Type of presentation: Poster

LS-6-P-5788 Immunolabeling bacterial biofilms

Webster P.[1], Kerwin J.[2], Wu S.[3]

[1]University of Southern California, Los Angeles, CA, USA, [2]UCLA, Los Angeles, CA, USA, [3]Lawrence Berkeley National Laboratory, Berkeley, CA, USA

Email of the presenting author: pwebster@usc.edu

Bacterial biofilms are composed of bacterial cells embedded in an extracellular matrix (ECM) of proteins, DNA and polysaccharide. Immobilization of extracellular antigens is an essential prerequisite for immunocytochemical studies. Immunolabeling is performed at ambient temperature so the optimal approach for preparing biofilms is by high pressure freezing, freeze substitution and embedding in a low temperature resin such as Lowicryl HM20 [1].

We used this approach to probe in vitro formed biofilms of non-typeable Haemophilus influenzae (NTHi), a human respiratory tract and middle ear pathogen. To identify suitable target proteins for study we performed a proteomic analysis of the ECM to identify biofilm-specific proteins, when compared with a similar list from planktonic (or non-biofilm) bacteria. Antibodies to biofilm proteins were applied to sections. However, most polyclonal antibodies reacted non-specifically to bacterial proteins, most likely because they were produced using bacteria-containing adjuvants. In order to successfully label biofilm sections we could only use polyclonal antibodies that had been targeted to purified bacterial proteins, or monoclonal antibodies to similar proteins expressed by eukaryotic cells.

Monoclonal antibodies were visualized with a secondary, polyclonal antibody followed by protein A-gold. To overcome the non-specific binding of secondary antibodies to bacterial proteins we added suspensions of lysed bacteria to antibody blocking solutions, an effective way to remove binding to bacterial proteins.

Eighteen biofilm-specific proteins were identified in ECM and all proteins were either associated with bacterial membranes or were cytoplasmic proteins. Immunocytochemistry showed two of the identified proteins, a DNA-directed RNA polymerase and the outer membrane protein OMP P2, present in bacteria and biofilm ECM. The amount of labeling present was affected by the age of the biofilm and the location within the biofilm. The different labeling patters could be illustrated by quantitative analysis, performed using a free stereology program [2].

Identification of biofilm-specific proteins present in the ECM of immature biofilms is an important step in understanding the in vitro process of NTHi biofilm formation. The presence of a cytoplasmic protein and a membrane protein in the biofilm ECM of immature NTHi biofilms suggests that bacterial cell lysis may be a feature of early biofilm formation.

1. Webster et al (2004) Ultrastructural preservation of biofilms formed by non-typeable Hemophilus influenzae. Biofilms 1: 165-182.

2. Tschanz etal (2011) A simple tool for stereological assessment of digital images: the STEPanizer. J Microsc 243: 47-59.

Acknowledgement: The work was supported by the NSF (grant #0722354), the NIDCD (5 P-30 DC006276-03), the Fritz Burns Foundation, the Hope for Hearing Foundation, the Deafness Research Foundation, the Hearst Foundation, and the Capita Foundation. Prof T. Murphy provided anti-OMP P2 (YKA) antibodies.

