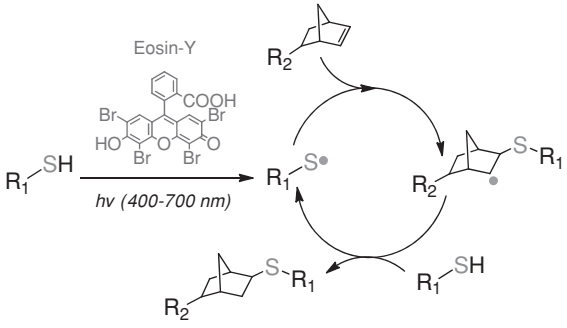
Visible-Light-Mediated Thiol-Ene Hydrogelation Using Eosin-Y as the Only Photoinitiator

Han Shih, Chien-Chi Lin*

The utility of visible-light-mediated polymerization in tissue engineering has been limited due to the necessary use of potentially cytotoxic coinitiator and comonomer. Here, we report a visible-light-mediated thiol-ene hydrogelation scheme using eosin-Y as the only photoinitiator. Under visible light exposure, rapid and highly tunable step-growth gelation is achieved using PEG-norbornene and a model cross-linker dithiothreitol. In addition to investigating the gelation kinetics and properties of thiol-ene hydrogels formed by this new gelation scheme, we also report high cytocompatibility of these hydrogels using human mesenchymal stem cells (hMSCs) and pancreatic MIN6 β-cells.



1. Introduction

Radical-initiated photopolymerizations have received significant attention for in situ cell encapsulation and controlled delivery of biological molecules.[1–6] The major benefit of radical-mediated gelation is its rapid and ambient gelation conditions, as well as high stability of the covalently crosslinked networks. A variety of synthetic macromers are increasingly developed for radical-mediated hydrogel synthesis.[7] The methods of radical-mediated photogelation, however, remain relatively unchanged for the past few decades. Mechanistically, a photoinitiator is required to initiate the chain-growth photocross-linking process. Following light exposure, a type I or cleavage-type photoinitiator readily absorbs photons and decomposes into two primary radicals to initiate gelation.[8,9] On the other hand, a type II photoinitiator abstracts a hydrogen from a coinitiator to generate secondary radicals and initiate cross-linking.[8–11]

Water solubility and molar absorptivity at cytocompatible wavelengths are commonly used to evaluate the suitability of a photoinitiator to initiate photopolymerization for hydrogel synthesis. Only a few photoinitiators are considered cytocompatible, including type I initiators Irgacure-2959 (I-2959)[12,13] and lithium arylphosphanate (LAP),[14] as well as type II initiator eosin-Y.[10,11,15] Commercially available I-2959 has low water solubility (<0.5 wt%) and low molar absorptivity at 365 nm ($\varepsilon < 10$ M^{-1} cm^{-1}). Added to these limitations is the fact that I-2959 cannot be used for visible-light-mediated photocross-linking due to its near zero molar absorptivity at wavelengths higher than 400 nm. Although LAP is highly water-soluble (>5 wt%) and has high absorbance at 365 nm ($\varepsilon \approx$ 200 M^{-1} cm^{-1}), its utility in visible light range is also very limited ($\varepsilon \approx 30$ M^{-1} cm^{-1} at 405 nm).[14] Type II photoinitiator eosin-Y, on the other hand, is highly water soluble and can be readily excited by visible light ($\varepsilon >$ 100 000 M^{-1} cm^{-1} at 515 nm). An example of this type of gelation is the synthesis of chain-growth poly(ethylene glycol) diacrylate (PEGDA) hydrogels. Unfortunately, a coinitiator [e.g., triethanolamine (TEOA)] and a comonomer [e.g., 1-vinyl-2 pyrrolidinone (NVP)] are required for generating sufficient radicals to achieve high and rapid functional group conversion. This prerequisite makes adjusting the compositions of a macromer precursor solution complicated and is perhaps the main reason why UV-mediated photopolymerizations, even with biosafety concerns, is still a preferred method for preparing hydrogels.

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In this contribution, we report an innovative step-growth hydrogel system formed by visible-light-mediated thiol-ene reactions with eosin-Y as the sole initiator. Mechanistically, the excitation of eosin-Y by visible light exposure caused hydrogen abstraction from the sulfhydryl groups to create thiyl radicals. These thiyl radicals initiated a rapid and orthogonal thiol-ene photo-click gelation reaction between sulfhydryl group on thiol-containing cross-linker and norbornene moiety on the multiarm PEG-norbornene macromer. Although it has not been used to cross-link hydrogels, this mechanism was recently employed to conjugate thiol-containing peptides to immobilized alkene moieties in hydrogels.[16,17] Using a low eosin-Y concentration and an inexpensive visible light source, we systematically studied the effects of cross-linking conditions on the resulting hydrogel properties. We also evaluated the cytocompatibility of these step-growth hydrogels with in situ encapsulation of human mesenchymal stem cells (hMSCs) and MIN6 β-cells.

