ROBUST BIOFILM DETECTION IN HUMAN CHRONIC WOUND TISSUE EMPLOYING MOLECULAR DIAGNOSTICS

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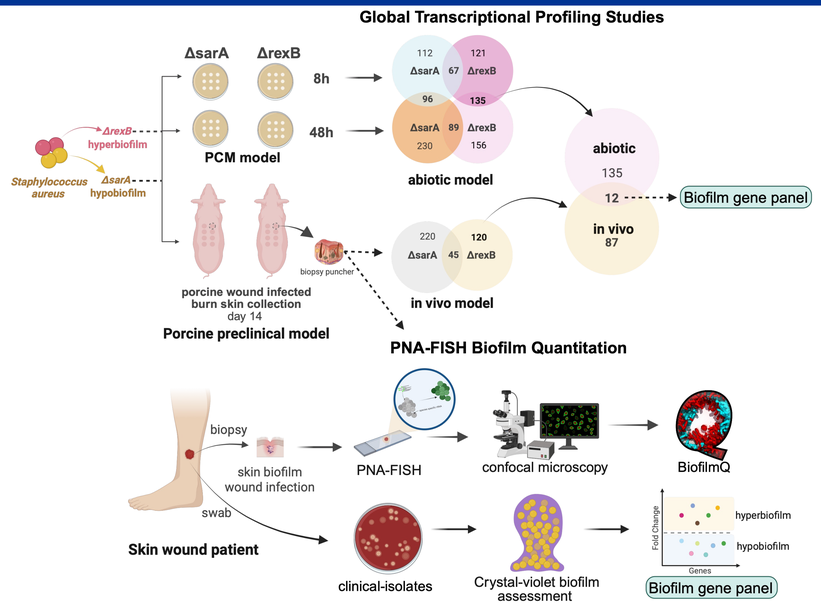
ABSTRACT

Background. Chronic wound biofilm infection (BFI) complicates healing, thus elevating the risk of amputation, sepsis, and death. Developing specific and sensitive clinical BFI assessment tools is of critical significance. RT-PCR is commonly used in clinical diagnostics. In deciding the diagnostic panel of genes to detect BFI, it is not enough to select biofilm-specific genes, and such candidates are known to change based on the biofilm microenvironment, *e.g.,* abiotic *versus* immune-supported wound *in vivo*. To identify BFI-diagnosing candidate gene panel relevant to the wound *in vivo,* microbial bulk RNA sequencing was performed using immune-competent porcine BFI-wound versus biofilm on abiotic surfaces. **Methods.** Global transcriptional profiling studies were performed in biofilm formed by two isogenic transposon mutants of *Staphylococcus aureus*: ΔrexB (hyperbiofilm) and ΔsarA (hypobiofilm). Both mutants were exposed to identical conditions *in vitro* (abiotic static biofilm model) and *in vivo* (preclinical porcine model) to identify microbial transcript signatures associated with BFI (n=4). Parallelly, we identified microbial transcript signatures from clinical chronic wound tissues (n=8). To gain insight into the spatial information on BFI in wound tissue, we developed a method integrating bacteria-specific PNA-FISH probes with BiofilmQ software to quantify biofilms. **Results.** Transcriptome analysis of *Staphylococcus aureus* mutants unveiled unique (abiotic *versus* porcine preclinical) transcript patterns that are uniquely associated with BFI (FDR p<0.05; FC ≥ 2; n=4). Notably, 20 genes were uniquely expressed in the *in vivo* wound environment and 18 genes consistently showed differential expression across biofilm conditions (FDR p<0.05; FC ≥ 2). Leveraging these biofilm-specific transcript panel signatures, BFI phenotypes of the clinical isolates from chronic wounds were successfully graded as low or high BFI. The *S. aureus*-specific PNA-FISH probes could detect *S. aureus* infections in wound tissue and display the structures of biofilms in the spatial context of the wound. Combined with BiofilmQ analysis, the biofilm abundance was quantified precisely in porcine and clinical chronic wound tissues. This molecular-based approach enabled us to robustly monitor the progression of BFI in clinical chronic wounds over successive visits. **Conclusion.** This work demonstrates that the bacterial gene expression profiles during BFI are distinct in an *in vivo* environment as compared to abiotic surfaces, indicating that the host immune-supported wound environment plays a crucial role in shaping wound tissue BFI. Such information is critical and should be considered while designing BFI diagnostic panels utilizing transcript-based molecular approaches. Integrating transcript panel methods with quantitative molecular imaging offers a powerful approach for BFI diagnostics in chronic wounds.

