Centers of Advanced Medicine
Center for Biofilm Science and Technology

Yoshimitsu Mizunoe, *Professor and Director*
Kazuhiro Hashimoto, *Professor*
Keishi Marumo, *Professor*
Shin Egawa, *Professor*
Koji Takada, *Professor*
Tetsuya Horino, *Associate Professor*
Noriyuki Murai, *Assistant Professor*
Midori Kono, *Assistant Professor*
Tadayuki Iwase, *Assistant Professor*
Ken-ichi Okuda, *Assistant Professor*
Seiji Hori, *Professor*
Katsuhiko Yanaga, *Professor*
Shoichi Uezono, *Professor*
Takeo Iwamoto, *Professor*
Ken Kaito, *Professor*
Jun Araya, *Associate Professor*
Ryuichi Nagahori, *Assistant Professor*
Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *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. The JCBST will promote research for the prevention and control of biofilm-associated infections. Research projects of the JCBST have focused on: 1) identification of ABC-JK2, a small molecular inhibitor of staphylococcal biofilm formation, 2) exploration of novel physiological functions of polyphenols, 3) imaging of biofilms in solution by atmospheric scanning electron microscopy, 4) importance of extracellular RNA in bacterial biofilms, 5) genotypic and biofilm profiles of *P. acnes* isolated from pacemakers without clinical signs of infection, 6) redundancy and complexity in biofilms.

Research Activities

Identification of ABC-JK2, a small molecular inhibitor of staphylococcal biofilm formation

In this study, we aimed to identify compounds that inhibit biofilm formation by S. aureus. One of the hit compounds, named anti-biofilm-compound JK2 (ABC-JK2), inhibited biofilm formation of several strains of S. aureus including MRSA and S. epidermidis at IC50 range of 12.0 to 22.5 μM. Metabolomic analysis showed that ABC-JK2 decreases intracellular levels of glycolytic metabolites. Microarray/quantitative real-time PCR revealed up-regulation of genes related to peptidoglycan biosynthesis and hydrolases. In addition, TEM observation showed that bacterial cell wall thickness and number of abnormal septa are increased in the presence of ABC-JK2. It was suggested that ABC-JK2 inhibits staphylococcal biofilm formation by affecting glycolysis and cell wall synthesis.

Exploration of novel physiological functions of polyphenols

Exploration of potential functions of food constituents provides an additional value for

health as well as offers applications for preventing diseases. 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. Its mode of action is now elucidated.

Imaging of biofilms in solution by atmospheric scanning electron microscopy
In this study, we visualized biofilms immersed in aqueous solution, including biofilms formed by the Gram-positive coccus Staphylococcus aureus and the Gram-negative bacillus Escherichia coli by recently developed atmospheric scanning electron microscopy (ASEM). Since ASEM allows a biofilm cultured on electron-transparent film windows to be observed by an inverted SEM from below, it was possible to study biofilm formation near the substrate and the ECM at high resolution. Membrane vesicles, delicate spiral flagella, straight curli fibres, and filamentous extracellular DNA networks were observed by ASEM with labelling methods such as labelling with positively charged Nanogold, heavy metals, and immuno-gold. Collectively, our results suggest that ASEM is a broadly applicable approach for microbial research and diagnostic purposes.

Importance of extracellular RNA in bacterial biofilms
We recently explored presence of extracellular RNA (eRNA) in Staphylococcus aureus biofilms. In this study, we analyzed its roles in biofilm development. The molecular size of the eRNA was estimated 20 to 100 nucleotides by urea-denaturing acrylamide gel electrophoresis. We observed localization of eRNA in the three-dimensional structure of biofilm by confocal laser scan microscopy. In addition, degradation of polysaccharides, which are major components of S. aureus biofilms, induced the release of eRNA from the biofilm. Furthermore, we demonstrated by surface plasmon resonance that purified polysaccharides bound to eRNA, indicating that polysaccharides are important to maintain eRNA in the biofilm. Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology.

Genotypic and biofilm profiles of P. acnes *isolated from pacemakers without clinical signs of infection*
Colonization of bacteria on the surfaces of cardiac pacemakers explanted from patients without clinical evidence of infection was consecutively analyzed. P. acnes was isolated from pacemakers without clinical signs of infection at high frequency (23%). Biofilm forming capacities of the P. acnes isolates and biochemical properties of the biofilms were different among strains regardless of the STs, however, extracellular DNA was suggested to be a factor commonly involved in biofilm formation of diverse P. acnes strains. High-resolution observation of nanostructures in the biofilms by transmission electron microscopy and atmospheric scanning electron microscopy visualized cytoplasmic components leakage along with cell lysis and fiber structures connecting cells in biofilm.

Redundancy and complexity in biofilms
Biofilm-forming capacity is determined by a self-produced extracellular matrix (ECM).

Our recent studies demonstrated that MR23, a clinical isolated strain of methicillin-resistant S. aureus, forms a robust proteinaceous biofilm. In addition, extracellular adherence protein (Eap), an S. aureus-specific secreted protein, was found to be abundant in ECM and to promote biofilm formation by S. aureus. However, deletion of the eap gene did not affect biofilm formation, suggesting the presence of other genes responsible for biofilm formation in MR23. To address this, a number of single and multiple gene-deletion mutants were constructed. Interestingly, simultaneous deletion of eap and sasG encoding a cell wall-anchored protein reduced biofilm formation, but single deletion of sasG did not. These results suggested that Eap and SasG have an overlapping function each other. Elucidation of their roles in biofilm formation may lead to the development of specific treatment for S. aureus infections.

Publications

Sugimoto S, Okuda K, Miyakawa R, Sato M[1], Arita-Morioka K[2], Chiba A, Yamanaka K[2], Ogura T[2], Mizunoe Y, Sato C[1] ([1]AIST, [2]Kumamoto Univ). Imaging of bacterial multicellular behaviour in biofilms in liquid by atmospheric scanning electron microscopy. *Sci Rep.* 2016; **6:** 25889.

Iwase T, Ogura Y[1], Hayashi T[1] ([1]Kyushu Univ), Mizunoe Y. Complete Genome Sequence of Klebsiella pneumoniae YH43. *Genome Announc.* 2016; **4:** pii: e00242-16.

Iwase T, Ogura Y[1], Hayashi T[1] ([1]Kyushu Univ), Mizunoe Y. Complete Genome Sequence of Klebsiella oxytoca Strain JKo3. *Genome Announc.* 2016; **4:** pii: e01221-16.