Centers of Advanced Medicine
Center for Biofilm Science and Technology

Yuki Kinjo, *Professor and Director*
Katsuhiko Yanaga, *Professor*
Shoichi Uezono, *Professor*
Takeo Iwamoto, *Professor*
Ken Kaito, *Professor*
Shinya Sugimoto, *Associate Professor*
Tadayuki Iwase, *Associate Professor*
Ken-ichi Okuda, *Assistant Professor*
Ryuichi Nagahori, *Assistant Professor*
Seiji Hori, *Professor*
Keishi Marumo, *Professor*
Shin Egawa, *Professor*
Koji Takada, *Professor*
Tetsuya Horino, *Associate Professor*
Jun Araya, *Associate Professor*
Akiko Tajima, *Assistant Professor*
Noriyuki Murai, *Assistant Professor*
Midori Kono, *Assistant Professor*

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

Detection of bacterial DNA from central venous catheter removed from patients by next generation sequencing
Catheter-related infection (CRI) is a serious challenge in clinical practice. This study examined how well next-generation sequencing (NGS) targeting 16S ribosomal DNA is able, compared with the culture method, to identify causative pathogens in CRI. The genera identified with NGS were consistent with those from conventional culture tests. The culture method and NGS showed high agreement, and, in patients suspected to have CRI, had a sensitivity of 80.0% and a specificity of 76.9%; meanwhile, the false-positive rate of NGS was as low as 4.0% in patients who were judged as having no infection symptom by the physicians and whose catheter was removed because it was no longer needed. In conclusion, NGS targeting 16S ribosomal DNA was able to analyze the bacterial composition of central venous catheter specimens and detect causative pathogens in patients with CRI and was, therefore, suggested as a promising diagnostic tool for CRI.

Mechanisms of RNA stabilization in staphylococcal biofilms

Staphylococcus aureus often causes serious infections, such as biofilm-associated infections due to formation of robust biofilm. Understanding the molecular mechanisms of how biofilms form is important to develop new strategies against 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 an extracellular polysaccharide-dependent manner. Although RNA is generally recognized to be unstable in the environment, how it is stably retained in the MR10 biofilm is unclear. To address this question, interaction between RNA and polysaccharides was analyzed in the MR10 biofilm and in vitro. Biochemical, microscopic, and molecular interaction analyses demonstrated that RNA directly interacts with and co-localizes with polysaccharides in the biofilm. Additionally, genome sequence analysis suggested that *nucA*, encoding extracellular thermonuclease with DNase and RNase activities, is completely deleted in MR10. As expected, the plasmid-born intact *nucA* from the strain Newman abolished biofilm formation of MR10. These results suggest that production of extracellular polysaccharides and deletion of *nucA* are key for the retention of RNA in the biofilm at an extremely high level.

Role of extracellular adherence protein in establishment of rugged and thick structures of S. aureus *biofilms*

MR23, a clinically isolated strain of methicillin-resistant *S. aureus*, forms a biofilm containing a large amount of secreted extracellular adherence protein (Eap). So far, we have found that (1) Eap and the cell-wall-anchoring protein *S. aureus* surface protein G (SasG) work redundantly to form an extensive biofilm, (2) cell-wall-anchoring is required for SasG to promote biofilm formation, (3) SasG binds DNA and protects it from degradation, and (4) simultaneous deletion of the genes *eap* and *sasG* significantly reduce mortality against silkworms. In this study, we analyzed the roles of Eap and SasG for determining the 3-dimensional structure of the biofilm. Confocal laser scanning microscopy with thioflavin T-staining of biofilms revealed that deletion of *eap* reduced the roughness but not the thickness of the biofilm, whereas the deletion of *sasG* did neither. In addition, double knockout of *eap* and *sasG* decreased both roughness and thickness. These results suggest that Eap and SasG work redundantly in terms of the amount of the biofilm, while only Eap contributes to the complicated biofilm structures. These findings indicate the importance of simultaneously analyzing multiple molecules, which could lead to a deeper understanding of the mechanisms of biofilm formation.

Imaging of biofilms in solution by atmospheric scanning electron microscopy

In this study, we visualized aqueous biofilms formed by the Gram-positive coccus *S. aureus* and the Gram-negative bacillus *Escherichia coli* by means of recently developed atmospheric scanning electron microscopy (ASEM). Membrane vesicles, delicate spiral flagella, straight curli fibres, and filamentous extracellular DNA networks were observed with ASEM and labelling methods, such as labelling with positively charged Nanogold and heavy metals. In addition, surface adherence of *Paracoccus* sp. and *Leptothrix* sp. was analysed with ASEM. Collectively, our results suggest that ASEM is a broadly appli-

cable approach for microbial research and diagnostic purposes.

Quality control of the type 8 secretion system in E. coli

The *E. coli* type 8 secretion system is involved in the secretion and formation of extracellular Curli amyloid fibers. We found that Curli biogenesis depends on the molecular chaperone DnaK, a bacterial Hsp70 homolog, via a quantity and quality control of RpoS, a stationary phase-specific alternative sigma factor regulating bacterial transcription, and CsgD, the master transcriptional regulator of Curli formation. DnaK also keeps CsgA and CsgB in a translocation-competent state by binding to their signal peptides prone to aggregation. We also found that certain periplasmic chaperones and proteases cooperatively maintain the quality and quantity of CsgA and CsgB in the periplasmic space. In addition, we identified epigallocatechin gallate as an effective inhibitor of Curli biogenesis. These results provide mechanistic insights into Curli biogenesis and robust biofilm formation.

Publications

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