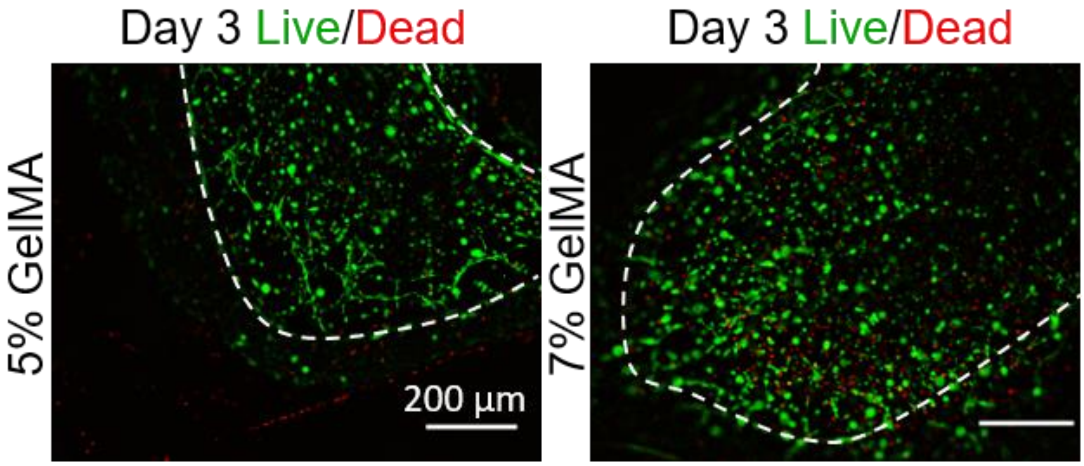

Figure S8: Live/dead viability assay of encapsulated cells in 5% and 7% (w/v) GelMA hydrogel scaffolds on day 3 after printing. Scale bars, 200 µm.

Reference

[1] Anton Paar, Flow of toothpaste out of a tube: Examples of the calculation of shear rates, 2019. https://wiki.anton-paar.com/en/examples-of-the-calculation-of-shear-rates/ (accessed 6 December 2019).