2. Experimental Section

2.1. Materials

Eosin-Y disodium salt, TEOA, and NVP were purchased from Fisher Scientific. Four-arm PEG (20 kDa) and Four-arm PEG-amine (20 kDa) were purchased from JenKem Technology USA. All other chemicals were obtained from Sigma-Aldrich unless noted otherwise.

2.2. Methods

2.2.1. Synthesis of PEG Macromers

Poly(ethylene glycol)-tetra-norbornene (PEG4NB) and PEGDA (10 kDa) were synthesized using established protocols (see Supporting Information for details).[18,19] Poly(ethylene glycol)-tetra-amide-norbornene (PEG4aNB) was synthesized by reacting norbornene acid (five-fold excess to amine groups) with PEG-tetra-amine in DMF using HBTU/HOBT as coupling reagents. After overnight reaction at room temperature, the product was precipitated in cold ethyl ether and purified with the same protocol for PEG4NB purification (see Supporting Information for details). ^{1}H NMR (Bruker 500) was used to confirm the degree of PEG functionalization (>90%).

2.2.2. Hydrogel Fabrication and Swelling

Step-growth thiol-ene and chain-growth PEGDA hydrogels were formed by radical-mediated photopolymerization using 0.1×10^{-3} M of eosin-Y under visible light exposure at an intensity of 70 000 Lux using a fiber optic microscope illuminator (AmScope). Coinitiator (0.75 vol% of TEOA) and comonomer (0.1 vol% NVP) were added in PEGDA precursor solution. To quantify hydrogel swelling, circular hydrogel discs were prepared from 50 μL precursor solution. Immediately after gelation, hydrogels were incubated in ddH_2O at 37 °C on an orbital shaker for 24 h to remove sol fraction. Gels were then dried and weighed to obtain dried polymer weights (W_{Dry}). The dried polymers were incubated in 5 mL of buffer solution (pH 7.4 PBS) at 37 °C on an orbital shaker. At equilibrium swelling (after 48 h), hydrogels were removed from the medium, blotted dry with Kimwipe, and weighed to obtain swollen weights ($W_{Swollen}$). Hydrogel mass swelling ratios (q) were determined by a ratio of $W_{Swollen}$ to W_{Dry}.

2.2.3. Rheometry

For rheometrical property measurements, hydrogel discs (8 mm in diameter and 1 mm in height) were fabricated as described previously. Strain sweep (0.1% to 20%) oscillatory rheometry was performed with swollen gels using a Bohlin CVO 100 digital rheometer. Shear moduli of the hydrogels were measured using a parallel plate geometry (8 mm) with a gap size of 800 μm and moduli in the linear viscoelastic region (LVR) were reported. In situ photo-rheometry was conducted in a light cure cell using a parallel plate geometry (25 mm) at room temperature. Time-sweep in situ photo-rheometry (10% strain, 1 Hz frequency, 0.1 N normal force, and a gap size of 100 μm) was performed to obtain the gel point, which was defined as the time when storage modulus (G') surpassed loss modulus (G'').

2.2.4. hMSCs Encapsulation

Desired density (5×10^6 cells/mL) of hMSCs were suspended in the following sterile polymer precursor solutions: (1) PEG4NB and dithiothreitol (DTT); (2) PEG4aNB and DTT; or (3) PEGDA, TEOA, and NVP. All precursor solutions contained 1×10^{-3} M of CRGDS and 0.1×10^{-3} M of eosin-Y. Precursor solutions (25 μL) were exposed with the same visible light source for 4 min at room temperature. Cell-laden hydrogels were incubated in low-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco), 1 ng mL^{-1} basic fibroblast growth factor (bFGF, Peprotech), and 1X antibiotic-antimycotic (Invitrogen) at 37 °C and 5% of CO_2. To quantify long-term cell viability, cell-laden hydrogels were incubated in 500 μL Almarblue® reagent (AbD Serotec, 10% in cell culture medium) at 37 °C and 5% of CO_2. After 14 h of incubation, 200 μL of media was transferred to a 96-well plate for fluorescence quantification (excitation: 560 nm and emission: 590 nm) using a microplate reader (BioTek, Synergy HT).

