Supporting Information

Improving gelation efficiency and cytocompatibility of visible light polymerized thiol-norbornene hydrogels via addition of soluble tyrosine

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Methods

Islets encapsulation and viability assay

CD1 mice (7- to 8-week old) were obtained from Charles River and islets were isolated following established protocol [1]. Permission for animal studies was approved by Indiana University School of Medicine IACUC (protocol #10235-MD/R). Islets were suspended in polymer solutions containing 0.1 mM eosin-Y, PEG8aNB, and di-cysteine peptide (i.e., Lys-Cys-Tyr-Ile-Gly-Ser-Arg-Cys-Lys or KCYIGSRCK) with and without L-tyrosine methyl ester. Precursor solution was exposed to the same visible light for 5 minutes to obtain cell-laden hydrogels (20 µl/gel), which were maintained in RPMI-1640 media supplemented with 10 % FBS, and 1 × antibiotic-antimycotic. To obtain qualitative cell viability, cells were stained with a live/dead staining kit (Calcein AM stained live cells green and Edithium homodimer stained dead cells red) and imaged with confocal microscopy (Olympus FV-1000 Laser Scanning Biological Microscope). For islets encapsulated gels, images were taken at a step size of 5 µm for a total depth of 50 µm per image.

mRNA isolation and rtPCR

In preparation for RNA isolation, cell-laden gels were rapidly frozen using liquid nitrogen and stored in -80 °C until use. Collected samples were processed following NucleoSpin® RNA protocols (Clontech). The concentrations of pure RNA were determined using NanoDrop 2000 Spectrophotometer (Thermo Scientific). RNA was reverse transcribed into cDNA using PrimeScript™ RT Reagent Kit (Clontech). SYBR Premix Ex Taq II kit (Clontech) was used for quantitative real time PCR (Applied Biosystems 7500 Fast Real-Time PCR machine) for

selected genes (Table S2). The results for gene of interests were internally normalized by a house keeping gene (i.e., β-actin), and further normalized to control (i.e., gels crosslinked without tyrosine on day 1).

Reference:

[1] N. D. Stull, A. Breite, R. McCarthy, S. A. Tersey, R. G. Mirmira, Journal of visualized experiments : JoVE 2012.

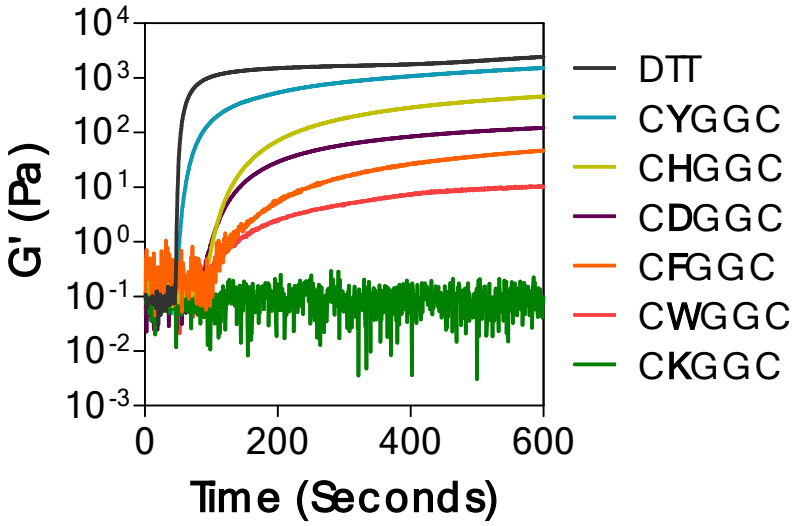

Figure S1. *In situ* photorheometry of thiol-norbornene photopolymerization between 5wt% $PEG4NB_{20kDa}$ and 5 mM of DTT or di-cysteine containing peptide in the presence of 0.1 mM eosin-Y. G': storage modulus. Light intensity: 70 kLux. n = 3. Error bars were omitted for clarity.

