Chitosan-based polyelectrolyte multilayer films for controlled gene delivery

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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, along with adenoviral and PEI-condesnsed vectors into multilayer films which have demonstrated successful *in vitro* cellular transfection of several cell lines (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. Our group proposes that using glycol-modified chitosan (glyc-CHI) in place of unmodified-CHI will improve the adhesion of MC3T3 pre-osteoblasts and primary murine bone marrow-derived progenitor cells to PE multilayer films, and further enhance their transfection efficiency. Here we characterize the build-up, physical film properties, osteogenic cell adhesion properties, and transfection profiles of 2D glycol-CHI/HA films with (and without) embedded PEI-plasmid DNA complexes. This work serves as a first step towards using these glyc-CHI/HA multilayer films to deliver osteogenic and angiogenic genes in a spatially- and temporally-controlled manner in bone 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 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. Multilayer films were then tested for cell adhesion using mouse pre-osteoblast MC3T3-E1 cells (ATCC, Manassas, VA), and P2-P4 murine bone marrow-derived stromal cells (BMSCs). Cellular adhesion was analyzed via light microscopy and quantified via ImageJ software (NIH, Bethesda, MD). Bolus and film-based transfection of MC3T3-E1 cells and P2-P4 BMSCs was evaluated via fluorescent microscopy and FACs analysis (using the BD FACSCalibur^{TM} system).

Results and Conclusions: Multilayer glyc-CHI/HA and unmodified CHI/HA films were formed and characterized. MC3T3-E1 cells and P2-P4 BMSCs exhibited increased adhesion to glycol-CHI/HA multilayer films composed of higher numbers of bilayers (see Fig. 1), compared to corresponding umodified-CHI/HA control films. Furthermore, MC3T3 and BMSC exhibit a well spread cell morphology on glyc-CHI/HA films, while exhibiting rounded morphologies and cell clusters on corresponding umodified-CHI/HA films. Work is currently under way to characterize the *in vitro* transfection of MC3T3 cells and P2-P4 BMSCs utilizing films incorporating PEI-DNA plasmid complexes on the surface, as well as embedded within the films in varying architectures. The build up, surface roughness and hydration properties of these PEI-DNA complex-containing films will also be analyzed The transfection profiles obtained will be compared to those from corresponding umodified-CHI/HA films and bolus transfections, and are anticipated to reveal higher transfection efficiencies from glyc-CHI/HA multilayers.

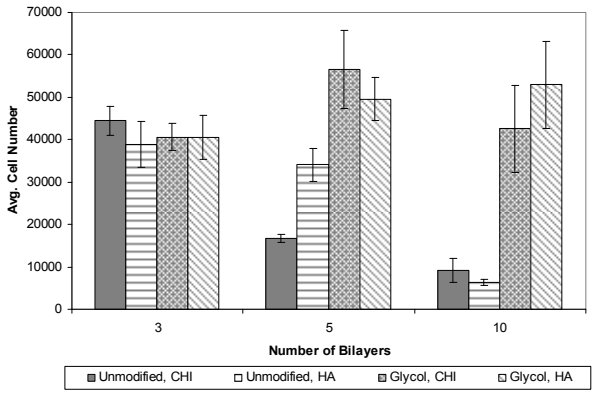

Figure 1: MC3T3 cell adhesion on: $[CHI/HA]_N CHI$ ("Unmodified, CHI"); $[CHI/HA]_N$ ("Unmodified, HA"); $(glyc\text{-}CHI/HA)_N glyc\text{-}CHI$ ("Glycol, CHI"); and $[glyc\text{-}CHI/HA]_N$ ("Glycol, HA") multilayer films, where N= 3, 5 or 10 bilayers.

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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