3. Results and Discussion

Thiol-norbornene photoclick hydrogels have increasingly been used in cell encapsulation studies.[18,20–22] Prior reports on this new type of hydrogels, however, all used a cleavage type photoinitiator (I-2959 or LAP) under 365 nm UV light exposure. Here, we report that thiol-norbornene gelation could be achieved using a visible light source (400–700 nm) with eosin-Y as the only photoinitiator (Figure 1A). Upon visible light exposure, eosin-Y was excited to abstract hydrogen from thiol-containing cross-linkers, such as DTT, thus forming thiyl radicals. These

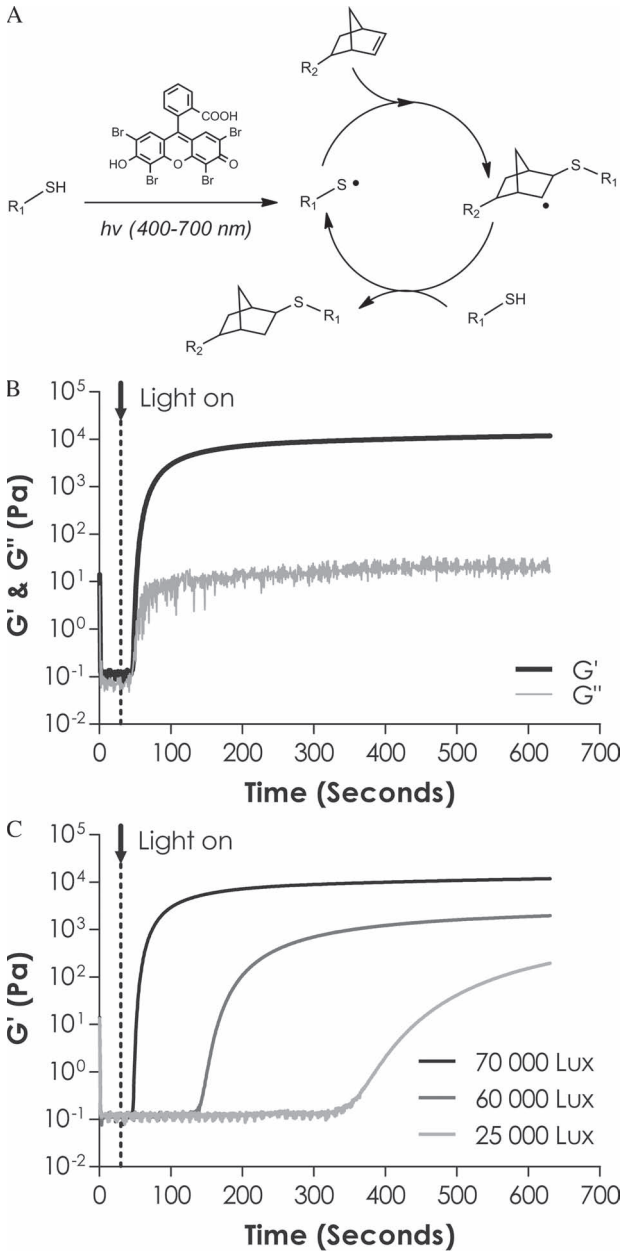

Figure 1. (A) Schematics of visible-light-mediated step-growth thiol-ene photo-gelation using PEG4NB and DTT. Eosin-Y was used as the only photoinitiator, which was excited by a visible light (400 to 700 nm) to initiate the photoclick reaction. (B) In situ photorheometry showing the crossover of *G′* and *G″* (10 wt% or 5×10^{-3} M PEG4NB, 10×10^{-3} M DTT, 0.1×10^{-3} M eosin-Y, 70 000 Lux). (C) Effect of visible light intensity on the gelation kinetics of thiol-ene hydrogels (light was turned on at 30 s, $N = 3$). Error bars in (B) and (C) were omitted for clarity.

radicals propagate through the norbornene moieties on multi-arm PEG macromers to form thioether bonds and carbonyl radicals. The termination of these carbonyl radicals is accomplished via abstracting hydrogen from other thiol-containing molecules.[23]

We first examined the gelation kinetics with in situ photorheometry in a light cure cell using 10 wt% PEG-$4NB_{20kDa}$ (20×10^{-3} M norbornene) and a stoichiometric-balanced thiol groups (from crosslinker DTT). 0.1×10^{-3} M eosin-Y was added in the precursor solution as the photoinitiator. As shown in Figure 1B, this visible-light-mediated step-growth thiol-ene reaction reached gel point rapidly (19 ± 2 s). The gel point was almost twice as fast as that in a conventional chain-growth PEGDA crosslinking reaction (37 ± 1 s) where equivalent macromer content (10 wt% $PEGDA_{10kDa}$ or 20×10^{-3} M acrylate), a coinitiator (0.75 vol%, TEOA) and a comonomer (0.1 vol%, NVP) were used (Figure S1 and Table S1, Supporting Information). Unlike chain-growth photopolymerizations of vinyl monomers (e.g., PEGDA), thiol-ene reactions are not oxygen inhibited,[24] and hence resulted in a fast gelation even without using comonomers. We also examined the gelation kinetics under different light intensities. Similar to other photopolymerization systems, significantly delayed gel points and decreased final shear moduli were obtained at lower light intensities (Figure 1C and Table S1, Supporting Information). We found that this eosin-Y-only, visible-light-mediated hydrogelation was unique to the thiol-norbornene system because gelation did not occur with PEG-tetra-acrylate alone unless coinitiator TEOA and comonomer NVP were also added. Furthermore, we found that the comonomer NVP, when added into the precursor solution, inhibited thiol-norbornene gelation. Further investigations are required to elucidate the underlying mechanisms.

