# Polypeptide Multilayer Films as Artificial Extracellular Matrices

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**Statement of Purpose:** The potential of polypeptide multilayer films for development of a variety of applications in biotechnology and medicine has only just begun to be realized (Haynie et al. Biomacromolecules 2005;6:2895-2913; Haynie DT. J Biomed Mater Res B Appl Biomater 2006;78B:243-252; Haynie et al. Nanomedicine 2006;2:150-157). Such films are prepared by layer-by-layer self-assembly (LBL). Control over cell behavior in vitro for sensor development and regenerative medicine is a major thrust of bioengineering research today. Here, we report initial results of a study on the application of polypeptide multilayer films as artificial extracellular matrices (ECMs).

**Methods:** Eight 32mer peptides were designed to test the relative importance of non-covalent interactions, namely, hydrophobic interactions and side chain hydrogen bond potential, in film assembly and cell behavior in vitro. In addition, peptides were designed to encode Arg-Gly-Asp (RGD), a signal recognized by integrin, or Cys (C), which forms disulfide bonds under oxidizing conditions. The peptides were prepared by a commercial source at >75 % purity (Celtek, USA). Four pairs of designed peptides were synthesized:

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KVGGKCGVKVGGKCGVKVGGKCGVKVGGKCGY – P1
EVGGECGVEVGGECGVEVGGECGVEVGGECGY – N1
KVGVKVGVKVGVKVGVKVGVKVGVKVGVKVGY – P2
EVGVEVGVEVGVEVGVEVGVEVGVEVGVEVGY – N2
KVGSKVGSKVGSKVGSKVGSKVGSKVGSKVGY – P3
EVGNEVGNEVGNEVGNEVGNEVGNEVGNEVGY - N3
KGRGDGKGKGRGDGKGKGRGDGKGKGRGDGKGKY – P4
EGRGDGEGEGRGDGEGEGRGDGEGEGRGDGEGEY – N4
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Other peptides included in the studied were poly(L-lysine) (PLL) and poly(L-glutamic acid) (PLGA) (Sigma, USA). Each chain was either positively charged (P) or negatively charged (N) for multilayer film assembly near neutral pH. Films were fabricated by LBL and characterized with regard to physical properties by quartz crystal microbalance (QCM), circular dichroism spectroscopy (CD), and atomic force microscopy (AFM) to determine peptide mass adsorbed, film internal structure, and film morphology, respectively. Murine 3T3 cells and mesenchymal stem cells were obtained from American Type Cell Culture (USA) to characterize films with regard to biological properties. Observables for this purpose included cell proliferation (MTT assay), cell viability (Live/Dead® assay, Molecular Probes), and cell morphology (F-actin staining with labeled phalloidin).

**Results/Discussion:** Analysis of polypeptide multilayer films by QCM has revealed that, although film thickness increases with number of layers adsorbed, different combinations of peptide display differences in assembly behavior [Fig. 1]. CD data (not shown) corroborate the QCM results. Moreover, CD has revealed that the internal structure of films consists largely of β sheets, the percentage depending on the peptides involved.

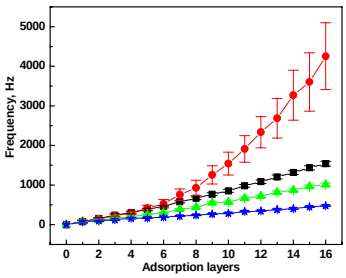


Figure 1. Assembly of combinations of designed peptides at pH 7.4, monitored by QCM. Frequency shift, which is proportional to mass deposited, is plotted against number of adsorbed layers. Error bars: standard deviation of 3 independent trials.

AFM has revealed significant differences in film morphology which depend on the peptides [Fig. 2]. Note the large differences in surface roughness.

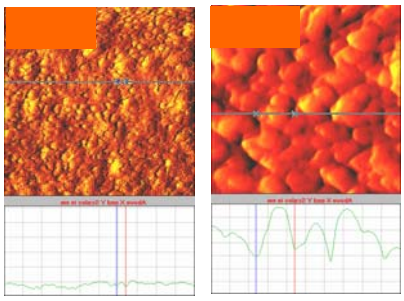

Figure 2. AFM images of two multilayer films made with the same positive peptide but different negative peptides. Top, height mode image; bottom, profilometric section. Vertical scale: 30 nm.

There are similarities and differences in attachment and proliferation of 3T3 cells and mesenchymal stem cells on artificial extracellular matrices as compared to standard tissue culture treated plastic [Fig. 3].

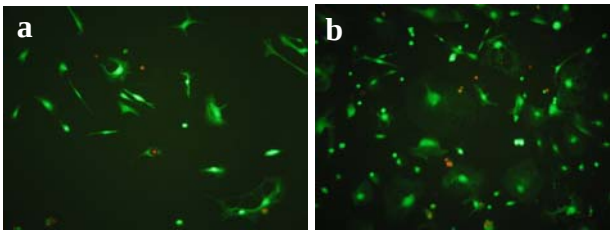


Figure 3. 3T3 cell culture on (a) tissue culture treated plastic and (b) on a PLL/PLGA multilayer film. Cells are stained with rhodamine-labeled phalloidin and visualized by fluorescence microscopy.

**Conclusions:** Details of peptide sequence influence multilayer film properties and cell behavior. The results of this study will be useful for comparing designed peptide cell culture coatings with related materials currently in the marketplace.