

## Employing “Wet SEM” Imaging to Study Co-Colonizing Mucosal Pathogens

D.L. Chance \* and T.P. Mawhinney \*\*

\* Departments of Molecular Microbiology & Immunology and Child Health, University of Missouri, Columbia, MO 65212

\*\* Departments of Biochemistry and Child Health, University of Missouri, Columbia, MO 65211

In this study we tested the applicability of “wet SEM” imaging [1, 2] to investigations of bacteria as they encounter their host and one another in mucosal infections. “Wet SEM” imaging, previously successfully applied to eukaryotic cells, tissues, and parasites, is performed with special finger-tight sealed capsules which permit imaging of hydrated, electron dense specimens through the thin electron-transparent membrane of the capsules by backscattered electron detection (BSE) in a standard scanning electron microscope (SEM) [1,2]. Much of our research in human airway infection, specifically chronic infection in cystic fibrosis (CF), targets understanding host-pathogen and pathogen-pathogen interactions [3-5]. CF and other difficult to treat airway infections often involve multiple organisms and bacterial- and/or host-secreted extracellular matrix materials (*i.e.* alginate, mucin). Focused on finding a convenient technique for high magnification surveying of CF mucosal pathogens in association with host materials and with one another, we evaluated the use of QuantomiX capsules (QuantomiX, Ltd, Israel) for mucin binding and mixed culture experiments.

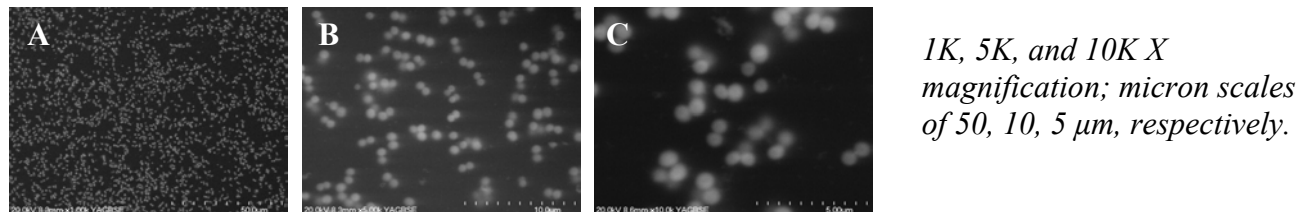
Bacteria used were CF patient isolates of *Pseudomonas aeruginosa* (non-mucoid and mucoid, nmPA, mPA) and *Staphylococcus aureus* (SA). In substrate-binding studies, bacteria suspended in a balanced salt solution were applied to uncoated or mucin-coated capsules and incubated 1h at 35°C. Immunofixation (IR) was with 4% paraformaldehyde, 0.1% glutaraldehyde, 0.08M sucrose, 0.02M calcium chloride, 0.1M sodium cacodylate buffer, pH 7.35, 0.1% ruthenium red (for matrix stabilization) [5], 1h, 22°C; secondary fixation, when indicated, was with buffered 1% osmium tetroxide (OsO<sub>4</sub>). For liquid co-culture growth experiments, mixtures of SA and PA in broth were incubated in QX-102 capsules 1 or 2 days prior to 1<sup>o</sup> and 2<sup>o</sup> fixation. For BSE-SEM analysis of agar-grown colonies of mucoid PA with SA, colonies were sampled by colony lift onto 3 mm carbon-coated nylon grids and fixed with IR, stained, and viewed in QX-302 tissue capsules. Various SEM settings and stains were tested and the most informative are presented here. To add electron density and contrast for BSE-SEM imaging, staining included uranyl acetate (5% UA, 15-30 min). After water rinses and application of imaging buffer, samples were visualized with BSE on an Hitachi S-4700 Scanning Electron Microscope (voltage 20 kV, emission current 20 μA, spot size 12).

As seen in Figure 1, UA staining of samples previously immunofixed with ruthenium red (IR) and osmicated provided adequate contrast to visualize with BSE-SEM bacteria bound to the capsule surface. Resolution was great enough to discern but not resolve two associated cocci. In mixed growth and binding experiments, this fixing and staining combination discriminated between the gram positive and negative organisms *S. aureus* (Gr+) and *P. aeruginosa* (Gr-), while fixation without UA staining did not provide sufficient contrast to image PA (not shown). As seen in the mucin binding assay of Figure 2, brief UA staining of previously IR-fixed specimens (with no osmication), provided: an outlining effect to bacteria, with clear distinction of Gr+ and Gr- bacterial cell wall density; ready detection of dividing organisms; and quick assessment of relative binding to different coating matrices. As seen in Figure 3, both liquid and solid media growth of mixed cultures

were also successfully imaged with BSE-SEM, though use of the two different capsule types. Resolution provided with BSE imaging through these membranes is great enough to permit discrimination between organism types, and provide visualization of growth and co-existence patterns. These data indicate that “wet SEM” imaging is a convenient and appropriate screening and analytical tool for the study of bacterial biology and for the evaluation of new treatment protocols.