We also evaluated the effect of macromer concentrations on network crosslinking using a gelation time of 4 min (*G′* higher than 95% of the final value, Figure 1B). As expected, there was an inverse correlation between macromer concentrations and gel points. Increasing macromer concentrations resulted in an increased in final gel moduli (Figure 2A) with accelerated gel points (Figure 2B). Furthermore, the gel fractions of thin hydrogels (thickness = 1 mm) were between 94% and 99% (Table S2, Supporting Information), indicating high gelation efficiency. Unlike chain growth PEGDA system where final gel moduli were affected by the necessary use of comonomer NVP,[15] the final moduli of visible-light-mediated step-growth thiol-ene hydrogels were more readily controlled by macromer content in the precursor solution (Figure 2B). Eosin-Y is a red dye used in a common histological staining (i.e., Hematoxylin and eosin staining). Due to its intense red color, the thiol-ene gels formed with 0.1×10^{-3} M eosin-Y appeared red-to-yellowish after gelation (Figure S2, Supporting Information). However, the color faded and the gels became transparent after swelling for 48 h, suggesting that the eosin-Y may release into the media. We observed similar phenomenon when higher eosin-Y concentrations were used for gelation (data not shown). As shown

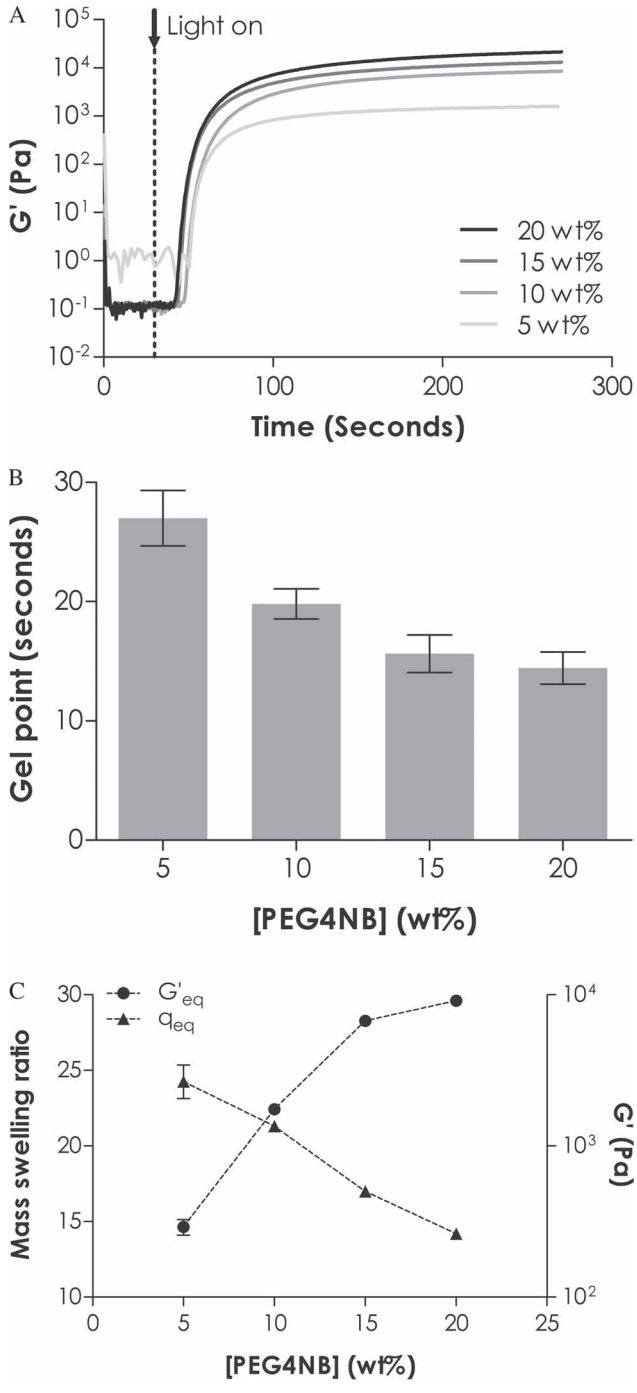

Figure 2. (A) Effect of macromer (PEG4NB) content on the gelation kinetics and (B) gel point. (C) Mass swelling ratio and elastic modulus of hydrogels at equilibrium swelling (Mean ± S.D., $N = 3$). Error bars in (A) were omitted for clarity.

in Figure 2C, the equilibrium mass swelling ratios and shear moduli of these step-growth hydrogels exhibited high dependency on macromer concentration, indicating the existence of network nonideality.[25] We have recently reported a similar trend in UV-mediated step-growth thiol-ene networks.[23] Furthermore, decreased network cross-linking efficiency was observed with thicker gel samples (3 mm), evidenced by decreased gel fractions and increased equilibrium swelling at lower macromer contents (Table S2, Supporting Information). We believe that this was due to higher light attenuation caused by red eosin-Y.

