Engineered Fibrin Nanoparticles for Efficient Drug Delivery to Biofilms

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Statement of Purpose: Fibrin-based bacterial biofilms such as those generated by *Staphylococcus aureus* (*S. aureus*) are a significant detriment to wound healing and medical device implantation, and are prevalent in diseases such as endocarditis and septic arthritis. The biofilm protects bacteria embedded within, and functions as a niche for bacterial survival and expansion. In the case of *S. aureus*, the bacteria hijack the host's clotting machinery to form a fibrin-based extracellular matrix by secreting the enzyme coagulase to activate prothrombin, which then generates thrombin to form an insoluble fibrin matrix. The fibrin-binding receptor ClfA present on the surface of *S.* aureus is used by bacteria to cause aggregation and subsequent biofilm development. This matrix functions as a barrier to stymie drug penetration and reduces metabolic and reproductive rates of bacteria, significantly limiting the efficacy of antimicrobial drugs and hindering healing in the affected area. Our group has fabricated Fibrin Based Nanoparticles (FBNs) which are capable of antibacterial drug loading and release, as well as integration with *S. aureus* biofilms. We hypothesize that FBNs will be incorporated directly by the bacteria as a component of their biofilms, leading to enhanced penetration of drugs and increased therapeutic effectiveness compared to free drug. Here we evaluate dual-loading of FBNs with biofilm-degrading and antimicrobial molecules to enhance their activity against biofilms.

Methods: FBNs were fabricated by forming a 5 mg/ml fibrin clot. The clot was then sonicated on ice and strained to remove impurities. The resulting FBNs were then frozen, lyophilized, and rehydrated at specific concentrations. FBNs were characterized using a NanoSight NS300 to obtain hydrodynamic radius and an Atomic Force Microscope to obtain topography information. Alexafluor 647-FBNs were incubated with Baclight Green-stained WT *S. aureus* and ClfA$^-$ *S. aureus* to study bacterial binding of FBNs in the absence of a fibrin(ogen) binding receptor. Alexafluor 647-FBNs and Alexafluor 594-fibrinogen were incubated with a GFP expressing *S. aureus* strain otherwise similar to WT (unlabeled and fluorescent fibrinogen added at the time of inoculation and FBNs 24 hrs later) and imaged using confocal microscopy to determine bacterial incorporation of FBNs and exogenous fibrinogen into a biofilm matrix. Lyophilized FBNs were loaded with 5 µM fluorescent vancomycin and/or 29 µg/ml tissue Plasminogen Activator (tPA). Loading and release parameters were determined for samples obtained over the period of a week. Vancomycin concentration was determined using a fluorescence plate reader assay while tPA concentration was determined using a clot degradation assay, and both assays were compared to standards.

Results: FBNs were found to have a mean hydrodynamic diameter of 225 ± 9.2 nm, dry diameter of 192 ± 81 nm, and dry height of 29.8 ± 3.26 nm. Using the two strains of Baclight Green-stained *S. aureus*, we determined that the fibrin binding receptor ClfA is crucial to enabling binding of bacteria to FBNs. The strain without the fibrin(ogen) binding receptors (Δ*clfA*) did not bind with FBNs, while the WT strain did. Using a GFP expressing *S. aureus* strain, we demonstrated the ability of FBNs and exogenous fibrinogen to be integrated into an *S. aureus* biofilm matrix. We then loaded FBNs with vancomycin and/or tPA; results of loading and release indicated a burst release followed by a sustained release profile for both drugs for up to 72 hrs.

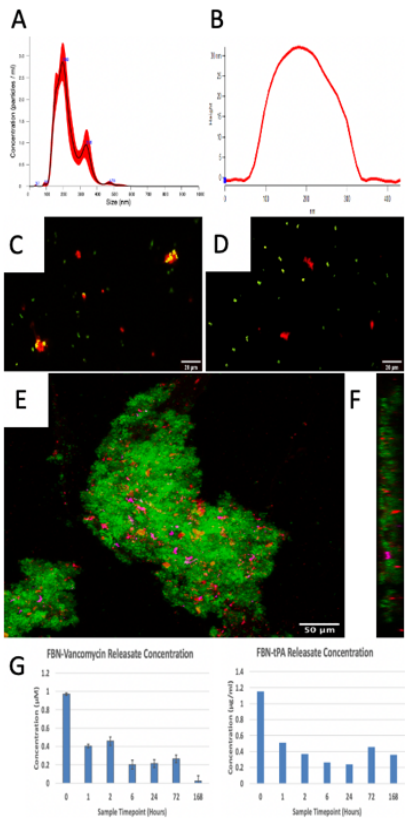

Figure 1: A) Nanosight analysis of FBNs; (B) AFM height trace of FBNs; (C) 647-FBNs incubated with Baclight Green-stained WT *S. aureus*; merged colors indicate binding; (D) 647-labeled FBNs incubated with Baclight Green-stained ClfA$^-$ *S. aureus*; image indicates lack of binding; (E) 647-FBNs, 594-fibrinogen, and unlabeled fibrinogen incubated with GFP *S.* aureus; image indicates binding of fibrin(ogen) molecules on the surface and throughout the colony. (F) Orthogonal view of the bacterial colony in image (F); (G) Results of FBN Vancomycin and tPA loading.

Conclusions: We have successfully engineered FBNs for efficient drug delivery into biofilms. We have demonstrated that *S. aureus* is capable of recognizing and binding FBNs in a similar fashion as native fibrin, which requires access to the fibrin(ogen) binding receptor ClfA found in WT *S. aureus*. We have shown that FBNs, when added to *S. aureus* biofilms in an exogenous fibrinogen matrix, can be found throughout and therefore deeply penetrate the biofilm community. Finally, we have characterized the loading and release kinetics of FBNs with antibacterial and thrombolytic drugs and determined that FBNs exhibit an initial burst release followed by 72 hrs of sustained release. This data indicates that FBNs are a suitable particle for incorporation into fibrin-based biofilms, and have the potential for loading and release of drugs to enhance the anti-biofilm properties of antistaphylococcal antibiotics such as vancomycin. Future studies will focus on conducting biofilm killing assays *in vitro* to assess the ability of loaded FBNs to combat bacteria within biofilms.