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RESEARCH ARTICLE

Enzymatic post-crosslinking of printed hydrogels of methacrylated gelatin and tyramine-conjugated 8-arm poly(ethylene glycol) to prepare interpenetrating 3D network structures

Supplementary File

The GelMA or GelMA/8PEGTA$_5$ solution at 37°C was transferred to a syringe (BD, 5 mL, Luer-Lock tip) with dispensing needle. The syringe was kept at 4°C for 10 min to form physical crosslinks and 20 min at room temperature for equilibrium. On testing, with given GelMA or GelMA/8PEGTA$_5$ solutions, a printing speed of 4 mm/s and an extrusion speed of 0.45 uL/s were applied.

	Size average, nm	Polydispersity index
GelMA	59.1±6.8	0.39±0.02
GelMA/8PEGTA$_5$	62.7±9.3	0.41±0.03

Quantification of crosslinking degree can be done according to the equations given below:

Flory–Rehner equation[S1,S2]:

$$Mc = -\rho V Q^{\frac{5}{3}} / (1 - \frac{1}{\chi_1})$$

Where, *Mc* is molecular weight between crosslinks (g/mol). The value of *Mc* represents the degree of crosslinking of the polymer. The larger the value, the longer the molecular chain between the crosslinking points, indicating the lower the crosslinking degree of the polymer. On the contrary, the smaller the *Mc*, the shorter the molecular chain between the crosslinking points and the higher the crosslinking degree.

ρ, polymer density (GelMA: 1.2 g/cm^3, PEG: 1.5 g/cm^3, GelMA/8PEGTA$_5$: 1.275 g/cm^3)

V, molar volume of the solvent (V_{water} = Mρ = 18 mL/mol)

$$Q = (\frac{m}{\rho} + \frac{m_1}{\rho_1}) / \frac{m_1}{\rho_1}$$

Q, swelling degree (approximate to water uptake shown in Table 1, GelMA: 17.3, GelMA/8PEGTA$_5$: 6.65).

x_1, Flory-Huggins interaction parameter (GelMA: ~ 0.497, PEG: ~ 0.47)

	M_c
GelMA hydrogel	2451
GelMA/8PEGTA$_5$-IPN	532

The fidelity quantitative analysis was evaluated with methodology introduced by Ouyang *et al.*[S3] on circularity of printed lattice structures using the equation:

$$Pri = \frac{\pi}{4} + \frac{1}{C} = \frac{L^2}{16A}$$

Pri = 1 indicates perfect square shape and higher fidelity. The bigger or smaller Pri value means low fidelity.

The optimization process was not intensively illustrated in this paper; therefore, we did not include the fidelity quantification in the paper. According to the above equation, the printed lattice structures of GelMA and IPN in Figure 6A are 0.95 ± 0.04 and 0.99 ± 0.02, respectively. The raw data and calculations are included in Table S1.

The fidelity quantitative analysis was evaluated using the methodology introduced by Ouyang *et al.*[S3] on circularity (Pri-value) of printed lattice structures (Table S1). According to our calculation, the printed lattice structures of GelMA and the IPN in Figure 6A are 0.95 ± 0.04 and 0.99 ± 0.02, respectively, whereas in case of an ideal square lattice shape, the value is 1. This indicates our IPN formulation as a new type of bioink delivers higher fidelity in extrusion-based 3D printing.

References

S1. Germershaus O, Werner V, Kutscher M, *et al*., 2014, Deciphering the mechanism of protein interaction with silk fibroin for drug delivery systems. *Biomaterials*, 35:3427–3434.

https://doi.org/10.1016/j.biomaterials.2013.12.083

S2. Malana MA, Bukhari JU, Zohra R, 2014, Synthesis, swelling behavior, and network parameters of novel chemically crosslinked poly (acrylamide-co-methacrylate-co-acrylic acid) hydrogels. *Des Monomers Polym*, 17:266–274.

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S3. Ouyang L, Yao, R, Zhao Y, Sun W, 2016, Effect of bioink properties on printability and cell viability for 3D bioplotting of embryonic stem cells. Biofabrication, 8:35020.

https://doi.org/10.1088/1758-5090/8/3/035020

Table S1. Fidelity evaluation data from above formulation in printed lattice structures.