Next, we evaluated the cytocompatibility of these visible-light-mediated thiol-ene hydrogels using hMSCs (encapsulated at 5×10^6 cells/mL). Our results revealed that visible-light-mediated thiol-ene hydrogels were highly cytocompatible for hMSCs following photoencapsulation (Figure 3A and S5, Supporting Information, ≈95% initial viability determined by live/dead staining) and prolonged in vitro culture (Figure 3B). On the other hand, the viability of hMSCs encapsulated in conventional visible-light-mediated chain-growth PEGDA hydrogels was relatively low and declined rapidly as time (Figure 3A and B). Although one may argue that the higher hMSC viability in thiol-ene hydrogels could be a result of hydrolytic degradation of

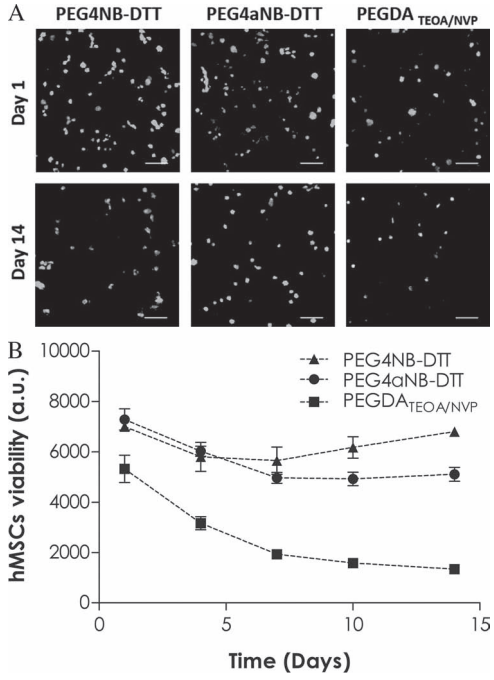

Figure 3. Cytocompatibility of visible-light-mediated thiol-ene photopolymerizations. (A) Representative confocal z-stack images of hMSCs stained with live/dead staining kit on day 1 and 14. hMSCs were encapsulated (5×10^6 cells/mL) in step-growth degradable PEG4NB-DTT (left column) or non-degradable PEG4aNB-DTT (middle column) hydrogels, as well as chain-growth nondegradable PEGDA hydrogels (right column). All gel were fabricated with 10 wt% PEG macromer, 1×10^{-3} M CRGDS, and 0.1×10^{-3} M eosin-Y. In chain-growth PEGDA photopolymerization, TEOA (0.75 vol%) and of NVP (0.1 vol%) were added to facilitate gelation. (Scale: 100 μm). Color images are available in the Supporting Information (Figure S5, Supporting Information) or upon request. (B) hMSCs viability measured by Alamarblue® reagent (Mean ± S.D., $N = 3$).

PEG4NB hydrogels,[23] our controlled experiments using hydrogels crosslinked by an amide-linked, nondegradable PEG4aNB macromer also supported higher degree of hMSC survival as compared with the chain-growth PEGDA hydrogel (Figure 3B). Interestingly, it was reported that visible-light-mediated chain-growth PEGDA gels supported survival of hMSCs.[26] In that particular study, however, the cells were encapsulated at an extremely high density (25×10^6 cells/mL), which might promote cell survival due to paracrine signaling. We also examined the cytocompatibility of hydrogels crosslinked by different concentrations of eosin-Y (0.1×10^{-3} and 1×10^{-3} M) but did not find significant cellular damage even at high eosin-Y concentration (Figure S3, Supporting Information).

An early and important application of visible-light-mediated chain-growth PEGDA hydrogels was the formation of conformal coating for isolated islets.[11] We were interested in comparing the cytocompatibility of our visible-light-initiated thiol-ene gels with the conventional PEGDA system by pancreatic β-cell encapsulation. We encapsulated radical sensitive MIN6 β-cells at 5×10^6 cells/mL in both systems and found that cell viability was significantly higher in the visible-light-initiated thiol-ene gels than the PEGDA system (Top panel, Figure S4A, Supporting Information). Furthermore, MIN6 β-cells formed spherical aggregates only in the thiol-ene hydrogels but not in conventional PEGDA hydrogels (Bottom panel, Figure S4A and Figure S4B, Supporting Information). We have recently reported a similar result using UV-mediated thiol-ene photopolymerization.[20] We believe the significant cell death (for both hMSCs and MIN6) in the chain-growth PEGDA system was a collective result of high concentrations of radical species,[27] formation of dense hydrophobic polyacrylate kinetic chains, and the potential cytotoxicity from TEOA and NVP.