OBJECTIVE

This study aims to develop transcriptome-based molecular imaging to quantify and assess biofilm infections in chronic skin wounds, advancing diagnostic and therapeutic strategies in wound care.

STUDY DESIGN



RESULTS

Dynamic Transcriptional Regulation Shapes Biofilm Phenotypes in *S. aureus*

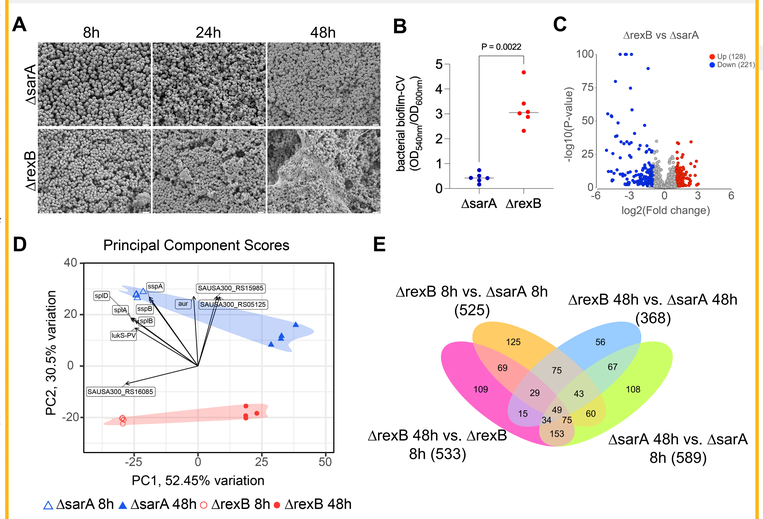


Figure 1. Dynamic changes in gene expression profile across early and mature stages of *S. aureus* biofilm formation. **A.** Scanning electron microscope (SEM) images showing static biofilm from isogenic strains of *S. aureus* USA300::rexB (ΔrexB) or USA300::sarA (ΔsarA) at 8, 24, and 48 hours on PCM membranes. **B.** S. aureus ΔrexB strain has higher biofilm production (normalized by growth). **C.** Volcano plot displaying upregulated and downregulated differentially expressed genes (DEGs) by comparing ΔrexB versus ΔsarA strains at 8 (early) and 48 hours (mature) on PCM membranes. Each dot represents one gene. The DEGs were considered to be those with a 2-fold change and a p-value less than 0.05. **D.** Principal Components Analysis of DEG of S. aureus ΔrexB and ΔsarA at 8 and 48 hours under static biofilm conditions. **E.** Venn diagram showing the overlapping differentially (FDR p<0.01 and fold change ≥2) regulated genes. Data are mean ± SEM (n = 4-6).