GelMA	Area	Perim.	*L2*	16A	Pri		
1	0.462	2.708	7.333	7.392	0.992		
2	0.673	3.168	10.036	10.768	0.932		
3	0.579	2.954	8.726	9.264	0.942		
4	0.577	2.946	8.679	9.232	0.94		
5	0.483	2.586	6.687	7.728	0.865		
6	0.629	3.094	9.573	10.064	0.951		
7	0.466	2.688	7.225	7.456	0.969		
8	0.68	3.225	10.401	10.88	0.956		
9	0.628	3.154	9.948	10.048	0.99	0.949	0.038
IPN	**Area**	**Perim.**	***L2***	**16A**	**Pri**		
1	0.689	3.321	11.029	11.024	1		
2	0.646	3.204	10.266	10.336	0.993		
3	0.589	3.093	9.567	9.424	1.015		
4	0.757	3.484	12.138	12.112	1.002		
5	0.774	3.479	12.103	12.384	0.977		
6	0.719	3.395	11.526	11.504	1.002		
7	0.639	3.135	9.828	10.224	0.961		
8	0.796	3.503	12.271	12.736	0.963		
9	0.638	3.202	10.253	10.208	1.004	0.991	0.019

The area and perimeter were determined with image processing tool Fiji (Image J).

Table S2. Control experiments of independent crosslinking of 8PEGTA$_5$.

	Polymer solution	Initiator	Crosslinking approach	Forming a gel (Yes/No)
Experiment 1	2 wt% 8PEGTA$_5$	4 U/mL HRP	0.03 wt% H_2O_2	Yes
Experiment 2	2 wt% 8PEGTA$_5$	0.5 wt% LAP	UV irradiation	No

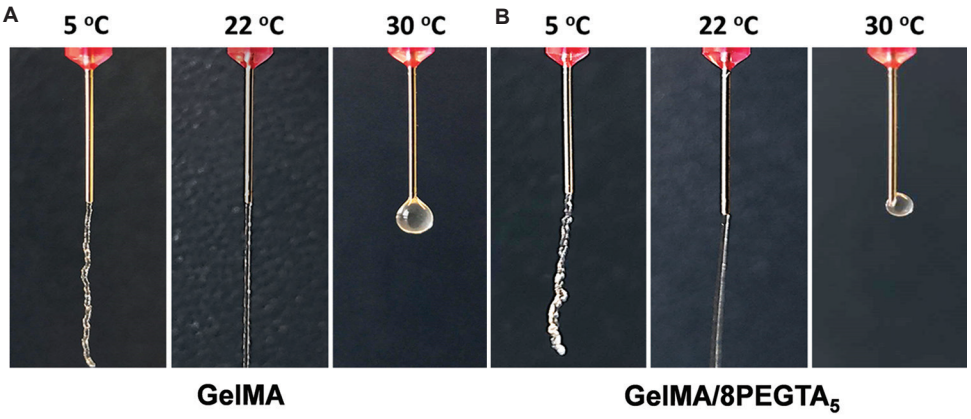

Figure S1. Printability of (A) 6 wt% GelMA and (B) 6 wt% GelMA/2 wt% 8PEGTA$_5$ inks at different temperatures.

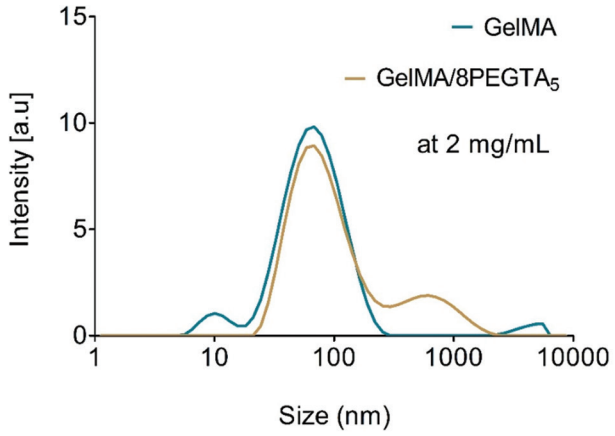

Figure S2. Size distribution profiles (intensity plots) of GelMA and GelMA/8PEGTA$_5$ aqueous solutions (2 mg/mL) at room temperature as measured with dynamic light scattering.