4. Conclusion

In summary, we have demonstrated an innovative approach for forming thiol-norbornene hydrogels by visible-light-mediated, eosin-Y-initiated-photoclick reactions. This gelation scheme preserves the rapid and efficient step-growth network cross-linking without the use of cytotoxic coinitiating components, thus ensuring high cytocompatibility for hMSCs and MIN6 β-cells. We believe that this gelation mechanism represents a significant improvement over existing visible-light-mediated gelation systems and should be of great interest to the field of biomaterials and regenerative medicine.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Visible light-mediated thiol-ene hydrogelation using eosin-Y as the only photoinitiator

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(Supporting Information)

1. Experimental Section

1.1. Synthesis of PEG macromers

Poly(ethylene glycol)-tetra-norbornene (PEG4NB) was synthesized by reacting 4-arm PEG with 5-norbornene-2-carboxylic acid (5-fold excess to hydroxyl group) in anhydrous dichloromethane (DCM) using N,N'-dicyclohexylcarbodiimide (DCC, 2.5-fold excess) as a coupling reagent. Norbornene acid was mixed with DCC and stirred at room temperature for 30 minutes. The resulting norbornene anhydride was filtered into an addition funnel and added to a flask containing 4-arm PEG (20 kDa), 4-(dimethylamino) pyridine (DMAP, 0.5-fold excess), and pyridine (5-fold excess) in DCM. The flask was kept in an ice bath and reacted for overnight. The product was washed with 5 % sodium bicarbonate solution twice and brine once, followed by precipitation in cold ethyl ether. The product was re-dissolved in minimum amount of DCM and re-precipitated in cold ether.

Poly(ethylene glycol)-diacrylate (PEGDA) was synthesized through reacting linear PEG (10 kDa) with acryloyl chloride (4-fold excess to hydroxyl group) in the presence of triethylamine (4.4-fold excess) in dry toluene. After overnight reaction, the solution was filtered through a thin layer of neutral aluminum oxide. Sodium carbonate was added to the solution and

the heterogeneous solution was stirred for 2 hours in the dark. The solution was then filtered through Hyflo filtration aid and the clear solution obtained was precipitated in cold ether.

1.2. Microwave-assisted solid-phase peptide synthesis (SPPS)

CRGDS peptide was synthesized in a microwave-assisted peptide synthesizer (CEM Discover SPS) using standard Fmoc-based solid phase peptide synthesis. Cleaved peptide was precipitated in cold ether, dried in vacuo, and purified by reverse phase HPLC (PerkinElmer Flexar system). Peptide identity was confirmed by mass spectrometry (Agilent Technologies Model 1200-6520). Analytical HPLC was used to confirm high purity of the peptides (>90%).

1.3. Gel fraction determination

Step-growth thiol-ene hydrogels were formed by radical-mediated photopolymerization using 0.1 mM of eosin-Y as the photoinitiator. The gelation was achieved with a fiber optic microscope illuminator (AmScope) at an intensity of 700 Lux (~ 10 mW/cm^2 at 515 nm). To quantify gel fraction, circular hydrogel discs were prepared from 50 μL precursor solution. Hydrogels were dried in vacuuo immediately after gelation to obtain dry polymer weight ($W_{Initial}$), which contains both sol and gel fractions. The polymers were then incubated in ddH_2O at 37 ^oC on an orbital shaker for 24 hours to remove sol fraction. Gels were dried again and weighed to obtain crosslinked polymer weights (W_{Dry}). Gel fraction was defined as the percent ratio of W_{Dry} to $W_{Initial}$.

1.4. MIN6 cell encapsulation and cytocompatibility

Desired density (5×10^6 cells/mL) of mouse insulinoma cells (MIN6) were suspended in polymer solutions (PEG4NB/DTT or PEGDA/TEOA/NVP representing step-growth thiol-ene and chain-growth hydrogels, respectively). Cell-laden hydrogels (25 μl) were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS, Gibco), 50 μM β-

mercaptoethanol (Sigma), and 1X antibiotic-antimycotic (Invitrogen). To quantify long-term cell viability, cell-laden hydrogels were incubated in 500 μL Almarblue® reagent (AbD Serotec, 10% in cell culture medium) at 37^{o}C and 5% of CO_2. After 14 hours of incubation, 200 μl of media were transferred to a 96-well plate for fluorescence quantification (excitation: 560 nm and emission: 590 nm) in a microplate reader (BioTek, Synergy HT).

Table S1: Characteristics of hydrogels formed by visible light-mediated thiol-ene photopolymerization. (10 wt.% PEG macromer and 0.1 mM of eosin-Y for all conditions. 0.75 vol.% TEOA and 0.1 vol.% NVP added for PEGDA gelation, N = 3, mean ± S.D.)