Host Immune-supported Wound Environment Plays a Crucial Role in Shaping *S. aureus* Transcripts in Wound Tissue BFI

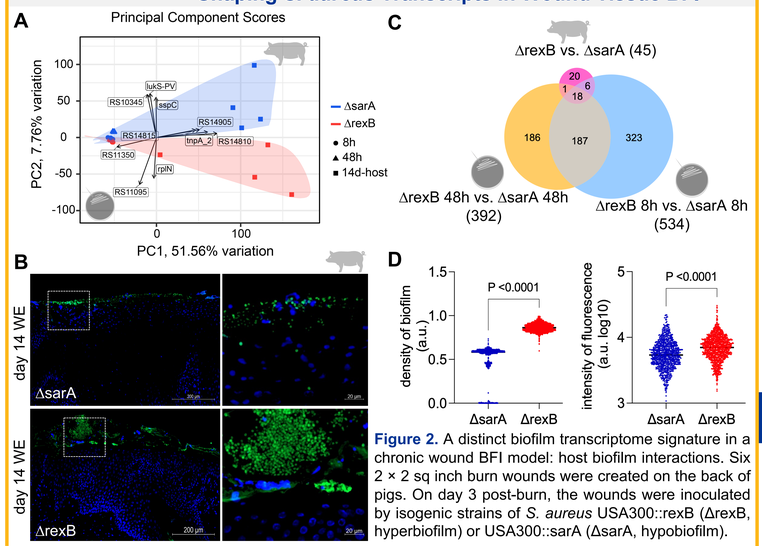


Figure 2. A distinct biofilm transcriptome signature in a chronic wound BFI model: host biofilm interactions. Six 2 × 2 sq inch burn wounds were created on the back of pigs. On day 3 post-burn, the wounds were inoculated by isogenic strains of *S. aureus* USA300::rexB (ΔrexB, hyperbiofilm) or USA300::sarA (ΔsarA, hypobiofilm).

On day 14 post-infection, the tissue was harvested for BFI bulk transcriptome study and PNA-FISH analysis. **A.** Principal Components Analysis (PCA) was performed on the DEGs from non-host at early and mature (8 and 48 hours) biofilms on PCM and host (porcine preclinical wound) mature BFI mature (14 days post-infection). **B.** Venn diagram displaying the overlapping DEGs (FDR p<0.05; fold change ≥ 2) for abiotic surfaces and in vivo biofilms. **C.** Representative confocal laser scanning microscopy images from burn wounds 14 days post-infection showing aggregates of *S. aureus* (PNA-FISH probe, green). Counterstaining was performed using DAPI (blue, nucleus). **D.** Quantitation of biofilm abundance in infected wound edge (WE) tissue on day 14 as determined using PNA-FISH and BiofilmQ software. Data are mean ± SEM (n = 4).

Biofilm Gene Panel Distinguished the Biofilm Phenotype of *S. aureus* Clinical Isolates

