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CORR Insights

CORR Insights®: Does Extracellular DNA Production Vary in Staphylococcal Biofilms Isolated From Infected Implants versus Controls?

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Where Are We Now?

Biofilm formation is considered an important factor in determining the virulence of most implant-related infections [2]. Despite studies attempting to clarify its construction and role [4, 5], the nature of bacterial biofilms remain only partially revealed. Extracellular polymeric substances, an important part of biofilm, contribute to the organization of biofilms. However, recently, extracellular DNA (eDNA) has materialized as a possibly relevant structural component of biofilms [12]. eDNA stabilizes biofilm matrix and gene-transfer mechanisms, conditions innate immune responses, prevents phagocytosis, reduces inflammation and promotes antimicrobial resistance [5]. Although eDNA has been widely recognized as a critical factor in biofilm formation, its implications in clinical settings is less understood.

In their current study, Zatorska and colleagues found that *Staphylococcus aureus* and *S epidermidis* had differential production of eDNA with time. The difference could be due to the mechanism of eDNA release in both strains. For example, *S aureus* eDNA originates from cell lysis and constitutes a necessary part of biofilm development; whereas for *S epidermidis*, the autolysin protein AtlE mediates eDNA release and biofilm initiation.

The most-important finding of this study is that clinical isolates of *S aureus* and *S epidermidis* produced substantially more eDNA than non-clinical control isolates. Interestingly, a previous study [8] demonstrated that clinical strains of *S epidermidis* and the biofilm forming strain RP62A produced an abundance of eDNA compared to weak biofilm forming strains. Another study [9] examining 55 clinical isolates of *S epidermidis* from postsurgical and biomaterial-related orthopaedic infections showed remarkable eDNA variability. These findings indicate the presence of eDNA and its structural role in the development of biofilms in clinical strains.

This CORR Insights® *is a commentary on the article* "Does Extracellular DNA Production Vary in Staphylococcal Biofilms Isolated From Infected Implants versus Controls?" *by Zatorska and colleagues available at:* DOI: 10.1007/s11999-017-5266-0.

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Where Do We Need To Go?

In view of these variations, it is important to establish uniform, standardized methods to study clinical isolates with respect to the growth conditions, biofilm development, and eDNA quantification. Specifically, the production of eDNA varies by bacterial strain, age of the biofilm, and growth conditions. Therefore, standardized methods of biofilm formation and eDNA quantification is required in order to better understand the importance of eDNA in clinical isolates.

The current study touches on many of the biofilm-associated, eDNA-specific issues that still need to be more-thoroughly addressed. We know that *S aureus* and *S epidermidis* readily colonize implantable medical devices by formation of biofilms that are often difficult to treat with conventional antimicrobials. One of the mechanisms of this antibiotic tolerance is due to the presence of eDNA in biofilms [3]. This study leaves us with important research questions: (1) What is the role of eDNA in orthopaedic device-related infections? (2) What factors contribute to the difference in the amount of eDNA in clinical and laboratory strains? (3) What impact will eDNA have on antibiotic tolerance and resistance in clinical settings?

Future studies should focus on the implications of eDNA in establishing biofilm-related infections and whether eDNA can be a possible diagnostic tool for the detection of clinical biofilms, as well as a target for successful treatment of Staphylococcal biofilm infections.

How Do We Get There?

In order to answer the above questions, we need standardized techniques for examining clinical biofilms and biofilm-associated eDNA. The Center for Biofilm Engineering at Montana State University [7] and a few other research laboratories such as Costerton Biofilm Center in Copenhagen, Denmark [11] and Singapore Centre for Environmental Life Sciences Engineering (SCELSE) in Singapore [10] are working in their capacity towards the development, validation, and standardization of biofilm methods. Standardized methods and clinically relevant models are extremely important for reliability of results [1, 6]. For example, if the standardized methods are not followed, there could be discrepancies between testing conditions, making it difficult to compare data across multiple research laboratories. Additionally, some in vitro models that are not relevant to an intended application fail when applied in vivo at the clinical level. Therefore, uniform and relevant standardized methodology must be followed while studying clinical biofilms.

Future studies should broaden the number and traits of species that we investigate concerning eDNA and biofilm properties and also focus on pathogens such as *Propionibacterium acnes* and its relationship with periprosthetic joint infections. Further, the use of eDNA-specific dyes such as TOTO-1, which is cell impermeable and has high DNA-binding affinity, instead of short-half-life SYTO 60 nonspecific dye is important in order to avoid interference of bacterial cellular DNA while quantifying eDNA. While working with clinical biofilms, multiple methods of biofilm estimation and eDNA quantification must be followed. For example, molecular techniques to quantify production of eDNA coupled with microscopy (fluorescent/confocal scanning microscopy) should be used while studying clinical samples.

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