**Effect of Polyelectrolyte Multilayer Assembly on Accessibility of Immobilized Growth Factor**

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**Statement of Purpose:** The layer-by-layer deposition technique has attracted considerable research interest in recent years due to numerous advantages for biomedical applications: ease of preparation under mild conditions; compatibility with physiological media; ability to incorporate bioactive molecules, extracellular matrix components, and biopolymers in the films; tunable mechanical properties; and spatio-temporal control over film organization. Several recent studies have explored polyelectrolyte multilayer (PEM) films designed as reservoirs of cytoactive factor(s) for controlled release applications. Another possibility that has received limited attention is immobilization of these factors within PEM films for highly localized therapeutic outcomes. Such an approach allows prolonged and controlled cellular contact with cytoactive factor(s) without adverse systemic effects that are typically associated with diffusion based delivery systems. The influence of polyelectrolyte type as well as number of layers has been well studied with respect to release kinetics of embedded cytoactive factors. The influence of the aforementioned parameters on accessibility of cytoactive factors immobilized within PEMs, however has not yet been studied. Furthermore, alteration in the bioactivity of cytoactive factors following immobilization to PEMs has not been studied. We are currently investigating the influence these key parameters on the accessibility and bioactivity of epidermal growth factor (EGF) covalently immobilized to PEMs using primary human keratinocytes. Our goal is to delineate the most efficacious design and assembly parameters for covalent immobilization of cytoactive factors within PEMs for therapeutic and biomedical device applications.

**Methods:** Optical bottom tissue culture treated black 96-well plates were sequentially treated with 200 µl of the positively charged polyelectrolyte (PE) poly(allylamine hydrochloride (PAH, MW ≈160 kDa) and the negatively charged PE poly(acrylic acid) (PAA, MW ≈90 kDa) solutions (1 mg/ml, pH 7) for 8 min per layer until deposition 10.5 bilayers ($[PAH+PAA]_{10}$+PAH). Between each PE treatment, the wells were rinsed three times for 1 minute each with ultrapure water (UPW). Control wells were temporarily sealed with adhesive tape. Remaining wells were treated with 200 µl of 1% bovine serum albumin solution for 1 hour followed by three rinses with UPW. Some wells were sealed as needed. Ten microliters of EGF covalently modified with the fluorescent dye Texas Red succinimidyl ester (TR) and the crosslinker sulfo-SANPAH (SS) were spread onto the wells and crosslinked for 5 minutes under collimated UV light (365 nm at 10 mW/cm$^2$). Wells were rinsed with UPW as above and 2.5, 4.5, or 6.5 PE bilayers were deposited over the EGF. Primary human keratinocytes were seeded (15,000/well) in serum-free Epilife medium without added growth factors. After 48 hours, cell proliferation on various surfaces was compared using a standard calcein-AM assay.

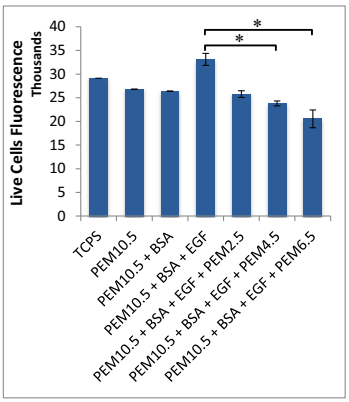


**Figure 1. Effect of number of PE bilayers on EGF accessibility.** EGF uncoated with any PE bilayers enhanced cell proliferation compared to all control surface treatments. Cell proliferation was reduced significantly when the EGF was covered with 4 or more PE bilayers.

**Results:** Keratinocytes with direct access to EGF (no PE bilayers above) showed increased proliferation compared to control surfaces of tissue culture polystyrene (TCPS), unmodified PEMs, and PEMs coated with BSA alone (Fig. 1). EGF bioactivity was preserved, as shown by proliferation, in spite of its modification with TR and SS and surface immobilization. However, coating the EGF with PEMs adversely affected keratinocyte proliferation, reaching significant reduction at and beyond 4.5 PE bilayers compared to uncoated EGF. However, a clear inverse relationship is apparent between EGF accessibility and/or bioactivity and number of PEMs coated over the immobilized EGF.

**Conclusions:** We have demonstrated that covalent immobilization of EGF onto PEMs does not significantly alter EGF bioactivity. Keratinocytes are able to detect and respond to uncoated EGF present on the PEMs. Coating the EGF with PEMs however, significantly affects keratinocyte proliferation, either by altering EGF accessibility or adversely affecting EGF bioactivity, or both. Increasing the number of PE bilayers deposited above the immobilized EGF may further reduce the proliferation. We are currently investigating this effect in our laboratory. It is possible that replacing the synthetic PEs used in the current study with biomimetic ones, such as amino acid polymers, may improve EGF bioactivity as well as allow cells to penetrate the PEMs and respond to EGF. Our results highlight the importance of elucidating the interaction between PEMs and embedded cytoactive factors for successful functional and therapeutic outcomes.



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