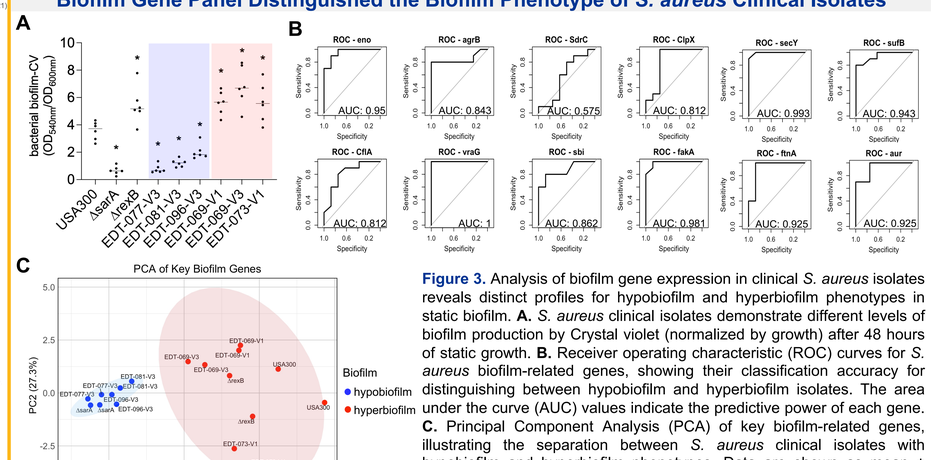


Figure 3. Analysis of biofilm gene expression in clinical *S. aureus* isolates reveals distinct profiles for hypobiofilm and hyperbiofilm phenotypes in static biofilm. **A.** *S. aureus* clinical isolates demonstrate different levels of biofilm production by Crystal violet (normalized by growth) after 48 hours of static growth. **B.** Receiver operating characteristic (ROC) curves for *S. aureus* biofilm-related genes, showing their classification accuracy for distinguishing between hypobiofilm and hyperbiofilm isolates. The area under the curve (AUC) values indicate the predictive power of each gene. **C.** Principal Component Analysis (PCA) of key biofilm-related genes, illustrating the separation between *S. aureus* clinical isolates with hypobiofilm and hyperbiofilm phenotypes. Data are shown as mean ± SEM (n = 8); p < 0.05.

Robustly Monitor the Progression of BFI in Clinical Chronic Wounds Over Successive Visits

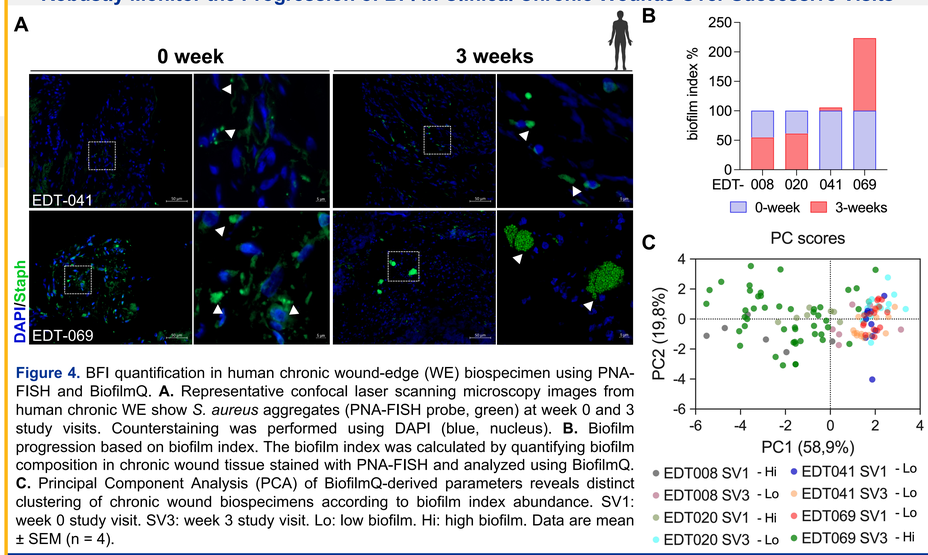


Figure 4. BFI quantification in human chronic wound-edge (WE) biospecimen using PNA-FISH and BiofilmQ. **A.** Representative confocal laser scanning microscopy images from human chronic WE show *S. aureus* aggregates (PNA-FISH probe, green) at week 0 and 3 study visits. Counterstaining was performed using DAPI (blue, nucleus). **B.** Biofilm progression based on biofilm index. The biofilm index was calculated by quantifying biofilm composition in chronic wound tissue stained with PNA-FISH and analyzed using BiofilmQ. **C.** Principal Component Analysis (PCA) of BiofilmQ-derived parameters reveals distinct clustering of chronic wound biospecimens according to biofilm index abundance. SV1: week 0 study visit. SV3: week 3 study visit. Lo: low biofilm. Hi: high biofilm. Data are mean ± SEM (n = 4).

CONCLUSION

This work demonstrates that bacterial gene expression profiles during biofilm infection (BFI) differ markedly in vivo compared to growth on abiotic surfaces, highlighting the pivotal role of the host immune-supported wound environment in biofilm development. Recognizing these differences is essential for advancing transcript-based diagnostic panels for biofilm infections. Furthermore, integrating transcriptomic panel approaches with quantitative molecular imaging represents a promising strategy for diagnosing biofilm infections in chronic wounds.