INTRODUCTION: A simple approach to the mechanical modulation of layer-by-layer (LbL) films is through manipulation of the film assembly. Here, we report results based on altering the salt concentration during film assembly and its effect on film rigidity. Based on changes in film rigidity, cell adhesion characteristics and transfection activity were investigated in vitro. LbL films consisting of reducible hyperbranched poly(amide amine) (RHB) have been implemented along with DNA for investigating fibroblast adhesion on [RHB/DNA] films with varying rigidities. Film mechanical properties along with cell adhesion, stress fiber orientation and transfection activity were investigated with regards to changes in the film rigidity.

METHODS: Molecular force probe (MFP) measurements were performed to measure the apparent Young’s modulus, E<sub>APP</sub>, of the films in situ. Cell adhesion and stress-fiber characteristics were investigated using total internal reflectance microscopy (TIRF-M). The average cell peripheral area, fiber density and average fiber length during 5 days of cell growth were investigated using either low-E<sub>APP</sub> (below 2.0 MPa) or high-E<sub>APP</sub> (above 2.0 MPa) films. Transfection studies were performed using gfpDNA and SEAP-DNA to investigate if changes in cell adhesion affect transfection activity. Cell proliferation and cytotoxicity studies were used to investigate cellular viability over a week.

RESULTS: These studies have shown that altering the salt concentration during film assembly of bioreducible LbL films can change the film modulus by 2 orders of magnitude, affecting both film thickness and roughness. Additionally, two different growth characteristics have been observed for films assembled in low or high salt concentration. AFM images show that film assembly within these two salt ranges has an effect on surface characteristics as well. Significant differences in cell attachment, growth and transfection activity are seen with film rigidities either < 2 MPa (low-E<sub>APP</sub>) or > 2 MPa (high-E<sub>APP</sub>). During cellular growth and proliferation, it is found that cells grown on high-E<sub>APP</sub> films have increased peripheral areas and stress-fiber density compared to cells grown on low-E<sub>APP</sub> films. These growth characteristics also have an effect on transfection activity which is found to be significantly higher for cells grown on high-E<sub>APP</sub> films compared to low-E<sub>APP</sub> films.

DISCUSSION & CONCLUSIONS: The results have shown that surface modification of bioreducible LbL films of controlled thickness and roughness promotes cellular adhesion, stress-fiber growth and increased transfection activity without the need for an additional adhesive protein pre-coating of the surface or chemical cross-linking of the film. In particular, it has been found that bioreducible LbL films with an apparent elastic modulus greater than 2.0 MPa have increased cell adhesion, cell spreading and transfection activity compared to films with low modulus.


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