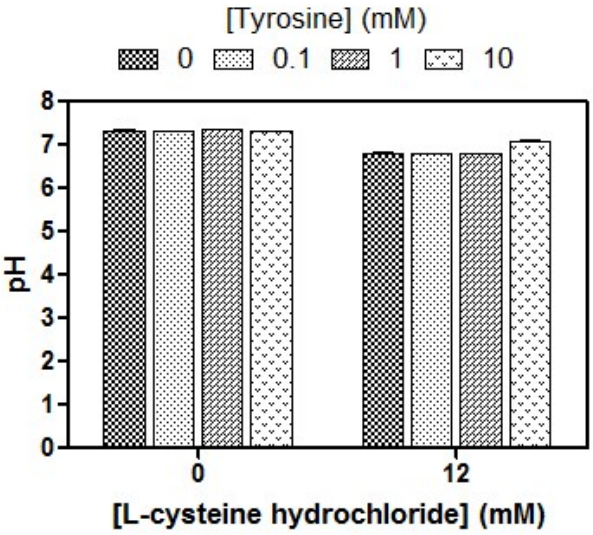

Figure S2. pH values of non-gelling solution containing different concentrations of tyrosine in the absence or presence of L-cysteine (n = 3).

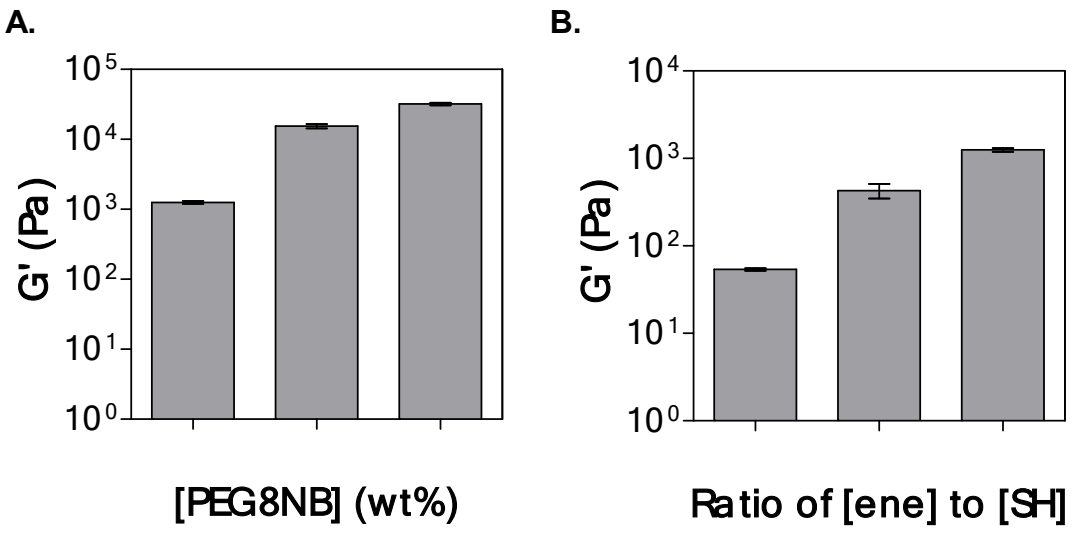

Figure S3. Effect of macromer concentration (A), and the ratio of norbornene to thiol (B) on the elastic modulus of PEG-peptide hydrogels. (3 wt% PEG8NB, 6 mM CGGGC, 0.1 mM eosin-Y, 400-700 nm light, intensity at 70000 lux, mean ± SD, n = 3).

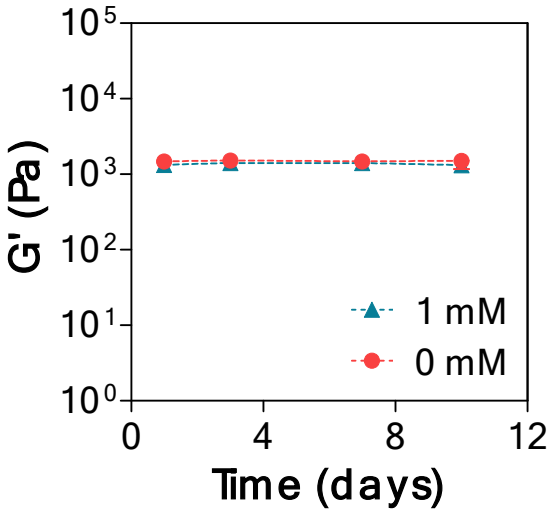

Figure S4. Effect of tyrosine concentration on the degradability of PEG-peptide hydrogels. All gel formulations contained β-cells at 5×10^6 cells/mL, 3.5 wt% PEG8aNB, 7 mM KCYIGSRCK, 0.1 mM eosin-Y, and 400-700 nm light at 70 kLux.

