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Biofilm Formation of *Staphylococcus epidermidis* With and Without Collagen Imaged Using Atmospheric Scanning Electron Microscopy and Antibacterial Effect of Ag-decorated Polymeric Particles Imaged by Transmission Electron Microscopy

Chisato Takahashi[1,2*], Keiichi Moriguchi[2], Miki Hori[2], Tatsushi Kawai[2], Mari Sato[3] and Chikara Sato[3]

[1.] National Institute of Advanced Industrial Science and Technology, Department of Materials and Chemistry, Innovative Functional Materials Research Institute, Nagoya, Aichi, Japan.
[2.] Aichi Gakuin University, School of Dentistry, Department of Dental Materials Science, Nagoya, Aichi, Japan.
[3.] National Institute of Advanced Industrial Science and Technology, Health and Medical Research Institute, Tsukuba, Ibaraki, Japan.
* Corresponding author: nagoya.u.takahashi@gmail.com

The biofilm formation of *Staphylococcus epidermidis* (*S. epidermidis*) is not well known although many researchers have reported that *S. epidermidis* biofilms are related to various infections [1,2]. Additionally, role of microvesicles and secretion of microvesicles of *S. epidermidis* have not revealed yet. Here, we studied the biofilm formation of *S. epidermidis* biofilms with and without collagen using atmospheric scanning electron microscopy (ASEM). The biofilm formation process was different in the cases of with and without collagen. To further study, antibacterial effect of pharmaceutical production against the biofilm was confirmed. In the present study, we applied Ag-decorated polymeric particles as nanocarriers of drug delivery system. We have prepared polymeric particles with high antibacterial effect to bacterial cells of biofilms [3,4]. However, time series of antibacterial activity of polymeric particles have not studied yet. Using TEM, we revealed efficacy of Ag-decorated polymeric particles to the bacterial cells under the biofilms from 30 min to 4 h and self-protection ability of *S. epidermidis* bacterial cells at nanoscale.

The bacterial cells in the present study were *S. epidermidis* (ATCC14990T). The biofilm formed in ASEM dishes after different cultivation times (17, 26, and 30 h) were fixed, stained, labelled, and imaged by ASEM. The details procedure was described elsewhere [5,6]. For observation of the biofilm formation, an inverted scanning electron microscope ASEM ClairScope™ (JASM–6200, JEOL Co., Japan) was used. For observation of Ag-decorated polymeric particles treated biofilm, TEM (1400 Plus, JEOL Co., Japan) was used. Biofilm of *S. epidermidis* bacterial cells were formed following a previously published procedure [3]. For preparation of Ag-decorated polymeric particles, PLGA (lactide:glycolide, 75:25; = molecular weight, 20,000 Da, Kuraray Co., Japan), $AgNO_3$ (Nacalai Tesque Inc., Japan), and $NaBH_4$ (Kishida Chemical Co., Japan) were used. Ag-decorated polymeric particles were prepared by the emulsion solvent diffusion method [7]. After confluent growth of the biofilm in a well, Ag-decorated particles were treated from 30 min to 4 h. The details procedure of ultrathin method in this study was described elsewhere [3].

We observed the *S. epidermidis* biofilm formation with and without collagen using ASEM. After 17 h of incubation, spherical shaped bacteria were observed in the both cases. Micro vesicles secretion from *S. epidermidis* bacteria was clearly observed in the both cases. MVs in the range of 100 nm - 1 µm were imaged using ASEM. After 30 h, the extracellular polymeric substance (EPS) film covered bacteria were observed in the case of the biofilm formation without collagen. On the other hand, a lot of nanotube–like structures were observed in the case of the biofilm formation with collagen. The sizes were several

hundred nm to several hundred μm in lengths and less than several dozen nm in diameter. In the case of the biofilm formation without collagen, sizes of nanotube–like structures were several hundred nm in lengths and several dozen nm in diameter. It was indicated that collagen has a role for growth of nanotube-like structures. To understand antibacterial activity of Ag-decorated PLGA particles to *S. epidermidis* biofilm, we observed Ag-decorated PLGA particles treated biofilm. It should be noted that the biofilm without any treatment was also observed. After 30 min treatment, Ag-decorated PLGA particles attached bacterial cells were observed. In this case, the damage between cell wall and cell membrane caused by Ag nanoparticles was observed. Ag nanoparticles have antibacterial activity that can induce the production of reactive oxygen species (ROS) such as superoxide radicals ($\bullet O_2-$), hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$). It is well known that the generated ROS inhibits the bacterial growth via lipid peroxidation and reaction with DNA, membrane proteins and enzymes [7]. After long time treatment such as 2 h and 4 h, the bacterial cells were drastically damaged and collapsed. On the other hand, Ag ejection from the bacterial cell was observed. In this case, Ag nanoparticles combined together and existed as agglomerate outside of the bacterial cells. The antibacterial activity of Ag-decorated PLGA particles and self-protection ability of bacteria were revealed in this study.

We revealed the biofilm formation of *S. epidermidis* under aqueous condition with and without collagen using ASEM. The mechanisms of the biofilm formation in the both cases were suggested based on ASEM results. Additionally, the antibacterial activity of Ag-decorated PLGA particles to the bacterial cells under the biofilm was visualized by TEM. According to treatment time of Ag-decorated PLGA particles, antibacterial activity against the bacterial cells was changed. At the same time, we found that Ag ejection from the bacterial cell. The self-protection of bacteria was also studied in the present study. This information can contribute for development of drug formation and treatments [8].

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