doi: 10.1017/S1431927609092228
Microsc Microanal 15(Suppl 2), 2009

Observing the Biofilm Matrix of *Staphylococcus epidermidis* ATCC 35984 Grown Using the CDC Biofilm Reactor

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Bacteria flourish in nearly every environment on earth. Contributing to their ability to grow in many esoteric locations is their development into a biofilm structure. A defining characteristic of biofilms is their production of an extracellular polysaccharide matrix that encapsulates the myriad, and often times plurality of cells within the biofilm. Importantly, traditional growth strategies using agar and broth largely select for planktonic (free-floating) cells whereas biofilms (sessile conglomerate of cells) develop more readily on surfaces exposed to shear forces in the presence of rich and renewable nutrients [1]. In an effort to more accurately model the growth environment of biofilms in nature, a Centers for Disease Control (CDC) biofilm reactor has been developed that mimics these shear forces and renewable nutrients. Notably, the matrix and/ or structures of biofilms grown using the reactor have not been reported using scanning electron microscopy (SEM). Therefore, SEM images were collected of *Staphylococcus epidermidis* ATCC 35984, a heavy matrix producer, grown using the reactor.

Formalin is the preferred fixative agent of many laboratories, yet a cationic dye/Glutaraldehyde/OsO_4 treatment is believed to preserve more of the biofilm matrix structure [2]. Therefore, biofilms that were grown in the reactor on a titanium substrate were fixed with either Ruthenium Red/Glutaraldehyde/OsO_4 or 10% buffered formalin and dehydrated. One sample was uncoated for imaging in low vacuum and two samples were coated with either gold or carbon for imaging in high vacuum. To collect the images, FEI's high resolution NOVA NanoSEM was used. In low vacuum mode, a secondary electron Helix detector was used whereas under high vacuum a backscatter vCD detector was used.

Previous imaging studies have used bacteria that were grown under stagnant broth or agar surface conditions [2-4]. Nevertheless, after being grown with the CDC biofilm reactor, the matrix components of *S. epidermidis* ATCC 35984 did appear to have similar matrix structures to these bacteria [2]. More specifically, after 48 hours of growth in the reactor the biofilm matrix consisted of complex networks of polymeric strands that form around the cells (Figure 1). However, in this study, large mushroom structures—characteristic of biofilms under shear force—were developed (Figure 2). The sharpest images were those of biofilms coated with gold and imaged under high vacuum (Figure 1). Furthermore, the matrix components were similar when imaged under high or low vacuum, yet high vacuum images were sharper (Compare Figures 1 and 3).

Similar to the findings of Erlandsen et al. [2], matrices that were stained/fixed using Ruthenium Red/Glutaraldehyde/OsO_4 appeared to preserve slightly more of the matrix than those fixed with 10% buffered formalin (Compare Figures 1 and 4). However, biofilms fixed with 10% buffered formalin did resolve matrix components displaying cell-surface and cell-cell attachments (Figure 4).

In conclusion, images of biofilms grown using the reactor suggest that a highly complex matrix is developed after 48 hours of growth. Although similar matrix structures are seen in bacteria grown using the traditional methods described previously, the attachment strength and matrix components of biofilms grown for longer periods of time in the two systems remains to be determined. Moreover, the development of large mushroom structures in the reactor is noticeably different than previous reports [2,4]. Results further indicate that, in this investigation, imaging under high vacuum is preferable after preservation of the matrix with a cationic dye/Glutaraldehyde/OsO_4 treatment.

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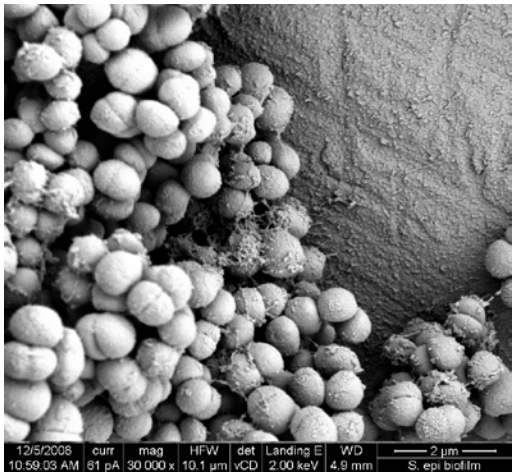

Figure 1: Complex matrix networks of the biofilm. Sample fixed with Ruthenium Red/Glutaraldehyde/OsO_4 and coated with gold.

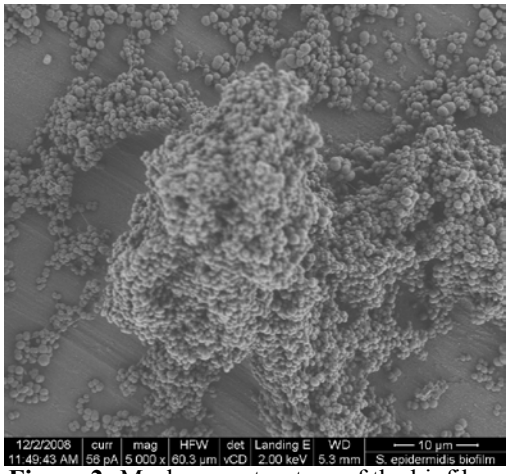

Figure 2: Mushroom structure of the biofilm. Sample fixed with 10% formalin and coated with carbon. Blurred portion indicates vertical rise of the biofilm toward the viewer.

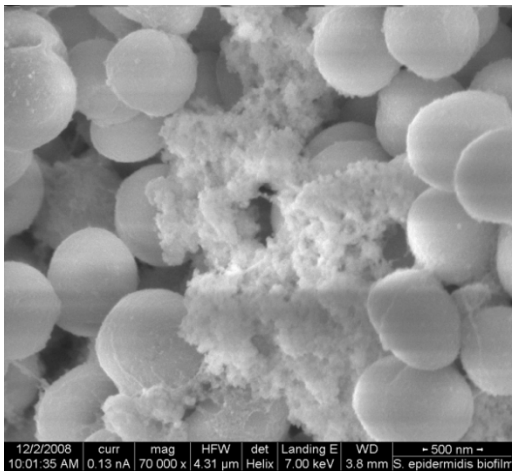

Figure 3: Low vacuum image of the biofilm matrix. Uncoated sample fixed with Ruthenium Red/Glutaraldehyde/OsO_4.

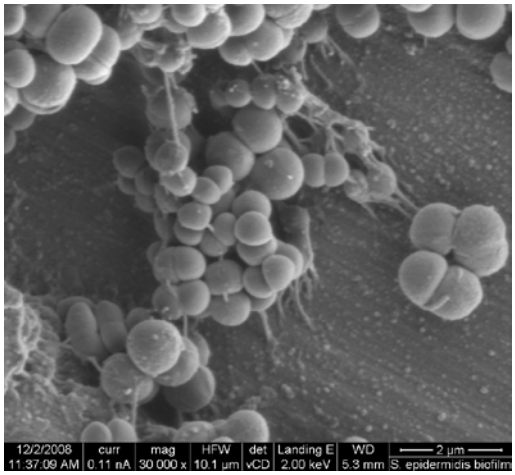

Figure 4: Cell-surface and cell-cell attachments of the matrix. Sample fixed with 10% formalin and coated with carbon.

https://doi.org/10.1017/S1431927609092228 Published online by Cambridge University Press