Supporting information for:

Comparison of Photocrosslinkable Gelatin Derivatives and Initiators for Three-Dimensional Extrusion Bioprinting

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GelNB ¹H NMR:

Figure S1. ¹H NMR of GelNB. Alkene associate peaks $(I_{6.11}, I_{5.77})$ were normalized to the methyl associated peak $(I_{0.74})$.

GelMA ¹H NMR:



Figure S2. ¹H NMR of GelMA. Alkene associate peaks ($I_{5.57}$, $I_{5.52}$, $I_{4.67}$) were normalized to the methyl associated peak ($I_{0.79}$).

GelNB Degree of Functionalization Equation:

Functionalization equations were derived using the general methods described by *van Hoorick et* al.¹ and the gelatin amino acid content provided by *Claaben et al*.² Equation S1 describes the degree of functionalization of GelNB, where DF_{NB} is the concentration of NB in mmol per g gelatin, and I_X are the integrals of peaks centered at the corresponding subscript on NMR spectra.

There are 2 mmol alkene H/ mmol norbornene, 6 mmol methyl H/ mmol methyl-containing amino acids, and 0.523 mmol methyl-containing amino acids/ g gelatin.

Equation S1
$$DF_{NB} = \frac{\frac{I_{6.11} + I_{5.77}}{2}}{\frac{I_{0.74}}{6*0.523}} = 1.57 * \frac{I_{6.11} + I_{5.77}}{I_{0.74}}$$

GelMA Degree of Functionalization Equation:

Equations S2 for the degree of functionalization of GelMA was derived using the same methods and values as in the derivation of **Equation S1**.

Equation S2
$$DF_{MA} = \frac{\frac{I_{5.57} + I_{5.52} + I_{5.29}}{2}}{\frac{I_{0.79}}{6*0.523}} = 1.57 * \frac{I_{5.57} + I_{5.52} + I_{5.29}}{I_{0.79}}$$

Computation of Extrusion Speed from Slic3r Generated G-codes:

Equation S3 was used to determine extrusion speeds (the speed at which the plunger compresses ink in the extruder) from Slic3r generated G-codes.

Equation S3
$$Extrusion Speed = \frac{FE}{\sqrt{(X_{current} - X_{previous})^2 + (Y_{current} - Y_{previous})^2}}$$

Where F is the print speed in mm min⁻¹ specified in Slic3r. For a given line of the generated Gcode, E is the extrusion length in mm specified in that line, X_{curent} and $Y_{current}$ are the nozzle positions in mm specified in that line, and $X_{presvious}$ and $Y_{previous}$ are the nozzle positions in mm specified in the line immediately prior to that line. Extrusion speed will be given in mm min⁻¹. The extrusion multiplier in Slic3r was used to linearly adjust the ratio of print speed:extrusion speed.



GelMA Biomaterial Ink Temperature Sweeps:

Figure S3. Rheological temperature sweeps of GelMA biomaterial inks prior to photocuring. Inks were deposited on the rheometer stage and allowed to soak at 40°C for three minutes before the temperature was decreased to 22.5°C. Physical gelation kinetics at 22.5°C were monitored as a function of time.

REFERENCES

1. Hoorick, J.; Gruber, P.; Markovic, M.; Rollot, M.; Graulus, G.-J.; Vagenende, M.; Tromayer, M.; Erps, J.; Thienpont, H.; Martins, J. C.; Baudis, S.; Ovsianikov, A.; Dubruel, P.; Vlierberghe, S., Highly Reactive Thiol-Norbornene Photo-Click Hydrogels: Toward Improved Processability. *Macromolecular Rapid Communications* **2018**, *39* (14), 1800181.

2. Claaßen, C.; Claaßen, M. H.; Truffault, V.; Sewald, L.; Tovar, G. E. M.; Borchers, K.; Southan, A., Quantification of Substitution of Gelatin Methacryloyl: Best Practice and Current Pitfalls. *Biomacromolecules* **2018**, *19* (1), 42-52.