#### References:

- [1] I. Barshack et al, *Ultrastruct. Path.* 28 (2004) 29.
- [2] S. Thiberge et al, *Proc. Natl. Acad. Sci. –USA*, 101 (2004) 3346.
- [3] D.L. Chance et al, *Microsc. Microanal.* 10 (Suppl 2) (2004) 238.
- [4] T. P. Mawhinney et al, *Recent Develop. Carbohydr. Res.* 2 (2005) 67.
- [5] D.L. Chance et al, *Microsc. Microanal.* 11 (Suppl 2) (2005) 968.
- [6] This work was generously supported by corporate sponsors, the Cystic Fibrosis Association of Missouri, and the Experiment Station Chemical Laboratories of the University of Missouri-Columbia (UMC); electron microscopy was performed at the UMC Electron Microscopy Core Facility, with valuable technical insights provided by Randy Tindall, Cheryl Jensen, and Lou Ross.



1K, 5K, and 10K X magnification; micron scales of 50, 10, 5 µm, respectively.

FIG 1. BSE-SEM images of *S. aureus* adherent to uncoated QX-102 capsule membranes. Fixatives and stain (F/S): IR, OsO<sub>4</sub>, UA.

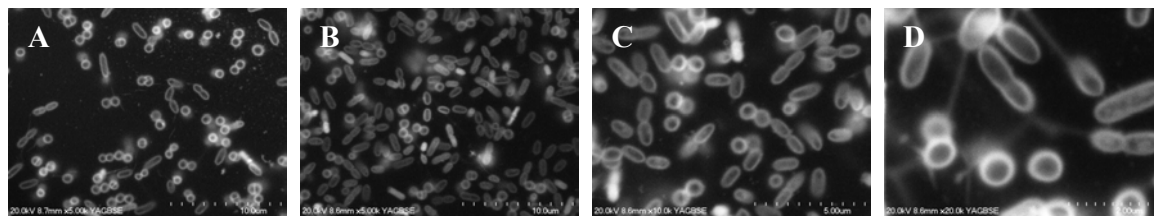


FIG 2. BSE-SEM images of mixtures of *S. aureus* and nonmucoid *P. aeruginosa* adherent to bovine submaxillary mucin (A), or human tracheobronchial mucin (B-D) coated membranes of QX-102 capsules. F/S: IR, (no OsO<sub>4</sub>), brief UA. 5K, 5K, 10K, 20K X, micron scales of 10, 5, 2 µm.

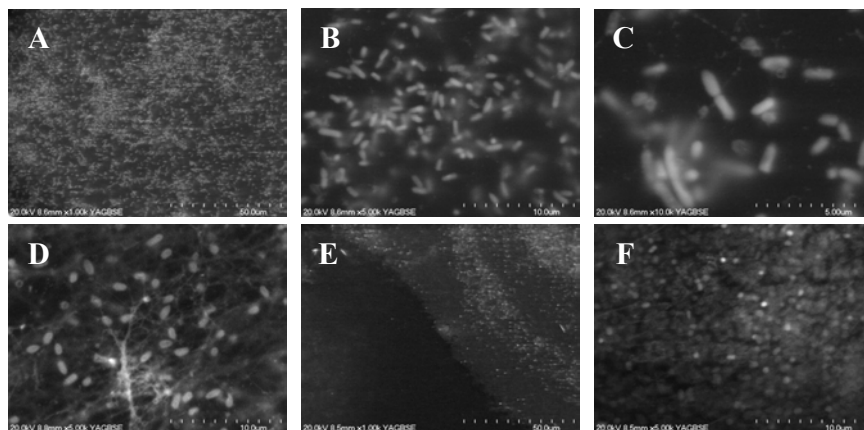


FIG 3. BSE-SEM images of mixed cultures of *S. aureus* and mucoid *P. aeruginosa*. (A-D) Liquid co-cultures with extracellular matrix in QX-102 capsules, (growth A-C, 1 d; D: 2 d. F/S: IR, OsO<sub>4</sub>, UA. 1K, 5K, 10K, 5K. (E,F) Mixed culture colony lift on nylon grid in QX-302 tissue capsule. F/S IR, UA. 1K, 5K.