Tetrahydropyran triazole phenyl-alginate and quaternized phosphocholine-chitosan conformal coating on human islets: an *in vitro* study on cell viability

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INTRODUCTION

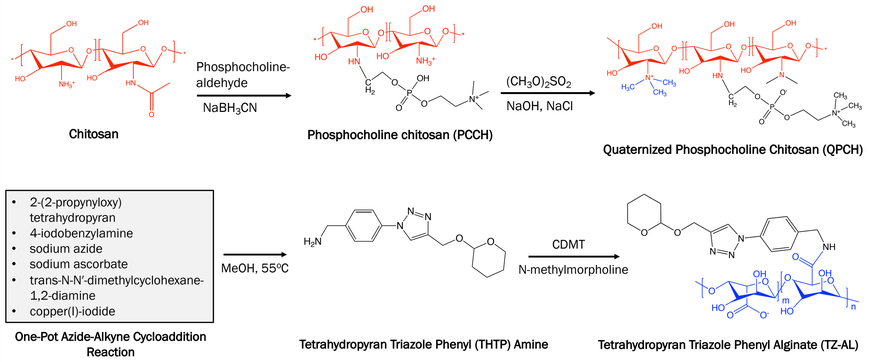
Pancreatic islet transplantation has been recently sought after as a promising alternative option for long-term treatment of Type 1 Diabetes (T1D) (1). One of the key components towards successful islet transplantation is mitigating the foreign body immune response induced by donor islets. This immune response can cause graft failure and requires administration of immunosuppressant drugs long term, which results in adverse side-effects. (2,3)

The main strategies used to tackle this challenge involve the formation of an immunoprotective barrier using biomaterials that can maintain the cell function and viability while shielding from pro-inflammatory immune reactions. (4) A key feature of such barrier must include minimizing implant volume to make sure cells can have adequate diffusion of nutrients, waste, and secreted hormones.(1) Conformal coatings have been shown in the literature to exhibit these properties by providing a nanoscale barrier between cells and their environment with adequate stability and immunoprotection. (5)

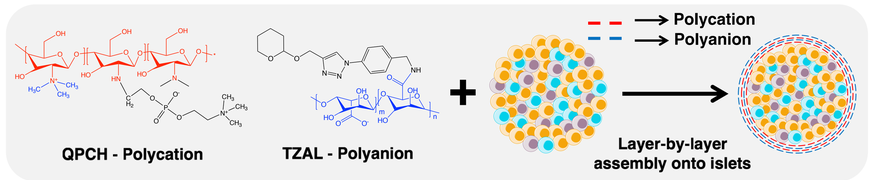
Here we have demonstrated the use of a novel combination of non-immunogenic materials, namely tetrahydropyran triazole phenyl-alginate (TZ-AL) (6) and quaternized phosphocholine-chitosan (QPCH), for multilayer film formation onto pancreatic islet surface and evaluated the subsequent metabolic and immunological activities of those islets *in vitro*. Ultimately the outcome of this research project aims to promote the use of islet transplantation as a standard clinical treatment option for T1D.

METHODS

Polymer Synthesis



Coating Deposition



Characterization

Polymer Analysis

- ^{1}H-NMR and ^{31}P-NMR to confirm polymer structure and purity.
- Macrophage Activation Assay using M0 macrophages derived from THP-1 monocytes to study the immunomodulatory properties of polymers.

Coated Islet Characterization

- Confocal microscopy to show coating deposition on islet cell surface.
- TEM used to study coating thickness, and morphology.
- Glucose-stimulated insulin secretion (GSIS) assay to assess the secretory function of coated islets.
- Live/Dead staining to assess cell viability of coated islets.

RESULTS

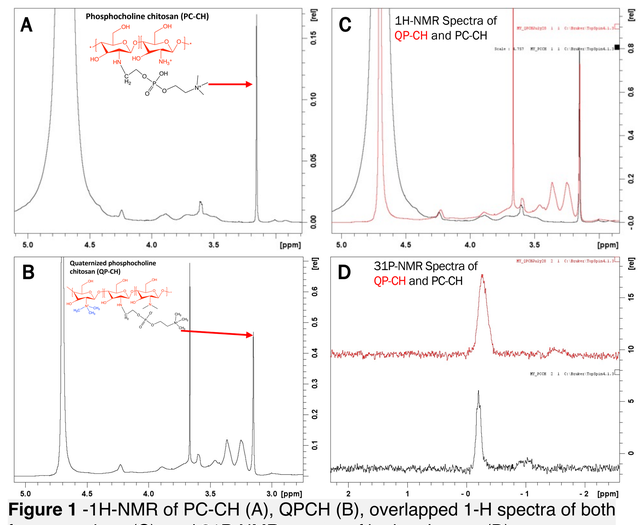


Figure 1 -1H-NMR of PC-CH (A), QPCH (B), overlapped 1-H spectra of both for comparison (C), and 31P-NMR spectra of both polymers (D)

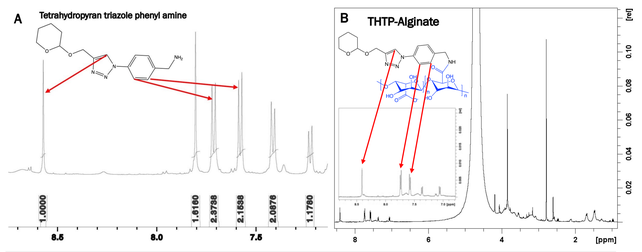


Figure 2 - 1H-NMR of THTP amine ligand (A) and THTP-Alginate (B)