Intensity ($mW/cm^2_{@515\ nm}$)	Macromer system	Gel point (seconds)	$G'_{@\ 600\ sec}$ (kPa)
3.5*	PEG4NB-DTT	366 ± 19	0.15 ± 0.04
8.5*	PEG4NB-DTT	114 ± 3	1.9 ± 0.5
10	PEG4NB-DTT	19 ± 2	12 ± 1.5
	$PEGDA_{TEOA/NVP}$	37 ± 1	17 ± 1.6

* At 600 seconds, this intensity did not yield complete gelation.

Table S2: Effect of gel thickness on gel fraction and equilibrium swelling ratio of hydrogels formed by visible light-mediated thiol-ene photopolymerization. (10 wt.% PEG4NB-DTT, N = 3, mean ± S.D.)

[PEG4NB] (wt.%)	Gel fraction (%)		Swelling ratio	
	Gel thickness (mm)		Gel thickness (mm)	
	1	3	1	3
5	94.0 ± 2.1	80.6 ± 3.1	24.3 ± 1.9	32.7 ± 0.9
10	99.3 ± 1.2	90.5 ± 3.3	21.3 ± 0.1	23.3 ± 0.8
15	98.4 ± 0.6	93.2 ± 3.6	16.9 ± 0.8	18.5 ± 0.7
20	98.7 ± 0.6	95.8 ± 1.3	14.2 ± 0.1	17.4 ± 0.6

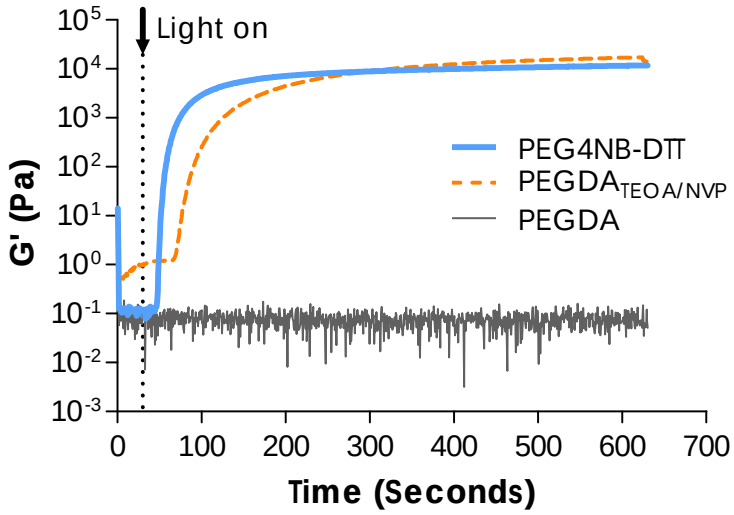


Figure S1: *In situ* photo-rheometry of PEG hydrogels formed by visible light-mediated photopolymerizations (step-growth PEG4NB-DTT or chain-growth PEGDA hydrogels). Visible light (700 Luz ~ 10 mW/cm^2 at 515nm) was turned on at 30 seconds. Gel compositions: 10 wt.% PEG macromer and 0.1 mM eosin-Y for all gel formulations. 0.75 vol.% TEOA and 0.1 vol.% NVP added for chain-growth PEGDA gelation. (N = 3; error bars are omitted for clarity)

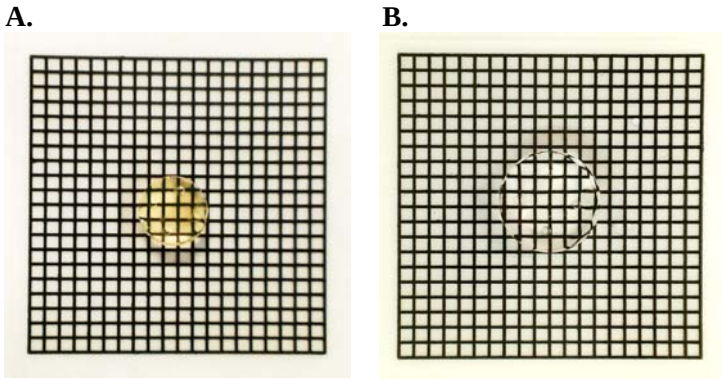


Figure S2: Photographs of visible light-cured thiol-ene hydrogels (A) before and (B) after swelling for 24 hours (5 wt.% PEG4NB with DTT as crosslinker, 0.1 mM eosin-Y as the initiator, total length of the grid = 2cm).

