Polymeric Nanostructure Layer-by-Layer Networks: Structural and Bio-functional relevance as Synthetic Mucus

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Statement of Purpose: Mucin, a key salivary film-forming glycoprotein in the oral cavity, provides a lubricating barrier that maintains hydration and protects the oral mucosa. When this layer is missing, patients may develop a perceptive feeling of dryness (e.g., xerostomia) with increased susceptibility to oral infections (e.g., dental caries, oral candidiasis) [1]. It is hypothesized that by synthetically recreating the lost mucus barrier the beneficial effects of naturally occurring mucus can be restored in these patients. By performing a series of complimentary mouth rinses of the protein streptavidin and biotin-functionalized polymeric nanocarriers (biotin-PNC), a layer-by-layer (LBL) 3-dimensional porous network (PNC-LBL) can be deposited over the oral mucosal surface. The self-assembled networks structurally resemble natural mucin networks when grown into a mesoscopic functional bio-interface. In order to translate these self-assembled PNC-LBL as synthetic oral mucus replacements, its ease of formation, structural and bio-functional properties need critical evaluation.

Methods: Biotinylated polymeric nanostructures (Biotin-PNC) of filamentous (FM) and spherical (S) morphology were formulated from poly(ethylene glycol)-*block*-poly(lactic acid) diblock copolymers (mPEG-PLA and biotin-PEG-PLA) using a co-solvent/evaporation method. The polyphenolic antioxidant curcumin was loaded into the Biotin-PNCs to serve as a fluorescence indicator and model hydrophobic drug. PNC-LBL networks were developed *in vitro* on multi-well plates by alternating incubations in suspension of Biotin-PNC and solution of streptavidin to form either filamentous (FM-LBL) or spherical (S-LBL) networks. PNC-LBL growth was tracked using fluorescence microscopy (via curcumin). Scanning electron microscopy (SEM) was used to study the network structure, and thickness of FM-LBL networks was measured using confocal laser scanning microscopy (CLSM). Surface hydration was measured using sessile drop contact angle studies on FM-LBL of different network thickness. FM-LBL destabilization and curcumin drug release were studied independently *in vitro* by incubating the FM-LBL under simulated oral salivary (SS) and protease (pronase) environment. Drug release was tracked using fluorescence spectrophotometry, while the network destabilization was measured by tracing radioactive ^{125}I-streptavidin loss from networks.

Results: Filamentous Biotin-PNC possess high projected surface area that provides better ligand (biotin) - receptor (streptavidin) contact. Hence, FM-PNC readily self-assembled into 3-D porous networks through layer-by-layer deposition with streptavidin as compared to spherical micelles, which had a reduced ability to form well-structured networks. As expected, network growth was not observed without biotin-streptavidin interactions. Visualization under SEM and fluorescence microscopy demonstrated the formation of nano-porous FM-LBL network with homogeneous coverage that resembles the nanoscale structure of natural mucus. On the other hand, S-LBL formed inhomogeneous networks with poor coverage. Thickness measurements of FM-LBL using CLSM showed a linear increase in barrier thickness at mesoscopic length scales (Figure 1(a)). The FM-LBL networks showed excellent surface hydration with consistently low contact angle. Further, the FM-LBL showed improved network stability under chemical environment, observed by tracking extent of ^{125}I-streptavidin destabilization with and without network formation. The polymeric PEG chains within the PNCs protected the streptavidin from destabilization and detachment from the network, ensuring structural robustness (Figure 1(b)). Drug release studies from FM-LBL demonstrated diffusion controlled release of curcumin, suggesting networks have remained predominantly intact, confirming their stability.

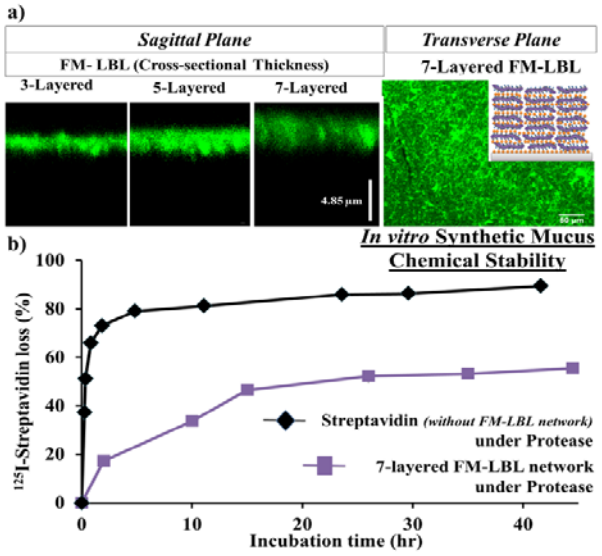

Figure 1: (a) Thickness measurements on filamentous layer-by-layer networks (FM-LBL) using confocal laser scanning microscopy (scale bar ~4.85 µm) and fluorescence microscopy imaging of 7-layered FM-LBL network (scale bar~ 50µm). (b) 7-layered FM-LBL destabilization under protease environment.

Conclusions: The ability to form 3-D porous networks via layer-by-layer self-assembly of polymeric nanostructures was demonstrated. Filamentous Biotin-PNC led to better structured nano-porous networks compared to spherical Biotin-PNC. The combination of excellent surface hydration and stability under simulated oral environment demonstrates the potential of FM-LBL for oral regenerative applications. Further, loading and release of curcumin from the self-assembled network demonstrates its ability to serve as a drug delivery vehicle.

References: Tabak L A. J Oral Pathol Med, 1982:11:1-17.