Exploring Surface Mediated Polymer Electrolyte and Nanostructured Calcium Phosphate Composite (NanoCaPs) Layers for Non-Viral Gene Delivery

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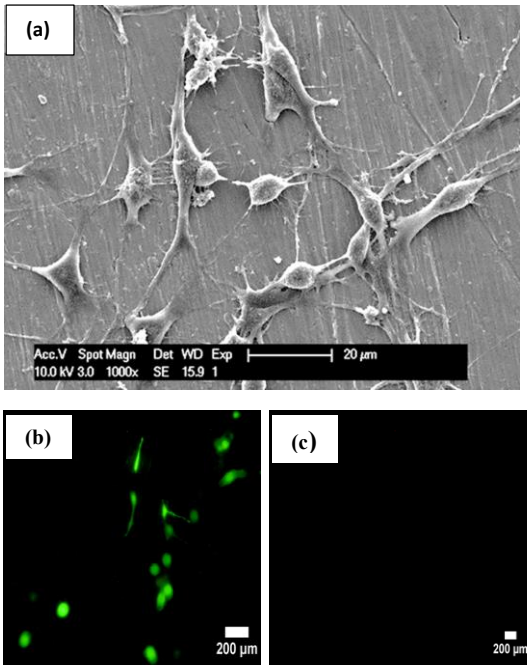
Statement of Purpose: Gene delivery using non-viral techniques are very desirable due to their convenience, ease of manufacturing, cost-effectiveness, and safe characteristics. Lack of a suitable carrier however currently limits the techniques. We have already developed nano-sized calcium phosphates aptly called "NanoCaPs" as a novel highly efficient gene delivery agent for plasmid DNA (pDNA) transfection [1]. Surface mediation using multilayer polyelectrolyte assemblies (MPA) to bind DNA is a nascent but promising approach still largely limited in genetic payloads. We have accordingly developed novel MPA-NanoCaPs-pDNA that display excellent biocompatibility and gene transfection potential. The matrices can also provide controlled release of pDNA creating the potential to provide nucleic-acid based therapeutics closely resembling traditional pharmaceuticals while enabling gene delivery for tissue engineering.

Methods: MPA films were synthesized using the Layer by Layer (LbL) approach using a dip coater. Titanium (Ti) was used as the substrate and MPA matrices were constructed using polycationic polymer (PC) and polyanionic polymer (PA). In a typical experiment, Ti-substrates were dipped in PEI solution to obtain a precursor layer with positive charge to initiate LBL assembly. Polyelectrolyte multilayers were deposited by alternatively dipping Ti-substrates in PA and PC solutions for a specific period and followed by washing with DI water and air drying at each step. Finally, five multilayer coatings i.e. $(PA/PC)_5$ were generated with final layer of PC followed by drying at room temperature and subsequently dipped in NanoCaPs-pDNA-GFP and pDNA-GFP (without NanoCaPs) solutions for a specific period. FTIR was used to confirm the presence of poly electrolytes on Ti and SEM was used for surface characterizations. In-vitro cell viability and cell attachments were evaluated using live-dead staining and SEM. For the transfection experiments, mouse embryonic fibroblast cell line-NIH3T3 cells were plated on different MPA coated substrates. The transfection efficiency of MPA-NanoCaPs-pDNA was compared with MPA-pDNA using fluorescence microscope.

Results: The formation of LbL films on the Ti-substrate with starting precursors was confirmed with FTIR. Live-dead results showed the films are highly cyto-compatible. The SEM results (Fig. 1a) show cell attachment, and normal morphology covering the entire coated surface. Fig 1b and Fig 1c show the cells transfected with pDNA expressing GFP (Fluorescing green) on the MPA-NanoCaPs-pDNA and MPA-pDNA. It is clear from Fig. 1c that MPA void NanoCaPs show no transfection.



(a)SEM micrograph showing attachment and growth of NIH3T3 cells seeded on MPA-NanoCaPs-pDNA composite matrix surface; Fluorescence microscopy of NIH3T3 cells seeded on MPA-NanoCaPs-pDNA (b) and MPA-pDNA (c).

Conclusions: Results indicate that MPA-NanoCaPs-pDNA matrices are cyto-compatible and promising non-viral gene delivery vectors. The study confirms that combination of a biodegradable polymer and pDNA without the addition of a transfecting agent yields an inefficient pDNA transfection into the cell. Therefore, our aim is to fabricate biodegradable and biocompatible MPA containing NanoCaPs for binding and packaging pDNA serving as an excellent transfection agent while also being used to coat different degradable and non-degradable scaffolds for possible soft and hard tissue regeneration.

References: [1]. D. Olton, et al. Biomaterials, 2007. 28(6): p. 1267-1279.