Influence of Poly-L-Lysine Molecular Weight on Antibacterial Activity of Polyelectrolyte Multilayer Films

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Statement of Purpose: Over 60% of hospital-acquired infections are device related.[1] With rising antibiotic resistance and occurrence of biofilms, treating these infections is becoming increasingly difficult. There is a need for device coatings that can inhibit bacteria attachment, preventing biofilms, while also killing planktonic bacteria. We have developed coatings with these properties using layer-by-layer (LbL) self-assembly of hyaluronic acid (HA), a polyanion, and poly-L-lysine (PLL), a polycation, which plays both a structural and functional role due to its antibacterial properties. We investigated the effect of PLL molecular weight (MW) on the antibacterial efficacy of (PLL/HA) films, finding that MW influences release-based killing while film hydrophilicity imparts resistance to bacterial adhesion.

Methods: *Film assembly.* $(PLL/HA)_{50}$ multilayer films (50 indicating number of bilayers) were assembled via LbL self-assembly. Different architectures of $(PLL/HA)_{50}$ films were assembled containing PLL with 33, 91, or 407 repeat units (6.9 kDa, 19 kDa, or 85.1 kDa, respectively), denoted PLL^{30}, PLL^{90}, and PLL^{400}, respectively. Coating thickness was examined via profilometry. *Examining film antibacterial efficacy.* Growth inhibition of planktonic bacteria was investigated by measuring bacterial density after a 24 hour incubation. Repeated exposure to fresh inocula of *Staphylococcus aureus* was tested every 24 hours. *S. aureus* attachment inhibition after a 2 hour incubation was tested via LIVE/DEAD staining and by agar incubation for 18 hours. *Measuring PLL in incubation solutions.* Concentration of released PLL was estimated using a microdilution assay and back-calculating from PLL minimum inhibitory concentration (MIC) against *S. aureus*. *Investigating PLL mobility within films.* $(PLL/HA)_{50}$ films were incubated with fluorescein-PLL (PLL^{F}) of the corresponding MW for 15 minutes and imaged using a laser scanning confocal.

Results: $(PLL/HA)_{50}$ films assembled with all three PLL MWs completely inhibited the growth of planktonic, gram-positive *S. aureus* and methicillin resistant *S. aureus* and gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* over a 24 hour exposure (>99.8% inhibition). Interesting differences between different MW PLL arose when films were repeatedly exposed to fresh *S. aureus*. PLL^{400} films lost their inhibitory effect after one 24 hour use, while PLL^{30} and PLL^{90} films were effective for 4 to 5 and 9 to 13 uses, respectively. PLL concentrations in the incubation solutions were generally at or above PLL MIC while films were effective (Fig. 1A), suggesting that extended PLL release plays an important role in allowing repeated effective use.

We hypothesized that differences in PLL release kinetics are related to higher PLL mobility in films assembled with lower MW PLL. Film cross-sectional confocal images confirmed reduced diffusion of PLL^{F} into the films with increasing PLL MW, with the most dramatic effect observed for PLL^{400F} as shown in the fluorescence intensity Z-profiles indicating maximum PLL^{400F} intensity at the top film surface farthest from the substrate (Fig. 1B). PLL MW also influenced the thickness and stability of films. PLL^{30} films (1.2 ± 0.2 µm thick) gradually eroded over 5 days, while the thickness of PLL^{90} films (3.6 ± 0.1 µm thick) only began to decrease significantly around day 10 (Fig. 1C), consistent in both cases with their loss in efficacy following repeated bacterial exposure. In contrast, PLL^{400} films remained stable over 16 days, indicating loss of efficacy after one use was not caused by film loss. $(PLL/HA)_{50}$ films also significantly reduced bacterial attachment and growth compared to uncoated substrates based on LIVE/DEAD staining (Fig. 1D) and overnight agar incubation. We hypothesize this effect is related to the hydrated nature of the films, indicated by an approximate 300 to 400% percent swelling upon dry film hydration in phosphate buffered saline and electrostatic repulsion between HA and negatively charged bacteria.

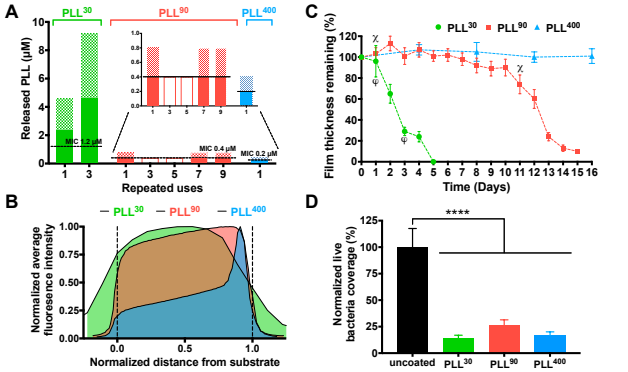

Figure 1. Antibacterial $(PLL/HA)_{50}$ film characterization. **A)** Concentration of PLL released into bacterial incubation solutions versus number of 24 hour uses reported as a range. Solid bar is lower limit, shaded bar is potential upper limit, empty bar indicates no detected bacterial growth inhibition. Inset: Zoom in on PLL^{90} and PLL^{400} films. **B)** Normalized fluorescence intensity in films versus normalized distance from substrate (0 indicating glass substrate, 1 representing top of film) upon incubation with PLL^{F}. **C)** Percent of film dry thickness remaining after incubation in bacteria media at 37˚C. **D)** Percent area covered with live *S. aureus* normalized to percent area of uncoated glass covered with live *S. aureus*.

Conclusions: We have shown that (PLL/HA) multilayers are inherently antibacterial without any modifications or additions. Our investigations demonstrated the role of MW in PLL mobility, PLL release, and film stability, which in turn influenced the number of effective repeated antibacterial uses for these coatings. Thus, the PLL films developed in this work may be tuned by varying PLL MW to prevent and treat device-associated infections.

References: [1] Salwiczek M. *et al*., Trends Biotechnol, 2014, 32(2):82-90. [2] Arciola C.R. et al. Biomaterials, 2012, 33(26):5967-5982.