Covalent layer-by-layer Antioxidant Films for Cellular Encapsulation

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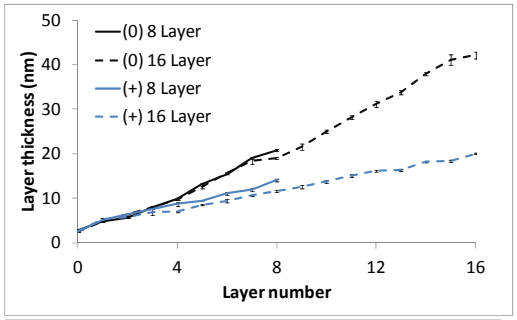
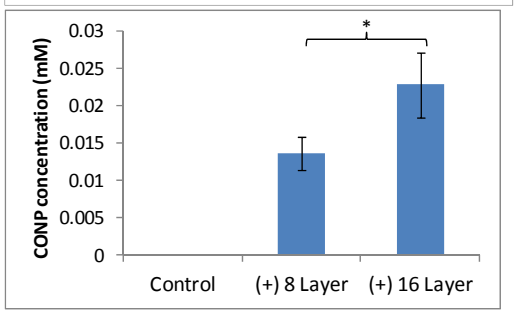
Introduction: Cellular transplantation via tissue engineering has tremendous potential. The long term survival of cellular grafts is commonly impaired due to inflammatory responses and the subsequent free radical generation at the implant site. Engineering a polymeric protective capsule around cells may minimize host responses, while supplementation of coatings with antioxidants may reduce free radical-induced cell death. In this study, we demonstrate, for the first time, the formation of layer-by-layer films, doped with cerium oxide nanoparticles (CONP), a potent, self-renewing antioxidant. Via functionalization of CONP with bioorthogonal linkers, covalently stabilized films with retained free radical scavenging capacity were generated.

Methods: *Fabrication of PAA-CONPs* 1M Cerium (III) Nitrate mixed with 100mM PAA salt (5,100 MW) was added drop-wise to 30% ammonium hydroxide, mixed overnight, and purified via centrifugation, sonication, and filtration. Nanoparticles were characterized by dynamic light scattering (DLS) and 3,3',5,5'- tetramethylbenzidine (TMB) oxidation. *PAA-CONP functionalization* H_2N-PEG-N_3 (PEG MW 300) and carbodiimide chemistry was used to functionalize PAA-CONP with azide (N_3), confirmed by ATR-FTIR and Kaiser's test. *Layer-by-layer assembly* N_3-functionalized silicon wafers were utilized to demonstrate layering of alternate phosphine-functionalized poly(amido amine) (PAMAM) dendrimers and PAA-CONP-N_3 via Staudinger ligation, measured by ellipsometry. PAMAM net charge was modulated with glutaric anhydride to assess effect of charge on film deposition and radical scavenging capacity.

Results: PAA-CONP nanoparticle size (3.5nm average radius) and activity was confirmed by DLS and oxidation of TMB, respectively. Following conjugation of PAA-CONP with azide, a negative Kaiser's test and detection of characteristic ATR-FTIR azide bands confirmed functionalization. PAMAM (G5) dendrimers, functionalized with complementary phosphines for conjugation via Staudinger Ligation, was employed as the interim layer. Both net positive and net neutral (neutralized via GA functionalization) was tested to parse out charge effects on layer formation. Layer-by-layer assembly with PAA-CONP-N_3 with complementary PAMAM-MDT was confirmed, with up to 16 layers covalently deposited. PAA-CONP-N_3 films assembled with net positive surface charged PAMAM-MDT resulted in tighter binding, likely due to the presence of both covalent and electrostatic interactions. Film assembly with surface neutral PAMAM-MDT was achieved, but resulting film layers were nearly two-fold thicker. This is likely due to steric hindrance and the absence of attractive electrostatic forces between layers.
Antioxidant reactivity of CONP within films was retained, as PAMAM-CONP films oxidized TMB in a concentration-dependent manner, with 16-layer films demonstrating nearly twice the CONP concentration as 8-layer films (*P<0.05).





Conclusion: Effective layer-by-layer assembly of azide-functionalized CONP nanoparticles and phosphine-functionalized PAMAM dendrimers was established, demonstrating potential for use as a protective coating for encapsulated cells, as well as implants. Positively-charged PAMAM-CONP layers demonstrated potent antioxidant activity. Further studies are necessary to evaluate antioxidant behavior of these layer-by-layer films.