Centers of Advanced Medicine
Center for Biofilm Science and Technology

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General Summary

The Jikei Center for Biofilm Science & Technology (JCBST) was established in April 2015 as a member of the Centers of Advanced Medicine of The Jikei University with the support of the Ministry of Education, Culture, Sports, Science and Technology-Supported Program for the Strategic Research Foundation at Private Universities.
Biofilms are intricate communities of microbes that form on biotic and abiotic surfaces. Within biofilms, microbes are embedded in a typically self-produced extracellular matrix composed of proteins, polysaccharides and/or DNA, which provides microbes survival advantages in stressful environments. Thus, biofilms formed on the surfaces of medical devices and tissues can often cause chronic so-called biofilm-associated infections. The JCBST, based on collaboration with basic and clinical research laboratories, aims to promote research for understanding molecular mechanisms of biofilm formation and for the prevention and control of biofilm-associated infections.

Research Activities

Norgestimate inhibits staphylococcal biofilm formation and resensitizes methicillin-resistant Staphylococcus aureus *to β-lactam antibiotics*
High-throughput screening identified norgestimate (NGM), which is a synthetic progestin, as an inhibitor of biofilm formation of staphylococcal strains, including MRSA. NGM inhibited production of polysaccharide intercellular adhesin and proteins in the extracellular matrix. Proteome analysis of *S. aureus* indicated that NGM represses the expression of the cell wall-anchored protein SasG, which promotes intercellular adhesion, and of the glycolytic enzyme enolase, which plays a secondary role in biofilm formation. Notably, NGM induces remarkable changes in cell wall morphology, characterized by increased thickness and abnormal rippled septa. Furthermore, NGM increases the expression level of penicillin binding protein 2 and resensitizes MRSA to β-lactam antibiotics.

Composition and structure of biofilms formed by Propionibacterium acnes *isolated from cardiac pacemaker devices without clinical signs of infection*
Culture tests using a simple stamp culture method pressing pacemakers against the surface of agar plates revealed frequent *Propionibacterium acnes* colonization on the surface of cardiac pacemaker devices. *P. acnes* was isolated from 7/31 devices, and the isolates were categorized by multilocus sequence typing into five different sequence types (STs) and unknown ST. An in vitro biofilm formation assay using microtiter plates demonstrated that 6/7 isolates formed biofilms. DNase I completely inhibited biofilm formation by all *P. acnes* isolates, whereas susceptibility to proteinase K and dispersin B varied among strains. Ultrastructural analysis of *P. acnes* biofilms revealed leakage of cytoplasmic components along with cell lysis and fibrous structures of extracellular DNA connecting cells.

Imaging of biofilms in solution by atmospheric scanning electron microscopy
In this study, we visualized aqueous biofilms formed by the Gram-positive coccus *Staphylococcus aureus* and the Gram-negative bacillus *Escherichia coli* by recently developed atmospheric scanning electron microscopy (ASEM). Membrane vesicles, delicate spiral flagella, straight curli fibres, extracellular adherence protein (Eap), and filamentous extracellular DNA networks were observed by ASEM with labelling methods such as labelling with positively charged Nanogold, heavy metals, and immuno-gold. ASEM observation suggested modes of actions of biofilm inhibitory small compounds and enzymes. Collectively, our results suggest that ASEM is a broadly applicable approach for microbial research and diagnostic purposes.

Exploration of novel physiological functions of polyphenols
In this study, we identified myricetin (Myr), a kind of polyphenol produced by plants, to effectively prevent biofilm formation by *E. coli* and *S. aureus* including methicillin-resistant strains, in a dose-dependent manner. In addition, a more effective Myr-derivative with approximately 10-fold higher activity than Myr was identified. Transmission electron microscopy and Western blot analyses demonstrated that the Myr-derivative prevented curli production by suppressing the expression of curli-related proteins. Taken together, these results provide valuable insights into the development of drugs to treat biofilm-associated infections.

Importance of extracellular RNA in bacterial biofilms
We recently explored that extracellular RNA (eRNA) exists in *S. aureus* biofilms and co-localizes with polysaccharides in the extracellular matrix. In addition, eRNA localized around cells in biofilms, suggesting that polysaccharides and other components are important for retention of eRNA in biofilms. In this study, we constructed a mutant strain defective in the synthesis of teichoic acids, which are universally present in Gram-positive bacterial outer surfaces. The mutant strain did not retain eRNA within the biofilm, indicating that teichoic acids are also involved in eRNA retention in the biofilm. NGS and mutational analyses demonstrated that formation of secondary structures and presence of uracil are crucial for eRNA to promote biofilm formation, suggesting diversity of biofilm-

associated RNA sequences. Indeed, human blood RNA promoted biofilm formation as purified eRNA did. Collectively, these findings provide novel insights into mechanisms of eRNA-dependent staphylococcal biofilm formation, host-microbe interaction, development of biofilm-associated infections.

Redundancy and complexity in biofilms

We previously demonstrated that MR23, a clinical strain of MRSA, forms a robust proteinaceous biofilm containing a large amount of Eap that is an *S. aureus*-specific secreted protein and promotes biofilm formation. However, deletion of *eap* did not affect biofilm biomass, suggesting presence of other genes responsible for biofilm formation in MR23. To address this, single and multiple deletions of genes involved in biofilm formation were conducted. The combined deletion of *eap* and the gene encoding the cell wall-anchored protein SasG reduced biofilm biomass, whereas single deletion of *sasG* did not. CLSM demonstrated that deletion of *eap* reduced roughness but not thickness of the biofilm, whereas that of *sasG* did not. In addition, combined deletion of *eap* and *sasG* remarkably decreased both roughness and thickness. The pathogenicity of Δ*eap* Δ*sasG* significantly decreased compared with wild type in a silk warm infection model. Our findings highlight the relationship between a secreted protein and a cell wall-anchored protein in *S. aureus* biofilm formation and pathogenicity.

Publications

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Reviews and Books

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