Effectiveness of Anti-biofilm Agents against *Staphylococcus aureus* biofilms

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Statement of Purpose

A biofilm is a community of bacterial cells that are no longer in a planktonic state and have attached themselves to each other and another surface, such as materials implanted within the body. Once attached, the microcolony of bacteria starts to behave as a single organism, secreting an extracellular polymeric matrix that makes traditional anti-bacterial treatments ineffective due to their inability to penetrate the polymeric matrix that coats the bacterial cells (*FEMS Immunol Med Microbiol* 65:146, 2012). Agents that specifically target biofilms (anti-biofilm drugs) are of great interest due to their potential therapeutic applications. These drugs often work by disrupting the polymeric coat to remove the protective barrier, detaching biofilms, preventing bacteria from attaching in the first place, or inhibiting communication between cells.

The goal of the present work is to evaluate *in vitro* the effectiveness of anti-biofilm agents in soluble form and after controlled release from a biodegradable polymer.

Methods

A biofilm assay was modified from that reported by Hochbaum *et al.* (*J Bacteriol* 193:5616, 2011). In short, biofilms of *Staphylococcus aureus* (ATCC 25923) were grown in tissue culture-treated 96-well plates in bovine heart infusion (BHI) media supplemented with 1% glucose and 2% NaCl. Assays were performed to test either biofilm inhibition or biofilm disruption. For biofilm inhibition, the required treatment was added to the BHI media at the same time as *S. aureus* were seeded. For biofilm disruption, the bacteria were seeded and allowed to grow for 24 hours at 37 °C before the media was refreshed and the required treatment added. The drugs investigated in this study included: lysostaphin, xylitol, lactoferrin, D-amino acids (phenylalanine, proline, tyrosine), L-amino acids (phenylalanine, proline, tyrosine), and vancomycin. Following treatment, the wells were washed with phosphate-buffered saline (PBS) and then stained with crystal violet. Ethanol was used to leach the crystal violet from the adherent biofilm and ultraviolet spectroscopy used to quantify the amount of biofilm present in each well. Poly(lactic-co-glycolic acid) (PLGA) microspheres were fabricated and loaded with lysostaphin (lyso) to investigate their ability to control the release of a powerful anti-biofilm agent.

Results and Discussion

The effectiveness of select anti-biofilm drugs at disrupting biofilms can be seen in Figure 1. Results are compared to effects of the commonly used and powerful antibiotic vancomycin. The endopeptidase lysostaphin was capable ofinhibiting the growth of new biofilms (not shown) and, more importantly, of disrupting an existing *S. aureus* biofilm.

Figure 2 shows the effectiveness of lysostaphin released from PLGAms as inhibiting the growth of *S. aureus* biofilms *in vitro* compared to known concentrations of drug and the microspheres alone.

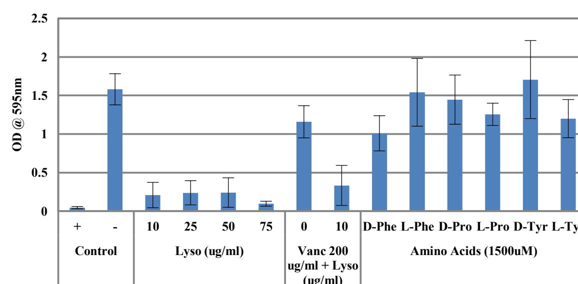

Figure 1: Biofilm disruption results comparing different anti-biofilm drugs and vancomycin.

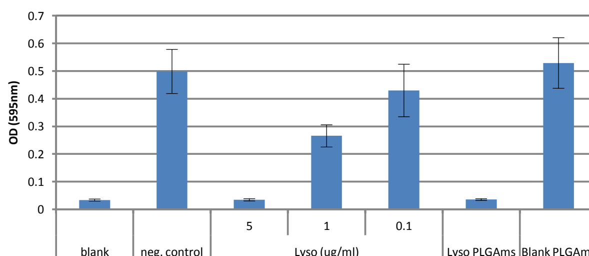

Figure 2: Biofilm inhibition results showing the effect of lysostaphin-loaded PLGA microspheres.

Conclusions

Lysostaphin has shown great potential as an anti-biofilm agent capable of disrupting an existing biofilm and inhibiting biofilm formation in the preliminary *in vitro* biofilm tests. This dual action of lysostaphin at a wide range of concentrations makes it a promising candidate for future anti-biofilm biomaterials that could eliminate an existing biofilm and prevent it from re-growing. The ability to load lysostaphin into a delivery vehicle further increases its potential therapeutic effectiveness by allowing for controlled release over time in a biomaterial device. The success of these initial trials warrants further investigation into the ability of lysostaphin to inhibit and disrupt biofilm formation after being incorporated into a therapeutic biomaterial.

Acknowledgements

This work was supported by a grant from the US Army Medical Research Acquisition Activity (W81XWH-09-1-0461). MEB was supported by NSF IGERT (DGE-0653710).

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