Modulation of osteoblast-like cells, MG-63, on polyelectrolyte multilayer films

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Statement of Purpose:

The layer-by-layer (LBL) assembly is based on the deposition of alternating layers of oppositely charged polyelectrolytes. A large number of layers can be assembled with this simple procedure. The surface properties of the resulting coating are dependant on a variety of assembly conditions, including nature and surface charge of the substrate, nature and molecular weight of the polyelectrolytes, pH and ionic strength of the coating buffer, duration of coating etc. The LBL surface modification method allows the coating of complicated geometries and is extremely versatile for biomedical devices.

Previously some studies suggest that the outer polyelectrolyte layer determines the biocompatibility properties of the multilayer film.[1] On the other hand, some showed that the molecular architecture of the multilayer polyelectrolyte films can control cell adhesion regardless of the outmost layer.[2] In the study, we investigated the cell adhesion, growth and functions on the polyelectrolyte films which were developed at different pH conditions and different layer numbers. We found that different coating conditions influence cell behavior.

Methods:

Poly(acrylic acid) (PAA) (Aldrich, MW ~100000) was prepared as 0.1% (v:v) solution, whereas Poly(allylamine hydrochloride) (PAH) (Sigma, MW ~70000) prepared as 1 mg/ml solution. Both types of polyelectrolyte solutions were adjusted to pH 2.0 or 6.5 with either HCl or NaOH, and filtered through a 0.45 μm membrane. 80μl of PAH was first deposited directly onto 96-well TCPS for 10 mins then rinse with deionized water twice. Afterward, 80 μl PAA with the same pH was deposited for 10 mins followed by the same rinse procedure. The coating cycle were repeated until the desire numbers of layers were assembled. The multilayer films were dried at in an oven 70ºC for 8 hours and store at 4ºC. MG-63 cells were plated in the LBL-coated plates at 10^4 cells/cm^2 in DMEM containing 10% FBS. After a certain period of time, the adherent cells were lysed with 0.1% Triton X-100. Cell number was determined by a BCA method. Alkaline phosphatase (ALP) activity was assayed as the release of *p*-nitrophenol from *p*-nitrophenylphosphate at pH 10.2 and the specific activity was calculated.

Results / Discussion:

The topmost layer on the LBL films with odd number of layers was positively-charged PAH, while that with even number of layers was negatively-charged PAA. The deposition process was identified by measuring surface charges on these LBL films. In general, MG-63 cells adhered better on the surfaces deposited at pH 6.5 than at pH 2.0 (Fig. 1), which is consistent with a previous study.[2] Mendelsohn et al. reported that highly ionically cross-linked films were cytophilic, while weakly ionically stitched conformations were cytophobic. Furthermore, cell adhered better on the surfaces with fewer layers. Cells spread on the pH 6.5 surfaces, while cells retained a round morphology on the pH 2.0 surfaces through the duration of the experiments. Furthermore, the cells grown on the pH 6.5 surfaces had higher ALP activities than those on the pH 2.0 surfaces (Fig. 2). The cells cultured on the pH 6.5 surfaces with 11, 20 and 21 layers expressed high ALP activities after 24 h, but after 72 h the ALP activity dropped to a level compared to those on the pH 2.0 surfaces. Our results indicate that the architecture of the LBL films modulates MG-63 cells' activity.

Conclusions:

Polyelectrolyte LBL deposition is a convenient method for surface modification of biomaterial surfaces. Cell behavior can be modulated by the number of layers and the condition of deposition.

References:

1. P. Tryoen-Toth et. al., *JBMR* 60:657, 2002.
2. J. D. Mendelsohn et al., *Biomacromolecules* 4:96, 2003.

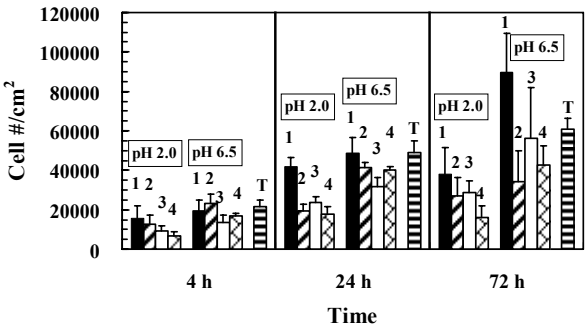

Fig. 1. Cell adhesion and growth after 4h, 24h and 72h incubation at various LBL surfaces. The pH shown in the figure indicates the deposition pH. 1. 10 layers; 2. 11 layers; 3. 20 layers, and 4. 21 layers; T, TCPS control.

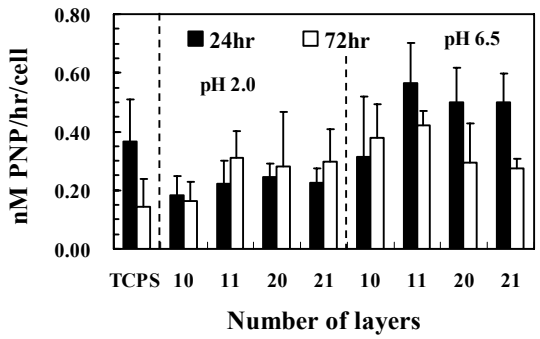

Fig. 2. Alkaline phosphatase activities of cell grown on different types of surfaces.