Visible light-mediated multi-scale thiol-ene hydrogels for 3D cell culture

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Statement of Purpose: Designing functional hydrogel scaffolds with tunable and predictable properties have been a major challenge facing tissue engineering. Recently, UV-mediated thiol-ene photo-click hydrogels have emerged as a versatile material platform for studying cell fate processes and for repairing damaged tissues.[1-3] The utility of thiol-ene hydrogels can benefit from a visible light-mediated mechanism that permits rapid gelation while prevents the use of potentially cytotoxic co-initiating components.[4] Here, we report a visible light-mediated step-growth gelation scheme for preparing cytocompatible thiol-ene photo-click hydrogels using eosin-Y as the only photoinitiator. In addition to investigating the gelation kinetics, we also demonstrated the wide applicability of this material platform with the encapsulation of human mesenchymal stem cells (hMSCs) and pancreatic MIN6 β-cells.[5] Furthermore, we introduced a simple yet highly tunable approach to form multilayer step-growth hydrogels via an interfacial thiol-ene photopolymerization mechanism.

Methods: Poly(ethylene glycol)-tetra-norbornene (PEG4NB, 20kDa) and di-thiol containing crosslinkers (dithiothreitol, DTT) were used to form hydrogels via a visible light-mediated (400-700 nm, 70,000 Lux, 4 min) step-growth photopolymerization with eosin-Y (0.1 mM) as the sole initiator. For comparison, PEG-diacrylate (PEGDA, 10kDa) hydrogels were prepared with a three-component initiator system containing 0.1 mM eosin-Y, 0.1 vol% N-vinyl-pyrrolidinone (NVP) and 0.75 vol% of triethanolamine (TEOA). Gelation kinetics, gel points, and elastic moduli were monitored by *in situ* photo-rheometry. Encapsulated hMSCs or MIN6 β-cells were stained with live/dead staining kit at day 1 to evaluate the cytocompatibility of the hydrogels. Dual-layer gels were formed by exposing visible light on pre-formed PEG4NB-DTT gels immersed in a macromer solution containing only PEG4NB and DTT. The dual-layer gel was imaged by a fluorescence microscope.

Results: Figure 1A shows the mechanism of visible light-mediated thiol-ene photopolymerization. Briefly, visible light sensitizes eosin-Y to extract hydrogen from thiols to form thiyl radicals, which initiate thiol-ene photo-click reactions and gelation. The visible light mediated thiol-ene gelation reached a faster gel point at 19±1 seconds, which was twice as fast as the conventional chain-growth PEGDA hydrogels (37±1 seconds). The final moduli of these gels were ~8 kPa after 4 minutes of light exposure (Figure 1B). In addition, the resulting thiol-ene hydrogels were more cytocompatible to hMSCs and sensitive MIN6 β-cells (Figure 1C and 1D, ~90 and ~80% initial cell viability for hMSCs and MIN6 cells, respectively). Furthermore, rapid step-growth thiol-ene gelation permitted homogeneity in cell seeding throughout the gels (Figure 1C and 1D, bottom panels).

We also found that eosin-Y retained its excitability to re-initiate thiol-ene photo-click reactions. This feature was explored to form a dual-layer step-growth hydrogel (Figure 2A). The thickness of the second gel layer was easily controlled by altering the concentration of eosin-Y, secondary light exposure time (Figure 2B), or macromer concentration in the coating solution (Figure 2C).

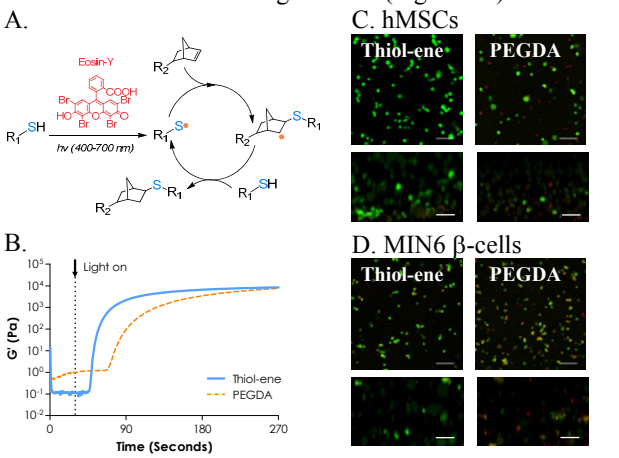

Figure 1. (A) Schematics of visible light-mediated step-growth thiol-ene photo-gelation using eosin-Y only. (B) *In situ* photo-rheometry. Confocal z-stack images of (C) hMSCs and (D) MIN6 cells-laden PEG4NB-DTT and PEGDA hydrogels stained with Live/Dead staining kit. (Top images: XY-view. Bottom images: XZ-view, scale: 100 μm)

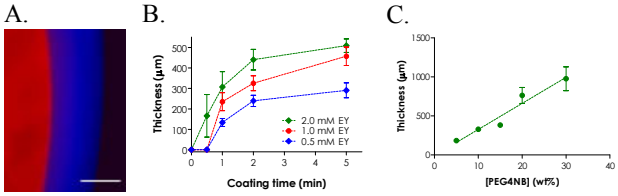

Figure 2. (A) Dual-layer thiol-ene gel (scale: 200 μm). (B) Effect of eosin-Y concentration, secondary light exposure time and (C) macromer content on gel coating thickness.

Conclusions: We have prepared cytocompatible step-growth thiol-ene hydrogels by visible light-mediated photo-click reactions. This gelation scheme produces gels with rapid crosslinking kinetics and improved cell seeding homogeneity over the conventional visible light-mediated chain-growth PEGDA hydrogels. In addition, the re-excitability of eosin-Y simplifies the process of forming multi-layer hydrogels. This material platform can be used for sequential delivery of multiple growth factors to promote vascularization. In sum, this gelation scheme represents an improvement over existing visible light-mediated gelation systems and should be of great utilities in scaffolding design for tissue engineering and regenerative medicine applications.

References: [1] Lin CC, Raza A, Shih H. *Biomaterials*. 2011;32:9685-95. [2] Shih H, Lin CC. *Biomacromolecules*. 2012;13:2003-12. [3] McCall JD, Anseth KS. *Biomacromolecules*. 2012;13:2410-7. [4] Sawhney AS, Pathak CP, Hubbell JA. *Biomaterials*. 1993;14:1008-16. [5] Shih H, Lin CC. *Macromolecular Rapid Communications*. 2012. doi: 10.1002/marc.201200605