An Investigation of NIH3T3 Cell Adhesion and Transfection on Glycol-Chitosan-Based Multilayered 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. The naturally-derived polysaccharides chitosan (CHI) and hyaluronic acid (HA) have been incorporated into multilayer film architectures which have been employed to successfully transfect several cell lines *in vitro* (1) and (2). However, additional studies have indicated that many cell lines exhibit decreased adhesion to CHI/HA multilayer films, particularly 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 examine the kinetics of this cellular adhesion in comparison to tissue culture plastic. Finally, we demonstrate that embedding plasmid DNA complexed with either PEI or Lipofectamine2000™ within these films allows for sucessful *in vitro* transfection of NIH3T3 fibroblasts and other cell lines. This work serves as a first step towards using these glyc-CHI/HA multilayer films to deliver various therapeutic genes in a spatially- and temporally-controlled manner in 2D and 3D tissue engineering applications.

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 DNA transfection complexes. Film deposition was monitored by quartz crystal microbalance (QCM), while film roughness and hydration properties were characterized via atomic force microscopy (AFM) and water contact angle measurement, respectively. NIH3T3 fibroblast adhesion and viability were analyzed via light microscopy and the MTT assay (Invitrogen, CA, USA), respectively. NIH3T3 cellular adhesion kinetics were examined with an electric cell impedance sensing system (ECIS, Applied Biophysics, NY, USA). Bolus and film-based transfection of NIH3T3 fibroblast cells was evaluated via fluorescent microscopy and FACs analysis (using the BD FACSCalibur™ system). The DNA release profile from the multilayer films was characterized via the PicoGreen assay (Invitrogen, CA, USA).

Results and Conclusions: NIH3T3 cells exhibited increased adhesion and viability on glycol-CHI/HA multilayer films composed of higher numbers of bilayers, compared to corresponding umodified-CHI/HA control films. These differences in cell adhesion were found to be predominately due to differences in film topography, roughness, and chemistry. ECIS analysis revealed that while cellular adhesion is delayed on Glyc-CHI films, it eventually reaches levels similar to tissue culture plastic controls. Preliminary results have indicated successful *in vitro* transfection of NIH3T3 fibroblasts from glyc-CHI/HA films, with Lipofectamine200™ complexes showing higher efficiencies than PEI-DNA plasmid complexes. Various film architectures are currently under investigation to produce different DNA release profiles. The build up, surface roughness and release profiles of these DNA complex-containing films are also currently under detailed analysis.

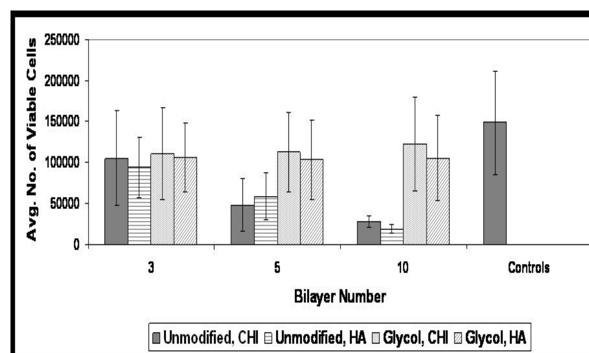

Figure 1: NIH3T3 cell viability on: $[CHI/HA]_N CHI$ ("Unmodified, CHI"); $[CHI/HA]_N$ ("Unmodified, HA"); $(glyc\text{-}CHI/HA)_N glyc\text{-}CHI$ ("Glycol, CHI"); and $[glyc\text{-}CHI/HA]_N$ ("Glycol, HA") multilayer films, where N = 3, 5 or 10 bilayers. Data presented are mean ± STD, n = 9.

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