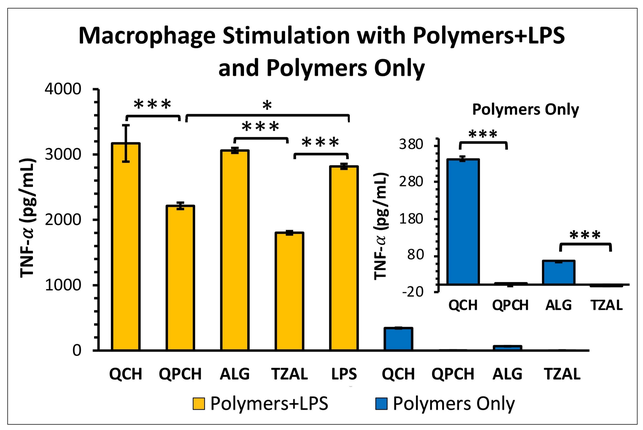


Figure 3 - ELISA quantification of TNF-α secretion from THP-1-derived macrophages stimulated using polymers with and without added LPS. Insert shows values for stimulation with polymers alone.*** $P < 0.005$, * $P < 0.05$

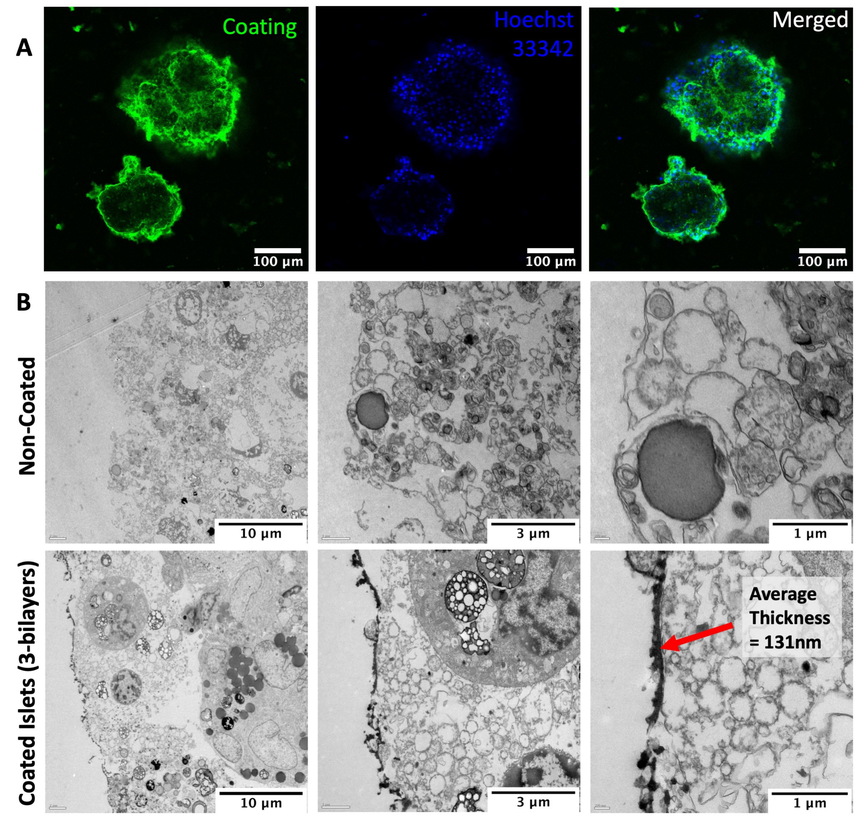


Figure 4 – Characterization of coating on islets (3-bilayer) using confocal microscopy with FITC-alginate as part of the bi-layer and Hoechst 33342 dye for staining the cell nucleus. (A). TEM analysis of coating thickness and morphology on donor human islets (B).

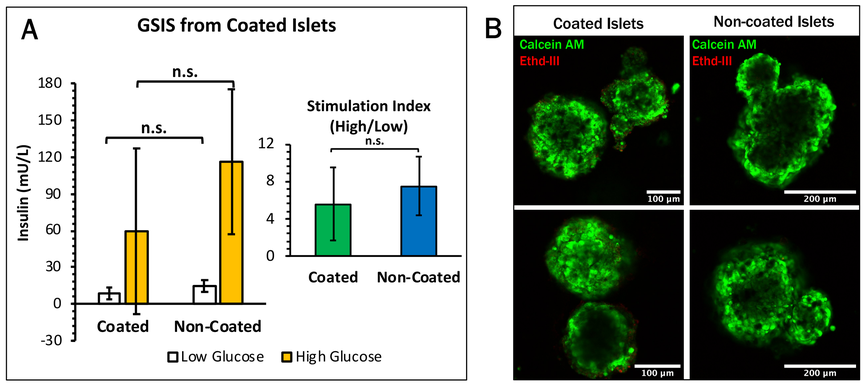


Figure 5 – ELISA quantification of glucose-stimulated insulin secretion (GSIS) from coated and non-coated islets and their stimulation index (high/low ratio) shown as an insert (A). Live/Dead assay of coated and non-coated islets with live cells indicated as green and dead cells indicated as red (B). n.s = $P > 0.05$

CONCLUSION

The study demonstrated successful synthesis of proposed polymers and successful coating deposition on islet surface with a thickness of ~130nm. Coated islets were shown to maintain cell viability and insulin secretion. The proposed polymers also show promising results towards immunomodulation of macrophages. Current experiments are assessing long-term cell viability of coated islets as well as assays to measure other immune markers.

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