Integration of Silver-Impregnated Polymeric Multilayers onto Biological Tissues to Provide Antibacterial Activity

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Statement of Purpose. Polymeric multilayers can be fabricated to incorporate a range of bioactive molecules, providing a general approach for controlling the surface properties of materials. We report a novel design of polyelectrolyte multilayers (PEMs) that can be prefabricated on an elastomeric stamp, and mechanically transferred onto soft medical devices and biological tissues such as the dermis of skin grafts (G'~ 200 kPa). Using this approach, we test the hypothesis that nanoscopic localization of silver-nanoparticles within PEMs onto model wound-beds can lead to antimicrobial activity at loadings of silver that are small compared to those found in conventional silver-containing wound dressings. This study provides the basis of new approaches to functionalize biological tissues without subjecting them to deleterious processing conditions.

Methods. Polyelectrolyte multilayer (PEM) films were prepared on poly(dimethylsiloxane) (PDMS) sheets by sequential incubation in 0.01 M solutions of poly(allylamine hydrochloride) (PAH) and poly(acrylic acid) (PAA), as described previously[1]. A monolayer of carboxylate-modified polystyrene microspheres, 2 μm in diameter, were deposited on $(PAH/PAA)_{10.5}$ multilayers by adsorption for 30 min, followed by rinses with water. Additional layers of polyelectrolytes were assembled over the microspheres, to obtain the final PEMs/microsphere composition, designated as: $(PAH/PAA)_{10.5}$(microspheres)$(PAH/PAA)_{10.5}$. Post-assembly, the PEMs were loaded with silver nanoparticles by immersion in 5 mM silver nitrate solution followed by chemical reduction of the impregnated silver ions using 1 mM sodium borohydride solution[1]. The silver loading was manipulated through control of the cycles of silver ion exchange and reduction, and measured using an inductively-coupled plasma emission spectrometer after extraction into 2% nitric acid[1].

PEMs were stamped onto dermis layers of cadaver skin (GammaGraft®, Promethean LifeSciences Inc, PA), a model of partial-thickness wounds, by bringing the PDMS stamps supporting PEMs into contact with skin-dermis. A pressure of ~200kPa was applied on the stamps in contact with the skin-graft for 30s and the stamps were subsequently peeled away. Antibacterial activity of the treated dermis of the cadaver skin was tested by incubating the dermis with buffer solutions containing 10^7 colony forming units (CFU) of *Staphylococcus epidermidis* or *Pseudomonas aeruginosa* for 24h-48h, as described elsewhere[1]. The viable bacterial cell counts were determined by the surface spread-plate method.

Results. Fabrication of the PEMs on the PDMS sheets was confirmed by fluorescent measurements. Transfer of the PEMs from the PDMS onto the skin-dermis was determined by microscopy using fluorescently labeled PAH and fluorescent microspheres in the PEMs. The key finding of this study is that the contact printing of PEMs of (PAH/PAA)n from elastomeric stamps onto substrates is dependent on the mechanical properties of the substrates. While PEMs of $(PAH/PAA)_{10.5}$ could not be stamped onto skin-dermis using methods described in literature[2] for contact printing on rigid substrates (G'~65 GPa), we found that incorporation of a layer of microspheres in the PEMs greatly facilitated their mechanical transfer onto skin-dermis using methods developed in this study. The loading of silver in the PEMs assembled on PDMS sheets was varied from ~0.30 μg/cm^2 to ~1.64 μg/cm^2. Loss of silver from the PDMS during stamping on the skin-dermis was use to calculate the transfer of silver onto the skin-dermis. Measurement of silver ions extracted into dilute nitric acid from stamped skin-dermis was used to determine the upper limit of silver ion concentrations that could be released from the stamped skin-dermis into aqueous solutions.

While untreated skin-dermis, or that stamped with PEMs containing ~0.30±0.03 μg/cm^2 silver exhibited no antibacterial activity, skin-dermis stamped with PEMs containing ~1.00±0.12 μg/cm^2 silver resulted in 10^6-fold decrease in bacterial counts (CFU) in 24h (**Fig. 1**). The amount of silver extracted from skin-dermis stamped with the latter set of PEMs was only 0.25±0.01 μg/cm^2, which is below the cytotoxicity limit for mouse fibroblasts cells, as shown in our previous study[1].

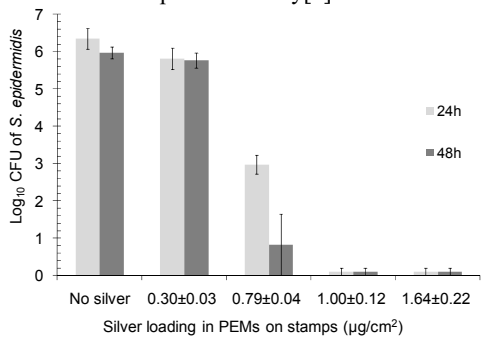

Fig. 1. Influence of silver loading in PEMs stamped onto skin dermis on the survival of *S. epidermidis* in buffers. (n≥4)

Conclusions. We have developed a general and facile methodology to functionalize the surfaces of biological tissues such as those found in wound-beds. The ability to directly stamp pre-fabricated PEMs onto biological tissues circumvents the exposure of the tissues to non-physiological processing conditions and chemicals and allows nanoscopic localization of non-cytotoxic levels of bioactive factors.

References.

1. Agarwal A. Biomaterials 2010; 31: 680-690.
2. Park J. Adv. Mat. 2004; 16: 520-525.