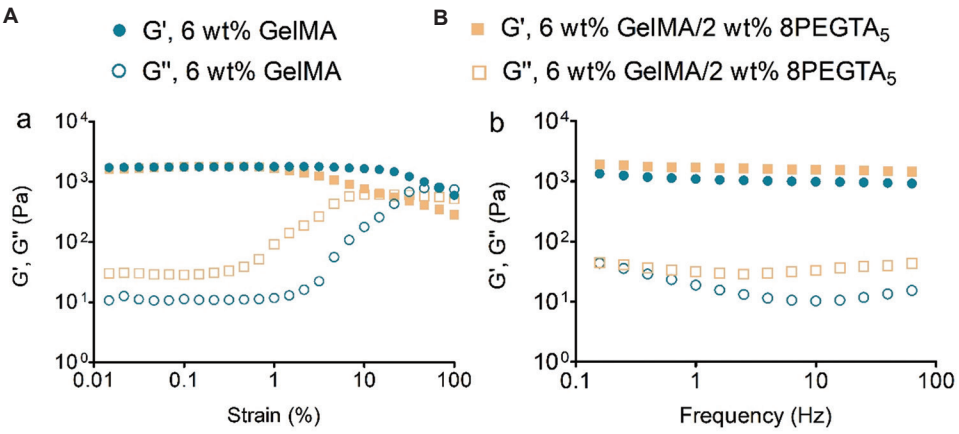

Figure S3. (A) Amplitude and (B) frequency sweep of 6 wt% GelMA and 6 wt% GelMA/2 wt% 8PEGTA$_5$ physically cross-linked hydrogels at 5°C.

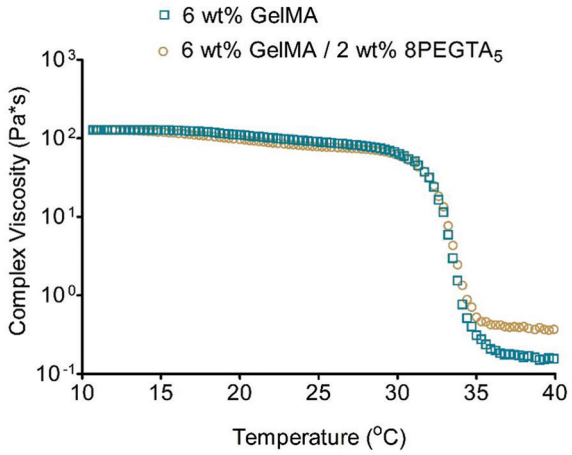

Figure S4. Complex viscosity of 6 wt% GelMA and 6 wt% GelMA/2 wt% $8PEGTA_5$ solutions as a function of temperature.

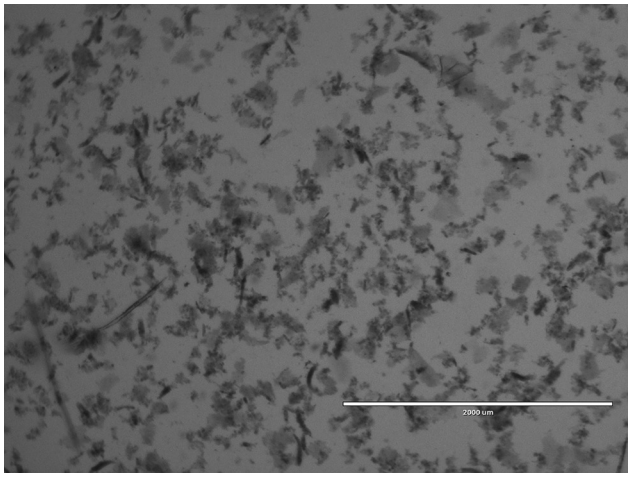

Figure S5. Optical microscopic imaging of IPN gels after degradation of 8 weeks in PBS.

