Influence of Genipin Cross-linking on Layer by Layer Polyelectrolyte Developed Biocompatible Surfaces and its Impact on Cell Adhesion

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Statement of Purpose: Designing biomaterial surfaces which are biocompatible, non-toxic, and selectively promote or inhibit cellular adhesion is a huge challenge; and until recently methods such as the Langmuir Blodgett technique and surface assembly modification (SAM) were used to achieve this. Development of the layer-by-layer (LbL) approach introduced a simple and versatile technique, involving assembly of polyelectrolyte films. However, cell adhesion studies carried out on LbL polyelectrolyte films have shown many of them to be non-adhesive for several cell lines. Amongst the numerous factors which cause this non-adhesive nature, it is suggested that the formation of smooth films inhibits cellular attachment, as demonstrated by Richert et al, who showed decreased chondrocyte adhesion on Ch/HA multi-layer films.[1] Many researchers have also elucidated to the fact that decreased cellular attachment may also be attributed to the gel-like character of these films.[2] Additional studies have suggested that, in order to increase cell adhesion, multilayer films require a more rigid surface, a specific range of surface roughness, and a certain degree of hydration.[1, 3] In order to encourage cell attachment, a number of avenues have been explored, these include; tailoring the chemical properties of surfaces, careful choice of polymer, and control of mechanical properties. Here, we aimed to increase cell attachment to Ch/HA and Ch/Alg multi-layer systems by cross-linking using a natural product, Genipin, at a range of concentrations. Studies have shown that mechanical factors influence cell types differently. Therefore we have also considered different cell lines, investigating how they attach to these films, pre and post cross-linking. **Methods:** LbL polyelectrolyte deposition was used to lay down $[Ch\text{-}Ha]_nCh$ (system 1) and $[Ch\text{-}Alg]_nCh$ (system 2) multi-layer films. Films composed of 3, 5 and 10 bi-layers, terminating in either Ch or Ha for system (1); or Ch or Alg for system (2) were prepared and subsequently cross-linked with genipin (0.5–44mM). Film deposition was monitored by quartz crystal microbalance (QCM) and all films were characterized by atomic force microscopy (AFM), and their hydrophob/philicity was determined by calculating the water contact angle. Resultant layers were tested for cell adhesion using mouse pre-osteoblast MC3T3-E1 cells, HEK 293T kidney cells, and Fr rat skin fibroblasts, supplied by American Type Culture Collection (ATCC, Manassas, VA). All cells were grown at 37°C, under a 5% CO_2 atmosphere for 1-4 days. After incubation, cells were fixed with 10% formalin and stained with 4', 6-diamidino-2-phenylindole dilactate (Dapi dilactate) (Invitrogen Corporation, USA). Fluorescence microscopy (Nikon, USA) was used to image the samples and count the number of cells per field. Cell adhesion was also monitored by light microscopy and using a CyQuant cell proliferation assay kit (Invitrogen, USA). **Results:** AFM characterization demonstrated an increase in surface roughness with LbL deposition and a linear increase with cross-linker (genipin) concentration. Contact angle measurements showed an increase in hydrophilicity after cross-linking, and QCM results showed two very different LbL growth regimes, which resulted in two significantly different membranes, with very different thicknesses. These differences, and the gel-like characteristics of the $[Ch\text{-}Ha]_nCh$ system were translated in the cell adhesion properties of these films. We found that different cell lines preferred varying degrees of cross-linking. Cell attachment and adhesion were pronounced in the $[Ch\text{-}Alg]_nCh$ cross-linked system, while cell adhesion was found to be less favored for the $[Ch\text{-}Ha]_nCh$ genipin cross-linked system.

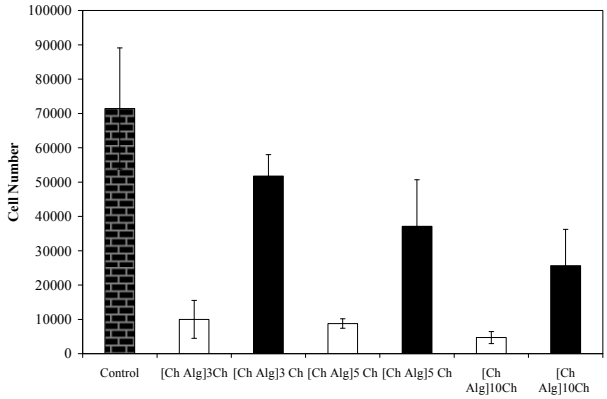

Figure 1. Fr rat fibroblast cell adhesion to $[Ch\text{-}Alg]_n$ LbL films, where ■ are films cross-linked with 44mM genipin and □ are uncross-linked multi-layers.

Conclusions: Genipin cross-linking increases the surface roughness of the multi-layers, as demonstrated by AFM, but the resultant surface is highly dependent on cross-linker concentration. The surface water contact angle is also dependent on the genipin concentration and is greatly reduced by the cross-linking reaction. Cross-linking the $[Ch\text{-}Ha]_nCh$ system does not promote increased MC3T3 cell adhesion, while HEK 293T cell adhesion is promoted at high degrees of cross-linking. $[Ch/Alg]_nCh$ system cross-linked with genipin produces a cell adhesive, biocompatible surface for the growth of rat fibroblastic skin cells. Optimal film thickness, chemistry, rigidity, and hydrophilicity vary for each cell type, making the design of a cell specific biocompatible surface very challenging.

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