**Enhanced Cellular Adhesion and Controlled Gene Delivery on Glycol-Chitosan-Based Polyelectrolyte Multilayer Films**

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**Statement of Purpose:** Layer-by-layer (LbL) polyelectrolyte (PE) films have been widely investigated for 2D controlled release of drugs, bioactive proteins, and plasmid DNA. Although the naturally-derived polysaccharides chitosan (CHI) and hyaluronic acid (HA) have been incorporated into multilayer films which have successfully transfected several cell lines *in vitro* (1,2), these films display decreased cellular adhesion as the number of bilayers are increased (3), thus suggesting transfection from these films may be far from optimal. Here our group demonstrates that films composed of glycol-modified chitosan (glyc-CHI) exhibit significantly improved cellular adhesion compared to corresponding films consisting of unmodified chitosan. We further demonstrate that embedding cationic PEI-plasmid DNA nanocomplexes within these films allows for sucessful *in vitro* transfection of NIH3T3 fibroblasts. This work serves as a first step towards using these glyc-CHI/HA multilayer films for controlled delivery of various therapeutic genes in 2D and 3D tissue engineering applications.

**Materials and Methods:** LbL polyelectrolyte deposition was used to form glycol-CHI/HA and unmodified-CHI/HA multilayer films composed of 3, 5 and 10 bilayers, with and without embedded PEI-DNA complexes. Film deposition was monitored by quartz crystal microbalance (QCM), while film roughness and hydration properties where characterized via atomic force microscopy (AFM) and water contact angle measurement, respectively. Total serum protein adsorption to the films was assessed via the BCA protein assay (Pierce, IL, USA). MC3T3-E1 murine pre-osteoblast adhesion and viability were analyzed via light microscopy and the MTT assay (Invitrogen, CA, USA), respectively. Bolus and film-based transfection of NIH3T3 fibroblast cells was evaluated via fluorescent microscopy and FACs analysis. The DNA release profile from the multilayer films was characterized via the PicoGreen assay (Invitrogen, CA, USA).

**Results and Conclusions:** MC3T3-E1 cells exhibited increased adhesion and viability on glycol-CHI/HA multilayer films composed of higher numbers of bilayers (see Fig. 1), compared to corresponding umodified-CHI/HA control films. These differences in cell adhesion were predominately due to differences in chemistry, since both types of films displayed similar: thickness, as measured via QCM; wettability, as measured via contact angle; surface roughness and topography, as measured via AFM (see Fig. 2); and protein adsorption, as measured via the BCA assay. Preliminary results have indicated successful *in vitro* transfection of NIH3T3 fibroblasts from glyc-CHI/HA films incorporating PEI-DNA plasmid complexes. Optimization of the transfection efficiency of these multilayers is investigated for various film architectures and complex N:P ratios. The build up, surface roughness and release profiles of these PEI-DNA complex-containing films are also analyzed.

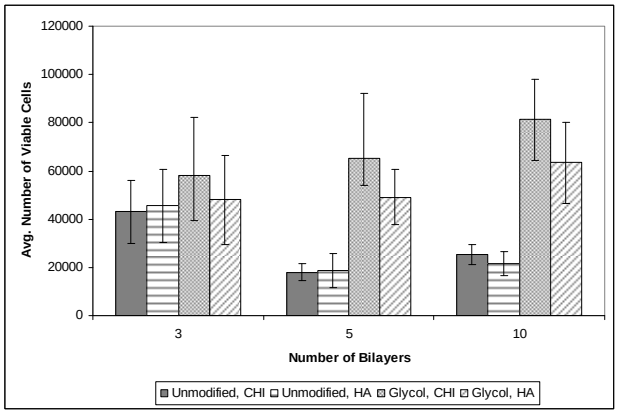


**Figure 1:** MC3T3 cell viability on: $[CHI/HA]_N CHI$ ("Unmodified, CHI"); $[CHI/HA]_N$ ("Unmodified, HA"); $(glyc\text{-}CHI/HA)_N glyc\text{-}CHI$ ("Glycol, CHI"); and $[glyc\text{-}CHI/HA]_N$ ("Glycol, HA") multilayer films, where N= 3, 5 or 10 bilayers.

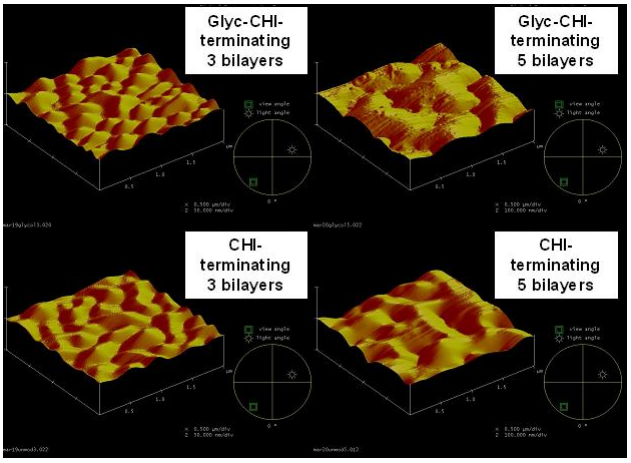


**Figure 2**: AFM imaging of the surface topography of: (top, left) $[Glycol\text{-}CHI/HA]_3 Glycol\text{-}CHI$;(top ,right) $[Glycol\text{-}CHI/HA]_5 Glycol\text{-}CHI$;(bottom, left)$[CHI/HA]_3$ CHI; and (bottom, right) $[CHI/HA]_5 CHI$ films. Scan size= 2 um, Z-range= 100 nm.

**References: (1)** Jessel N. PNAS. 2006: 103(23):8618-21. **(2)** Meyer F. Biochim Biophys Acta. 2006: 1758(3):419-22 **(3)** Schneider A. Biomed. Mater. 2007: 2(1):S45-51.

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