Targeted Cationic Peptide for the Disruption of *Staphylococcus epidermidis* Biofilm Formation

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Statement of Purpose: *Staphylococcus epidermidis* is a coagulase negative, gram positive bacterium implicated in the nosocomial infections of many blood-contacting biomaterials. One method by which *S. epidermidis* adheres to a biomaterial involves the bacterial surface protein SdrG binding to the β6-20 amino acid sequence (NEEGFFSARGHRPLD) located on the fibrinogen Bβ chain. Following initial adhesion, *S. epidermidis* produces a positively charged exopolysaccharide known as polysaccharide intercellular adhesin (PIA), which associates with the negatively charged bacteria to form cell-cell adhesions essential to biofilm formation. Antibacterial treatments are typically unsuccessful once a mature biofilm has formed, and as a result the infected material often needs to be removed. We have engineered a targeted, cationic peptide with the ability to disrupt the bacteria-PIA electrostatic interactions that lead to biofilm formation.

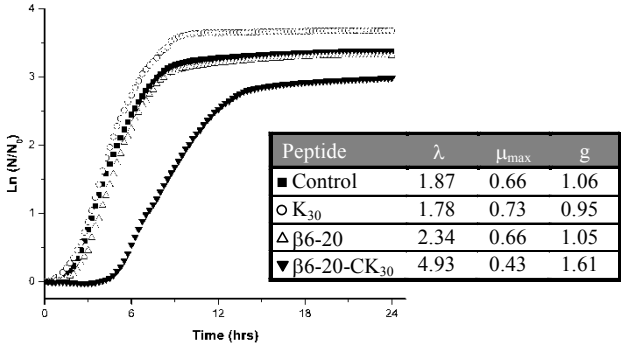
Methods: The β6-20 targeting peptide and two versions of polylysine (K_{30} and CK_{30}) were synthesized by solid phase peptide synthetic methods using fmoc amino acids and a Knorr resin. Peptides were subsequently purified by reverse phase high performance liquid chromatography (RP-HPLC). The targeted, cationic β6-20-CK_{30} peptide was prepared using sulfosuccinimdiyl 4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (Sulfo-SMCC) to crosslink the β6-20 and CK_{30} peptides in a 1:1 fashion.

S. epidermidis strain RP62A was cultured in tryptic soy broth (TSB) and adjusted to a concentration of $0.5x10^8$ cfu/ml in TSB. Bacteria were seeded in 96-well polystyrene culture plates for 2 hours, after which non-adherent bacteria were rinsed away. PBS, β6-20, K_{30}, or β6-20-Ck_{30} was then added for 30 minutes, followed by a final rinsing step and an additional 24 hour incubation period, during which optical density readings at 550 nm were taken every 15 minutes for bacterial quantification. Wheat germ agglutinin conjugated to Alexa Fluor 488 (WGA-488, Invitrogen) was used to visualize PIA content of biofilms. A modified version of the Gompertz equation was used to characterize the growth curve data:

$$N(t) = A * exp\left\{-exp\left[\left(\frac{\mu_{max} * e}{A}\right)(\lambda - t) + 1\right]\right\}$$

Where A is the asymptotic maximum concentration, μ_{max} is the maximum specific growth rate, and λ is the lag time (defined as the intercept between the x-axis and the tangent line through the point μ_{max}). Generation time, g, is defined as $\ln(2)/\mu_{max}$.

Results and Discussion: This study aims to selectively deliver a cationic peptide to the surface of *Staphylococcus epidermidis* that will interfere with the process of biofilm formation. Figure 1 compares growth curves of *S. epidermidis* collected after exposure to various peptides. Both lag times and generation times associated with the β6-20 and K_{30} peptides showed little difference from the control where only PBS was added. However, the β6-20-CK_{30} greatly altered the growth of the bacteria, as demonstrated by an increased lag time (4.93 hrs) and generation time (1.61 hrs).



Peptide	λ	μ_{max}	g
■ Control	1.87	0.66	1.06
○ K_{30}	1.78	0.73	0.95
△ β6-20	2.34	0.66	1.05
▼ β6-20-CK_{30}	4.93	0.43	1.61

Figure 1: Effects of 25μM peptide on bacterial growth. Lag time (λ), maximum specific growth rate (μ_{max}), and generation time (g) were determined by curve fitting using the modified Gompertz equation (R^2>0.99. n=3 for all curves).

Figure 2 illustrates the difference in PIA content of biofilms grown for 24 hours. When the β6-20-CK_{30} peptide was added to the bacteria (left), PIA content was greatly reduced relative to the bacteria grown in the absence of peptide (right).

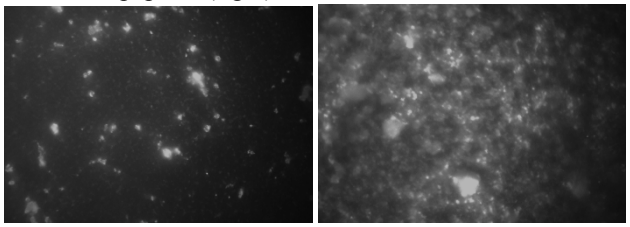


Figure 2: WGA-488 staining of polysaccharide intercellular adhesin (PIA). The addition of β6-20-CK_{30} (left) greatly reduces the amount of PIA present after 24 hours when compared to the control (right).

Conclusions: The β6-20-CK_{30} cationic targeting peptide inhibits bacterial growth and biofilm formation, as demonstrated by growth curve data and PIA staining. In addition, the β6-20 and K_{30} peptides on their own are unable to inhibit the growth of *S. epidermidis* biofilms, verifying that specific targeting plays a key role in the inhibition. With its ability to disrupt biofilm formation, the β6-20-CK_{30} peptide has the potential to be combined with traditional antibiotics for the improved treatment of *S. epidermidis* infections.

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