Effect of LL-37 Peptide in Disrupting Biofilms

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Statement of Purpose: Biofilms are often found on biomedical devices and are very difficult to eliminate; no effective approach has been reported. Our previous studies have shown that LL-37, a cationic antimicrobial peptide (CAMP) and a component of the innate immune system, has high potency against both intra- and extra-cellular bacteria.[1] Unlike conventional antibiotics, amphiphilic CAMPs like cathelicidin LL-37 are comprised of hydrophobic and hydrophilic residues aligned on opposite sides of the peptide, which may facilitate their penetration through biofilms to disrupt them.[2,3] In this study, our **aim** was to establish biofilms using *Staphylococcus aureus* (*S. aureus*) and to determine the effectiveness of LL-37 in disrupting the biofilms.

Hypothesis: We **hypothesized** that LL-37 would be effective in disrupting *S. aureus* biofilms.

Methods: Five *S. aureus* strains were examined including bioluminescent strains (Xen36, Xen29, Xen8.1), ATCC25923, and clinical 1004. Four sets of experiments were carried out: (i) Optimizing bacterial concentration in establishing biofilms using *S. aureus* Xen36. (ii) Establishing biofilms using the five bacterial strains and correlating bioluminescent intensity of the bioluminescent strains with colony forming units (CFUs) at different time points. (iii) Examining the effect of LL-37 concentrations in disrupting biofilms. (iv) Comparing the effect of LL-37 with conventional antibiotics in disrupting *S. aureus* biofilms.

Results: We found that a concentration of 10^2 CFU/mL of *S. aureus* Xen36 could provide detectable bioluminescent intensity, and the growth profiles of 10^2, 10^4, and 10^6 CFU/mL had similar patterns at the time points studied (data not shown). The biofilm-forming capacity of the various *S. aureus* strains were observed by cultivating the biofilms on polystyrene and stainless steel surfaces. We found that all *S. aureus* strains examined formed biofilms but their biofilms had very different morphologies (**Fig. 1**); biofilms formed quicker on polystyrene surfaces compared to stainless steel ones (data not shown). The capacity of LL-37 to disrupt *S. aureus* biofilms was determined and we found that LL-37 was highly potent in the disruption of *S. aureus* biofilms compared to the other CAMP (i.e. lactoferricin-B) and the conventional antibiotics such as clindamycin, vancomycin, and cefazolin, and LL-37 reduced the CFUs more than 70% in the clinical 1004, ATCC25923, and Xen36 *S. aureus* strains (**Fig. 2**).

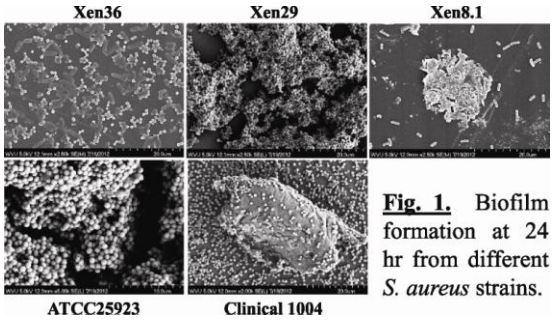

Fig. 1. Biofilm formation at 24 hr from different *S. aureus* strains.

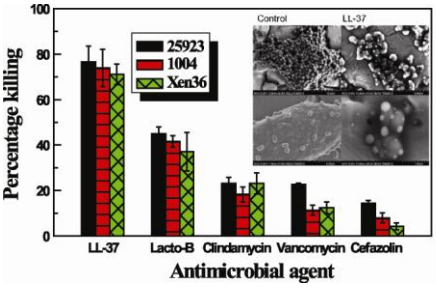

Fig. 2. Effect of LL-37, lactoferricin B, clindamycin, vancomycin, and cefazolin on bacterial killing in biofilms. Inset shows the disruption of *S. aureus* biofilms by LL-37.

Discussion: Our previous studies have demonstrated that LL-37, compared to conventional antibiotics, is more potent and faster at eliminating both extra- and intra-cellular *S. aureus*.[1] LL-37 was also found to exhibit synergistic antibacterial activities with β-defensin and lysozyme in both neutral and acidic environments.[4] However, it was unknown whether LL-37 would be more effective in disrupting *Staphylococcal* biofilms compared to conventional antibiotics. We have now demonstrated *in vitro* that LL-37 could disrupt *S. aureus* biofilms and that it was significantly more effective in disrupting *S. aureus* biofilms compared to commonly used conventional antibiotics. The disruption of biofilms was believed to take place through the lysis of the *Staphylococci* which leads to destabilization of the biofilm matrix. Administration of LL-37 therefore may have the potential to eliminate the need for surgical removal of infected biomedical devices.

Conclusions: We found that Xen36 was stable and had a high *in vitro* bioluminescent signal, biofilms were developed on stainless steel discs with the bioluminescent bacteria, and LL-37 disrupted the bioluminescent biofilms and was much more effective compared to commonly used conventional antibiotics (e.g. clindamycin, vancomycin, and cefazolin).

Significance: *S. aureus* has been commonly found to grow biofilms on biomedical devices and has been a significant clinical concern. Unfortunately, very few therapeutic approaches have been reported as effective in disrupting or eliminating such biofilms. In this study, we found that LL-37 was much more effective in disrupting *S. aureus* biofilms compared to conventional antibiotics and could potentially contribute to biofilm removal clinically.

References: [1] Noore J, Noore A, Li B. *Antimicrob Agents Chemother* 2013;57(3):1283-90. [2] Burton MF, Steel PG. *Nat Prod Rep* 2009;26:1572-84. [3] da Silva BR, et al. *Peptides* 2012;36(2):315-21. [4] Chen X, et al. *J Dermatol Sci* 2005;40:123-32.