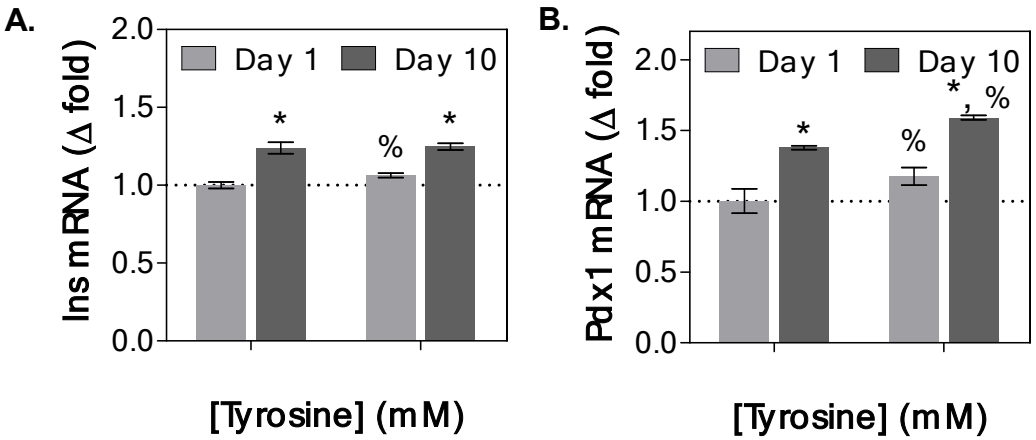

Figure S5. Effect of tyrosine on the mRNA expression of MIN6 β-cells. mRNA level of (A) Ins and (B) Pdx1 evaluated by qPCR. Actβ was used as a house keeping gene. * indicates comparison between day 1 and day 10, while % indicates comparison between 0 to 1 mM of tyrosine, $p < 0.05$. All gel formulations contained β-cells at 5×10^6 cells/mL, 3.5 wt% PEG8aNB, KCYIGSRCK, 0.1 mM eosin-Y, and 400-700 nm light at 70 kLux. Ratio of [thiol] to [norbornene] was 1 and 0.85 for 0 mM and 1 mM of tyrosine, respectively.

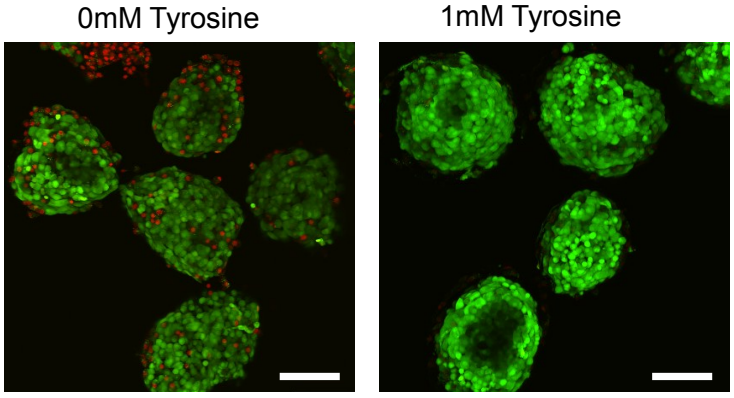

Figure S6. Effect of tyrosine on the cytocompatibility of CD1 mouse islets. Representative confocal z-stack images of islets stained with live/dead staining kit on day 1. Gel formulations: 3.5 wt% PEG8aNB, KCYIGSRCK, 0.1 mM eosin-Y, and 400-700 nm light at 70 kLux (Scales: 100 μm).

