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 DNA which provides microbes survival advantages in stressful environments. Thus, biofilms formed on the surfaces of medical devices and tissues can often cause what are known as chronic 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 preventing and controlling biofilm-associated infections.

Research Activities

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 means of recently developed atmospheric scanning electron microscopy (ASEM). In addition, ASEM reveals polyfunctional nanofibril appendages that mediate attachment, filamentation, and filament adaptability in Fe/Mn-oxidizing bacterium *Leptothrix cholodnii*. Collectively, our results suggest that ASEM is a broadly applicable approach for microbial research.

Functional analysis for periplasmic proteases that degrade bacterial amyloid precursor proteins
Bacterial extracellular amyloid fibers, called Curli, are involved in biofilm formation and the colonization of *E. coli*. Previously, we reported that cytoplasmic molecular chaperone DnaK kept CsgA and CsgB, the major and minor structural components of Curli, in a translocation-competent state by binding to their signal peptides prone to aggregation. We also found that certain periplasmic proteases degraded CsgA and CsgB in the periplasmic

space. These findings provide new insights into the regulation of bacterial amyloid fibers.

Microscopic and bacterial analyses of biofilms formed in clogged biliary stents from patients

Endoscopic biliary stenting is the most common treatment for patients who have jaundice associated with malignant hepatobiliary tumors. However, recurrent jaundice is a major complication of biliary endoprosthesis insertion. Thus, stent removal and replacement with a new stent frequently occur as a consequence of device blockage caused by microbial biofilm formation and biliary sludge accumulation in the lumen. In this study, we are analyzing microbial biofilms formed in the stents by ASEM and confocal laser scanning microscopy. We are also characterizing bacteria isolated from a biliary stent removed from a patient.

Screening of S. aureus *biofilm inhibitors and mechanism of action studies*

Formation of *S. aureus* biofilms on medical devices can cause severe or fatal infectious diseases. To develop new methods for preventing and treating biofilm-associated infections, we have performed high-throughput screening of small compounds that inhibit biofilm formation by *S. aureus*. One of the screened compounds, JK1, suppressed production of extracellular polysaccharide that is important for biofilm formation. A pull-down assay of JK1-immobilized beads revealed that JK1 specifically binds to a protein that is involved in cell-wall synthesis of *S. aureus*. Substitution of amino acids in the active center of this protein significantly reduced both enzymatic activity and JK1-binding activity. Therefore, JK1 appears to bind to the active center of the target protein. We also evaluated the biofilm inhibitory activity of JK1 using a continuous flow biofilm model on a microfluidic device and revealed that JK1 was effective under flowing conditions.

Promotion of biofilm formation by external RNA

S. aureus often causes life-threatening infections, such as biofilm-associated infections, due to formation of robust biofilm. Understanding the molecular mechanisms of how biofilms form is important for developing medical countermeasures for these infections. We have previously found that RNA is a component of the biofilm formed by MR10, which is a clinically isolated strain of methicillin-resistant *S. aureus* and forms a robust biofilm in a polysaccharide-dependent manner. Microscopic, biochemical, and molecular interaction analyses showed that RNA directly binds with and co-localizes with polysaccharides in the biofilm. Additionally, RNA purified from human blood promoted biofilm formation under static and flow conditions. These results suggest that RNA serves as a biofilm scaffold during infection and that *S. aureus* might utilize blood RNA to form a strong biofilm on implanted medical devices or tissues, causing chronic infections.

Publications

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