Interfacial thiol-ene photochemistry for forming orthogonal immuno-isolation coating on pancreatic islets

Han Shih[1], and Chien-Chi Lin[1,2]
[1] Weldon School of Biomedical Engineering, Purdue University, West Lafayette, IN 47907, USA
[2] Department of Biomedical Engineering, Purdue School of Engineering and Technology, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, USA

Statement of Purpose: Type I diabetes mellitus (T1DM) is an autoimmune disease caused by the destruction of insulin-producing β-cells by auto-reactive T-cells. Although islet transplantation holds the promise of permanently reversing T1DM, significant shortage of donor islets, as well as the need for lifelong immunosuppressant therapy limits the clinical prevalence of this approach. An effective microencapsulation strategy should serve as an immuno-isolation barrier to protect the transplanted islets from host immune response.[1] Recently, our lab introduced a visible light-mediated step-growth gelation scheme for preparing highly cytocompatible hydrogels. Apart from the photoinitiator eosin-Y and di-thiol-containing cross-linker, no additional cytotoxic co-initiator or co-monomer is required to achieve rapid gelation.[2] Here, we capitalize on this new visible light-initiated thiol-ene photo-polymerization scheme and prepare orthogonal interfacial conformal coatings on pancreatic MIN6 β-cell aggregates and isolated murine islets.

Methods: MIN6 β-cell aggregates or isolated mouse islets were stained with photoinitiator eosin-Y and incubated with PEG-di-thiol (PEGdSH). After mild washes, stained aggregates/islets were immersed in a solution containing 8-arm poly(ethylene glycol)-norbornene (PEG8NB, 20kDa) and crosslinker dithiothreitol (DTT). Interfacial thiol-ene photocrosslinking was initiated by visible light (400-700nm) exposure. Macromer M.W. and light exposure time were controlled to tune the thickness of the conformal coating, which was characterized by image analysis (n > 200). Coated islets were stained with live/dead staining kit and imaged with confocal microscope to evaluate the cytocompatibility of the coating chemistry. Glucose-responsive insulin secretion (GSIS) from coated or non-coated islets was conducted and the amounts of insulin secretion were quantified by mouse insulin ELISA kit.

Results: Figure 1A shows the scheme of visible light-mediated step-growth thiol-ene interfacial coating. A thin layer of thiol-ene hydrogel coating was obtained after 30 seconds of visible light exposure (Figure 1B) and the thickness of the coating could be controlled by altering molecular weight of PEGdSH, PEG8NB concentration, and polymerization time (between 13.0±0.6μm to 43.3±0.8μm, Figure 1C). We also utilized this interfacial thiol-ene photochemistry to prepare conformal coating on isolated islets (Figure 2A). Live/dead staining result showed a few dead cells on the surface of the islets (Figure 2B). However, the coated islets preserved their ability to secrete insulin to a level comparable to the non-coated islets after 1 day of culture *in vitro* (Figure 2C), indicating the coating process did not damage the critical cellular function.

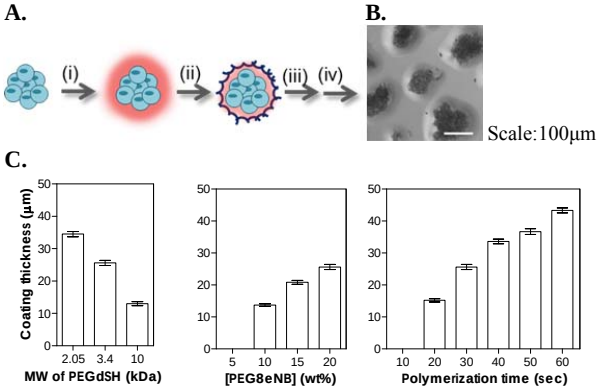

Figure 1. (A) Schematic of interfacial thiol-ene photochemistry. (i) β-cell aggregates were stained with eosin-Y, (ii) incubated with PEGdSH, (iii) washed with HBSS, and (iv) suspended in PEG8NB-DTT solution, followed by visible light-initiated thiol-ene photopolymerization. (B) Thiol-ene gel coated MIN6 β-cell aggregates. (C) Effect of PEGdSH molecular weight, PEG8NB concentration, and polymerization time on thiol-ene coating thickness.

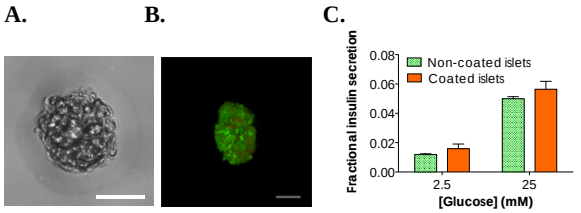

Figure 2. (A) Phase-contrast and (B) confocal image of live/dead stained coated islets. (C) Glucose-stimulated insulin secretion from coated or non-coated islets. (20wt% PEG8NB-DTT, day 1, scale: 100 μm)

Conclusions: In summary, step-growth immuno-isolation barriers on pancreatic β-cell aggregates and islets were successfully formed by interfacial thiol-ene photochemistry. This conformal coating strategy has high tunability in coating thickness and preserves the viability and function of the coated islets. Current work is focusing on transplanting coated islets in diabetic mice, as well as determining the long-term efficacy of the transplanted coated islets on maintaining euglycemia.

References: [1] Sawhney AS. Biomaterials 1993;14:1008-16. [2] Shih H. Macromolecular Rapid Communications 2013; 34: 269-73. [3] Shih. ACS Applied Materials and Interfaces 2013; 5: 1673-80.