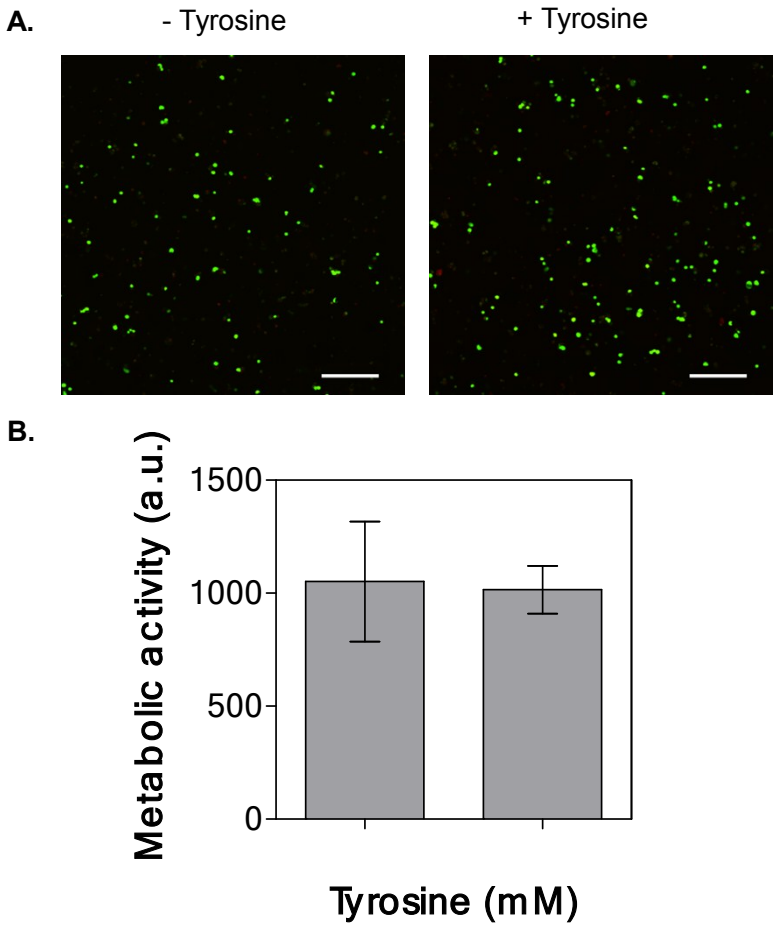

Figure S7. Effect of soluble tyrosine on the viability of MIN6 β-cells post gelation. Cell-laden hydrogels were incubated in media containing tyrosine for one hour before transferring back to regular media. (A) Representative confocal z-stack images of MIN6 cells stained with live/dead staining kit on day 1 (Scales: 100 μm). (B) Cells viability as assessed by Alamarblue® reagent. Gel formulations: 3.5 wt% PEG8aNB, KCYIGSRCK, 0.1 mM eosin-Y, and 400-700 nm light at 70 kLux.

Table S1. Mouse primer sequences used in real time PCR.

Gene Symbol	Primer Sequence	Reference
Actβ	TGAGAGGGAAATCGTGCGTG	[1]
	TGCTTGCTGATCCACATCTGC	
Ins	TGGCTTCTTCTACACACCCAAG	[2]
	ACAATGCCACGCTTCTGCC	
Pdx1	CGGACATCTCCCCATACGAAG	[3]
	CCCCAGTCTCGGTTCCATTC	

[1] Ejarque, M., et al., *Neurogenin3 cooperates with Foxa2 to autoactivate its own expression.* J Biol Chem, 2013. 288(17): p. 11705-17. [2] Iype, T., et al., Mechanism of insulin gene regulation by the pancreatic transcription factor Pdx-1: application of pre-mRNA analysis and chromatin immunoprecipitation to assess formation of functional transcriptional complexes. J Biol Chem, 2005. 280(17): p. 16798-807. [3] Jaroch, D.B., et al., *Mouse and human islets survive and function after coating by biosilicification.* Am J Physiol Endocrinol Metab, 2013. 305(10): p. E1230-40.