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STEM Observation of eDNA as a Dominant Component of EPS in *Pseudomonas aeruginosa* Biofilm

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Extracellular polymeric substances (EPS) are biofilm self-produced matrices that provide a protective shed for biofilm bacteria[1]. eDNA represents an important structural and functional component of EPS. Here we show that thread-like eDNA fill the space between bacteria throughout *Pseudomonas aeruginosa* prototypical strain PA01 biofilm (Figure 1).

Biofilm structures have been investigated by diverse microscopic methods, including fluorescence light microscopy, atomic force microscopy, transmission electron microscopy, and scanning electron microscopy [2-5]. Among them, confocal laser scanning microscopy is a powerful tool often used to study biofilm spatial structures. However, the resolution of confocal laser scanning microscopy limits its application to ultrastructural study of EPS. Most observation of eDNA and other EPS in biofilm are limited by resolution. In this work scanning transmission electron microscopy (STEM) is applied to the structural study of *in vitro P. aeruginosa* PA01 biofilm.

The biofilms were cultured on polycarbonate membrane in agar plate at 37°C for 48 hours. Biofilm discs were chemically fixed with 2.5% glutaraldehyde and 2% paraformaldehyde in 0.15M cacodylate buffer, and then *en bloc* stained with 2% reduced osmium tetroxide. After dehydration and infiltration, samples were embedded in 100% durcupan resin and incubated at 65°C for 2 days. The resin embedded biofilm discs were processed to 90nm ultra-fine sections by using a Reichert-Jung Ultracut E ultramicrotome (Leica, Wetzlar, Germany). The thin sections were picked up with a loop and put on 400 mesh copper grids. The grids were air dried and then coated with amorphous carbon (t=3nm) on both sides. Electron microscopy data were collected in STEM mode on a Tecnai F20 S/TEM (Thermo Fisher Scientific, Hillsboro) operating at 200kV.

In *P. aeruginosa* PA01biofilm, eDNA was reported as the major component of EPS[6]. Our results showed that thread-like EPS presented throughout the PA01 biofilm, with especially large concentrations in the region close to the culture base (Figure 1). Recently, Turnbull et al. reported that explosive lysis of *P. aeruginosa* contributed significantly to the eDNA contents of biofilm [7]. Our images also indicated the thread-like structures had morphological similarity to the bacterial internal content (Figure 1), suggesting that the thread-like structures are DNA.

P. aeruginosa biofilm is frequently associated with chronic wound infection. Using STEM imaging techniques, we were able to gain an insight into the structures of *P. aeruginosa* PA01 biofilm. The structure provided the biofilm architecture for better understanding the biofilm EPS organization, which is likely to serve as structure basis for further therapeutic intervention [8].

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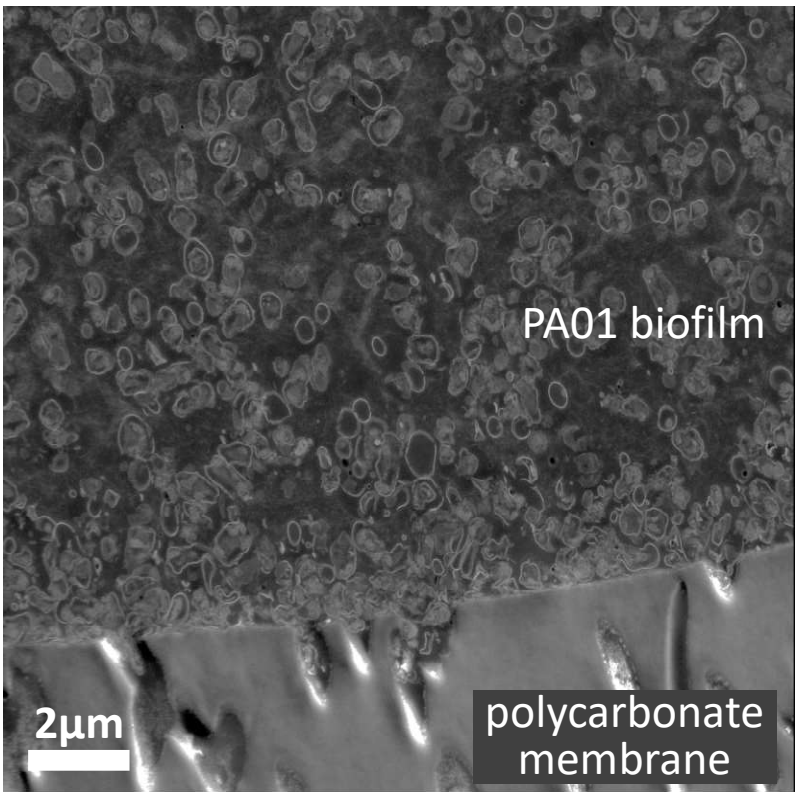

Figure 1. STEM image of PA01 biofilm showed thread-like eDNA structures. Scale bar = 2µm. The EM sample section is 90nm thick. The culture base is polycarbonate membrane.

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