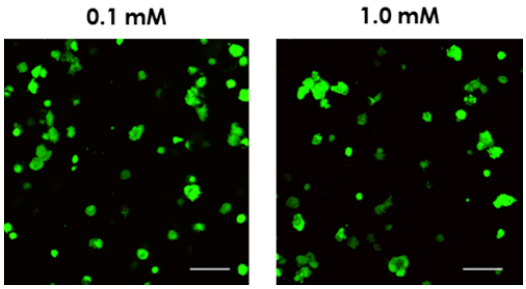
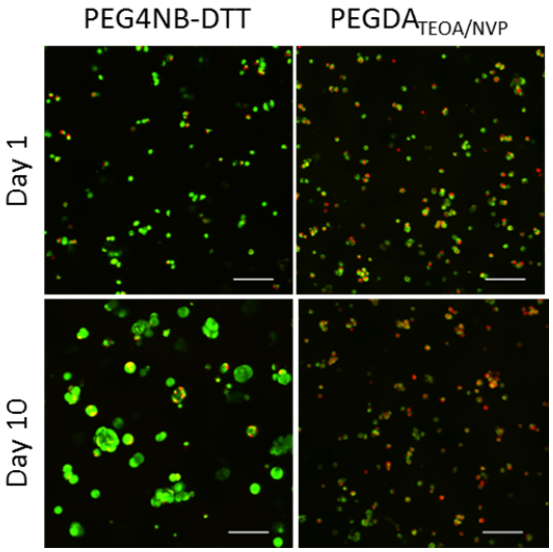


Figure S3: Confocal z-stack images of hMSCs stained with Live/Dead staining kit (day 1 post-encapsulation). hMSCs were encapsulate in 10 wt.% PEG4NB-DTT hydrogels crosslinked using 0.1 or 1.0 mM eosin-Y (cell packing density: 5×10^6 cells/mL , scale: 100 µm).

A.



B.

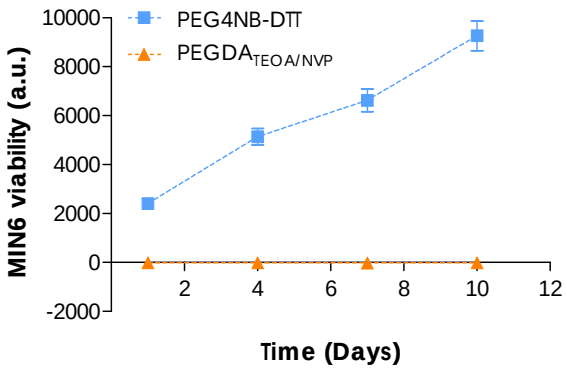


Figure S4: (A) Confocal z-stack images of MIN6 cells stained with Live/Dead staining kit. Cells were encapsulated in PEG4NB-DTT or PEGDA hydrogels using 0.1 mM eosin-Y (scale: 100 µm). (B) MIN6 viability quantified by Alamarblue® reagent. (10 wt.% PEG hydrogels, cell packing density: 5×10^6 cells/mL, 0.75 vol.% TEOA and 0.1 vol.% of NVP were used in PEGDA hydrogels, N = 3, mean ± SD)

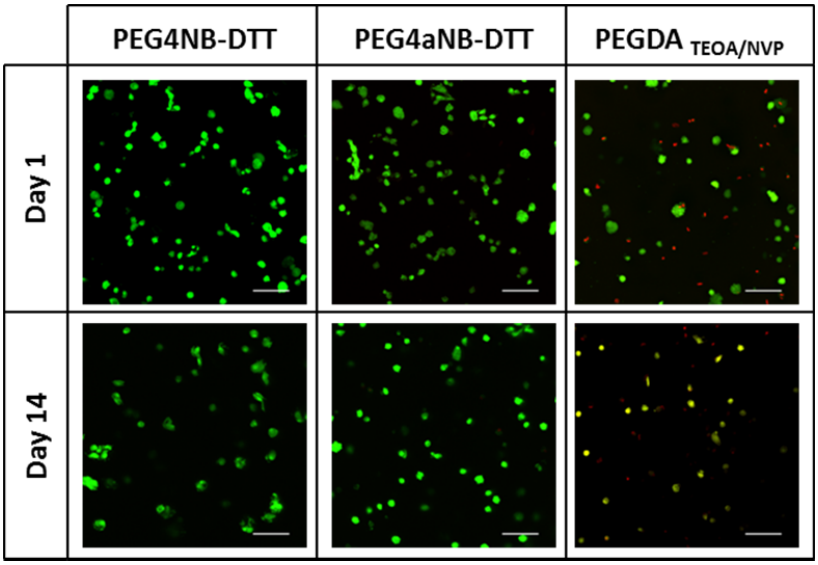


Figure S5: Representative confocal z-stack images of hMSCs stained with Live/Dead staining kit on day 1 and 14. hMSCs were encapsulated (5×10^6 cells/mL) in step-growth degradable PEG4NB-DTT (left column) or non-degradable PEG4aNB-DTT (middle column) hydrogels, as well as chain-growth non-degradable PEGDA hydrogels (right column). All gel formulations contained 10 wt.% PEG macromer, 1 mM CRGDS, and 0.1 mM eosin-Y. In chain-growth PEGDA photopolymerization, TEOA (0.75 vol.%) and of NVP (0.1 vol.%) were added to facilitate gelation. (Scale: 100 μm).