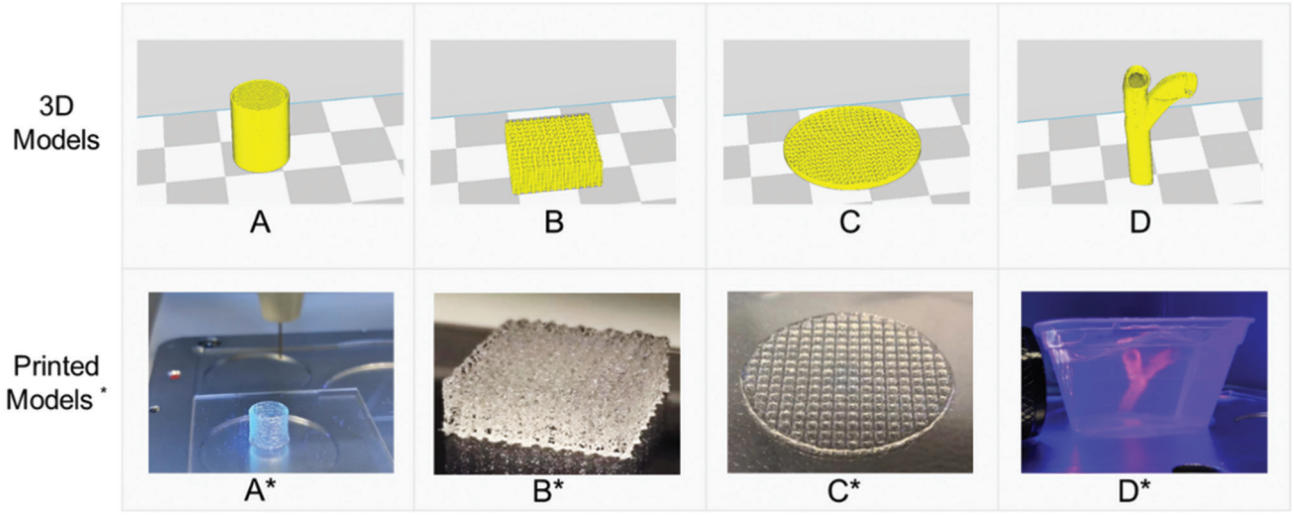

Figure S6. Photographic images of representative 3D constructs printed with bioinks of GelMA/8PEGTA$_5$ hydrogels before enzymatic crosslinking (A, cylindrical tubular; B, cubic grids; C, cylindrical grids; and D, blood vessel). Scale bar: In 3D models, one cubic square is 10 ×10 mm.

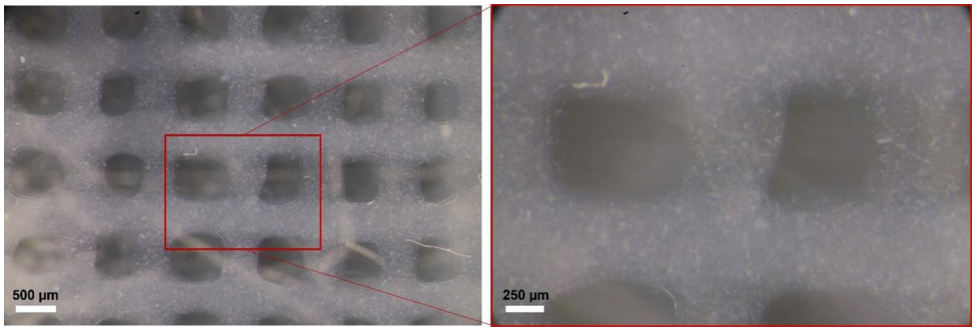

Figure S7. Stereo microscope images of GelMA/8PEGTA$_5$-IPN with MG-63 cells after 21 days in culture.

Video clip S1. Examples of printing 3D objects using a 6 wt% GelMA/2 wt% PEGTA$_5$ solution and intermediate or continuous UV crosslinking. Last examples were printed using a gel bath from SunP Biotech. All scaffolds can be enzymatically post-crosslinked.