Covalent Layer by Layer (CLbL) Assembly of Hydrogel Coatings via Chemoselective Staudinger Ligation

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Introduction

The encapsulation/immunoisolation of cells within semi-permeable membranes has many potential applications in tissue engineering. Of particular interest has been the encapsulation of insulin-secreting cells for the long-term treatment of diabetes. While encapsulation is capable of providing protection of donor cells from the host immune system, most microencapsulation approaches results in capsules 300-800µm in diameter. Unfortunately, the relative size of these coatings imposes consequential mass transport limitations and produces transplant volumes not suitable for infusion into the portal vein of the liver. New approaches such as conformal coating, selective withdrawal, and layer-by-layer assembly can generate polymer coatings from 5-50µm. Particular challenges in fabricating thin coatings are incomplete encapsulation, coating defects, and layer stability. In our laboratory, we have developed functionalized polymers capable of forming stable, covalent linkages via Staudinger Ligation, a spontaneous, chemoselective, and cell-compatible reaction between an azide and phosphine. In this study, we sought to translate these polymers to form covalent layer by layer (CLbL) coatings for cellular encapsulation. Herein, we describe the formation of nano-thin coatings using hetero-bifunctional and multi-arm poly(ethylene glycols) (PEGs) and/or multi-functional alginates.

Materials and Methods

Purity, functionalization, and characterization of the polymers were assessed using attenuated ATR-FT-IR spectroscopy, mass spectroscopy, and ^{1}H-NMR spectroscopy. NHS-PEG-Azide **[1]** was fabricated from NH2-PEG-COOH. 4-arms-phosphine **[2]** and 8-arms-phosphine PEG **[3]** were fabricated from 4-arm and 8-arm amine-PEG, respectively. Azide-alginate **[4]** and azide-alginate-FITC **[5]** were fabricated via substitution of the carboxylic acid on the alginate backbone with an azide and/or FITC via aqueous carbodiimide chemistry. (further details in polymer synthesis in *ref. 1&2)*. Nano-scale layers were formed on idealized aminated or azide surfaces, either glass microbeads or silicon wafers. The initial layer was formed via incubation with NHS-PEG-azide **[1]** in PBS for 45 mins or azide silane on glass. Subsequent layers were formed via step-wise incubation with complimentary polymers (ie **[2 or 3]** followed by **[4]**) for 1-3 hrs. Characterization of layer formation was achieved via confocal fluorescent imaging of **[5]**, or ellipsometry (dried wafers). Surface uniformity and roughness was assessed via atomic force microscopy (AFM). Islets were isolated from Lewis rats using standard methods and incubated in polymers in full culture media, with the exception of the initial layer **[1]**.

Results

Step-wise incubation of idealized aminated or azide surfaces, beads and silicon wafers, with functionalized, complimentary polymers resulted in the formation of covalently linked nano-scale polymeric layers, as shown in Figure 1. Ellipsometry data illustrate build up of layers from 5 to 18 nanometers, generated via layering of polymers, **[3], [4], [3],** and **[4]** on azide-silane surface. Confocal microscopy images illustrate the uniformity of coating, in addition to the specificity of binding, where unfunctionalized polymers did not exhibit sustained binding. Resulting coatings were highly stable, with no decrease in fluorescent intensity observed following treatment with highly ionic solutions or over the course of 60 days. Co-incubation of polymer solutions with islets resulted in no observational effects of viability, as evaluated via Live/Dead staining.

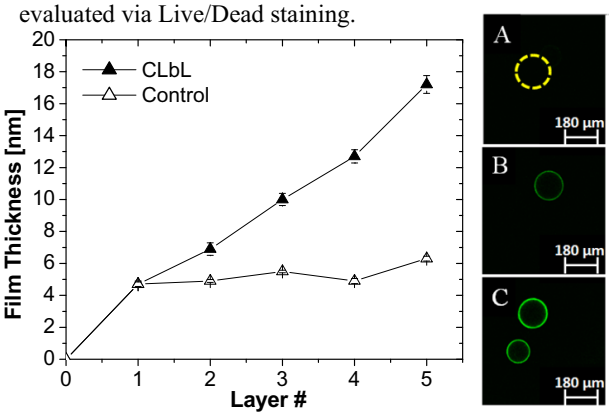

Fig. 1. Left graph: Ellipsometry measurements of polymer film thickness, with alternating layers of **[3]** and **[4]**. Right image: Confocal images of nano-layers (polymers fluorescently labeled) on aminated beads: one layer of Polymer **[1]** (**A);** three total layers of Polymers **[1, 2 & 5] (B);** multiple layers of polymer **[1, 2 & 5] (C).**

Discussion and Conclusions

We have illustrated the capacity of Staudinger functionalized polymers to undergo CLbL assembly on idealized surfaces. The resulting layers exhibit superior strength and stability over ionic LbL assemblies. These properties are likely highly desirable for cellular encapsulation and vivo longevity. Given the generality of the coating procedure, it can benefit numerous areas of cellular transplantation, particularly cells of high metabolic activity, such as islets.

References

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