Targeted Binding of Material-Based *Staphylococcus epidermidis* Infections

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Statement of Purpose: *Staphylococcus epidermidis* is a coagulase negative, gram positive bacteria that has been implicated in the infection of many blood contacting biomaterials. One method by which *S. epidermidis* attaches to these surfaces is through plasma proteins such as fibrinogen, fibronectin, and vitronectin when they are encountered in biomaterial-based thrombus formations. It has been shown that the surface protein SdrG is present in nearly all clinical *S. epidermidis* isolates (McCrea, K.W. Microbiol-UK. 2000;146:1535-1546), and that this protein is responsible for the bacteria's ability to bind with fibrinogen (Pei, L. Infect Immun. 1999;67:4525-4530.). Specifically, the SdrG protein binds to a small region of the fibrinogen Bβ chain that can be readily synthesized in a laboratory setting (Davis, S.L. J Biol Chem. 2001;276:27799-27805.).

Development of a targeting ligand specific for *S. epidermidis* will allow for non-invasive *in vivo* identification and drug delivery directly to the site of infection. In these experiments, the potential for using a fibrinogen-based peptide as a ligand is explored. The peptide NEEGFFSARGHRPLD is synthesized and labeled at the amino terminus with a gold probe to provide visibility in a scanning electron microscope (SEM), and it is shown that the peptide is able to specifically target *S. epidermidis*.

Methods:

The fibrinogen-based peptide NEEGFFSARGHRPLD, representing amino acids 6-20 of the fibrinogen Bβ chain (referred to as the β6-20 peptide), was synthesized by standard solid phase peptide synthetic methods on an ABI 433a peptide synthesizer (Applied Biosystems, Foster City, CA), using 9-fluoronylmethoxycarbonyl (fmoc) amino acids on a Knorr resin. After purification by HPLC, the peptide was labeled for imaging using sulfo-N-Hydroxy succinimide NanoGold (Nanoprobes, Inc; Yaphank, NY). NanoGold is a discrete, uncharged gold label 1.4nm in diameter containing a single sulfo-N-Hydroxy succinimide moiety which is able to covalently bind to primary amines.

S. epidermidis strain RP-62A was used in all experiments in this study. The bacteria were cultured in a Tryptic Soy Broth (TSB) solution, after which the cells were rinsed and their concentration adjusted to $1x10^8$ cfu/ml. Glass substrates were then seeded with the bacteria solution and incubated at 37ºC, followed by binding with either unlabeled peptide, NanoGold labeled peptide, or both unlabeled and NanoGold labeled peptide. The 1.4nm NanoGold particles were then enhanced to approximately 50nm in order to make them clearly visible in the SEM using GoldEnhance (Nanoprobes, Inc; Yaphank, NY).

Sample surfaces were imaged using a Hitachi S-4500 Scanning Electron Microscope.

Results/Discussion:

To test the binding ability of the β6-20 peptide, the NanoGold labeled peptide was incubated directly with the growing bacteria. Using SEM, NanoGold beads are visible as small, bright circular objects approximately 20-50nm in diameter. The presence of NanoGold particles on the *S. epidermidis* (Figure 1) indicates that the peptide was able to bind to the bacteria.

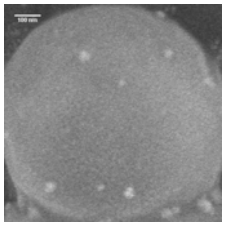

Figure 1 - NanoGold labeled peptide bound to *S. epidermidis*. Scale bar is 100nm.

In order to demonstrate the specificity of the β6-20 peptide, unlabeled peptide was first added to the bacteria, followed by a rinsing step and the subsequent addition of the NanoGold labeled peptide. The resulting SEM analysis shows a complete lack of NanoGold particles visible on the bacteria. This indicates that the unlabeled peptide occupied all of the potential β6-20 binding sites prior to the addition of the NanoGold labeled peptide, suggesting that the β6-20 peptide is able to specifically bind to *S. epidermidis*.

Conclusions: In these experiments, NanoGold labeling and SEM have been used in order to demonstrate that the fibrinogen-based β6–20 peptide, NEEGFFSARGHRPLD, is able to bind specifically to *Staphylococcus epidermidis* in a ligand-receptor manner. Additional control studies are currently underway, and future work will serve to further characterize the binding capabilities of this peptide. The overall goal of this project is to develop a peptide which can be used to deliver therapeutic agents directly to sites of biomaterial-based infections. There is also the potential that this peptide could instead carry an imaging agent that would allow for the non-invasive identification of *S. epidermidis* infections *in vivo*. If successful, a similar technique could be applied to both identify and treat infections of other bacterial species.