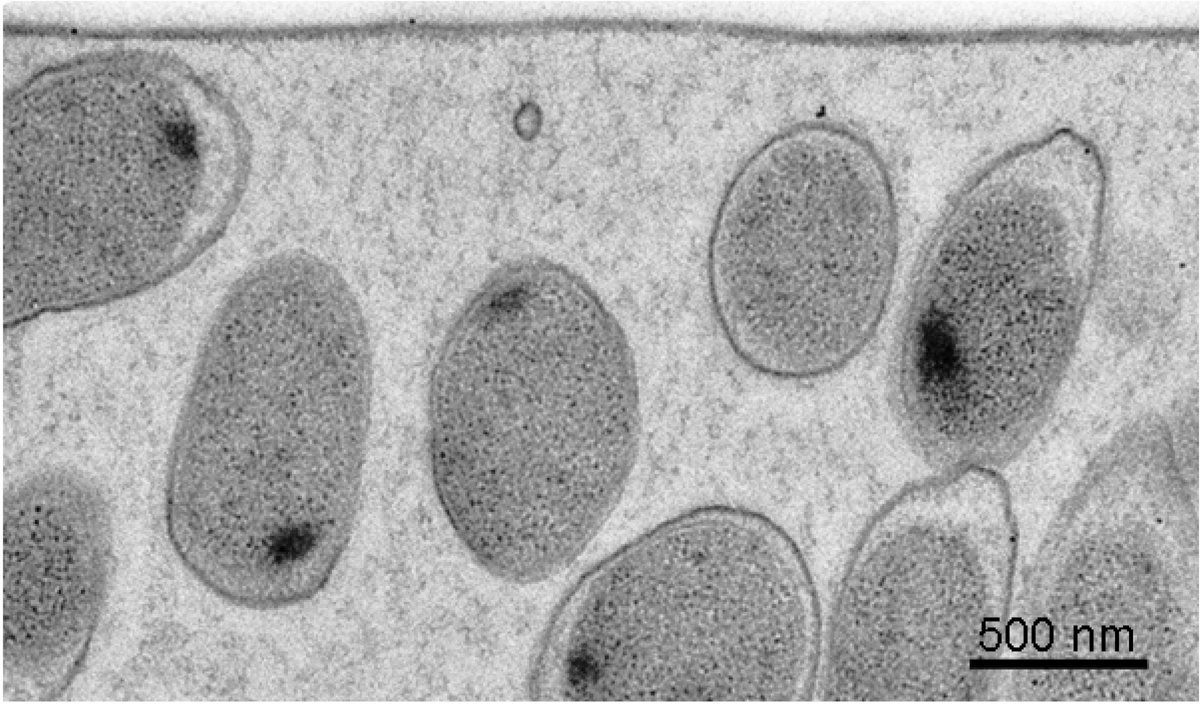
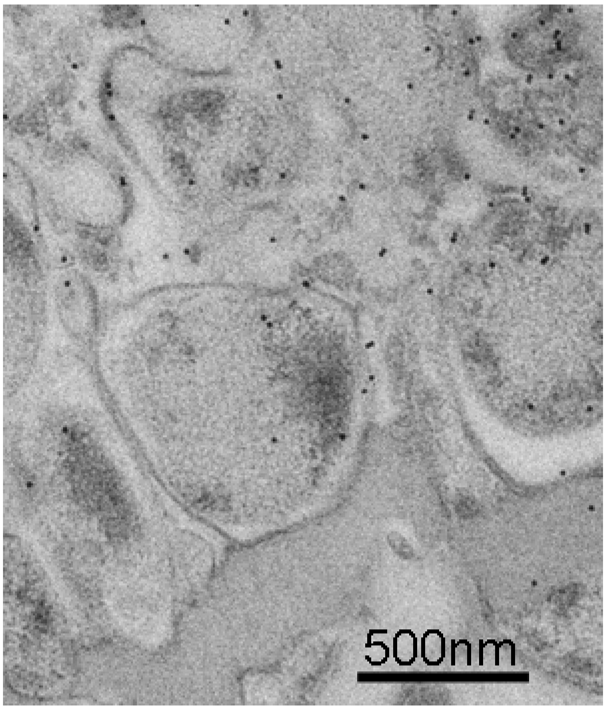


Fig. 1: A cross section through an cryo-preserved NTHi biofilm. Bacteria and extracellular material are all preserved, including the top of the biofilm. The section was labeled with anti-OMP P2 polyclonal antibodies and 10nm protein A gold. The antibody labeling is low in this upper region of the biofilm



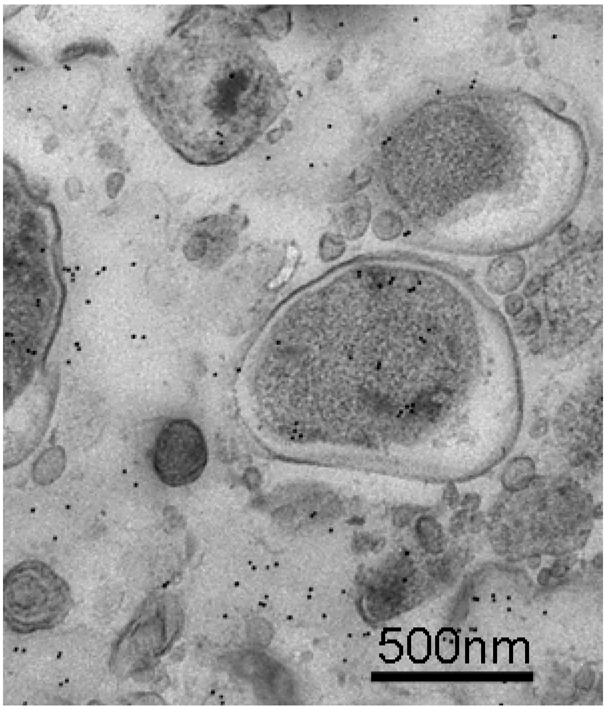


Fig. 2: At the base of of the biofilm the anti-OMP P2 label is more abundant, and is associated with bacterial cells and small vesicles in the ECM. These structures can be easily removed by conventional preparation protocols but are retained by cryo-preservation approachesl. Amorphous material at the base of the biofilm does not label.

Fig. 3: Monoclonal antibodies (mabs) to a DNA-directed RNA polymerase show abundant labeling at the base of the biofilm. Label is mostly associated with the amorphous material between cells and at the base of the biofilm. the mabs were visualized using a RxM secondary in PBS containing 1% fish skin gelatin and lysed bacteria, and 10nm protein A-gold.