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Morphological Effects of Quaternary Ammonium Compounds on Staphylococcal Biofilms

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A wide range of antimicrobial agents are being explored to control bacterial colonization of surfaces and biofilm formation. Among these are quaternary ammonium compounds (QACs). We are interested in exploring the mechanism of action that QACs have on killing staphylococcal bacteria commonly implicated in biomaterials-associated infection. Here we take advantage of recent developments in scanning electron microscopy (SEM) to image *S. epidermidis* before and after exposure to a particular QAC. These images indicate that the QACs disrupt the cell wall.

Staphylococcal biofilms were grown in 35 x 10 mm non-coated bacterial suspension dishes for 24 hrs. The biofilms were then washed with phosphate buffer. An amount corresponding to the miminum (planktonic) bactericidal concentration (1xMBC) of Ethoquad ® C/25 (Cocoalkylmethyl [polyoxyethylene (15)] ammonium chloride) QAC (150 μg/mL) was then added to the culture medium and it was incubated for 12 hrs. The biofilms were then fixed using 2% glutaraldehyde and stained with OsO4. Confocal images were taken of biofilms using the procedure above but without glutaraldehyde fixation or OsO_4 staining.
Leaving each biofilm in its original polystyrene culture well, the fixed and stained biofilms were embedded in epoxy using standard electron-microscopy protocols. A Leica Ultracut UCT microtome was then used to uniformly expose the biofilm/polystyrene interface. SEM imaging and focused-ion-beam (FIB) machining were performed using a Zeiss Auriga FIB-SEM using 1.90 keV electrons and an in-lens backscattered detector (EsB). FIB milling was done using 30 keV Ga ions at a current of 600 pA. Data obtained were reconstructed in 3D from 400 serial images collected at slice intervals of 20 nm using the Avizo® Fire 3D analysis software.

Figure 1 shows typical results SEM images using the in-lens backscattered detector. A considerable morphological difference can be observed between the untreated control (fig. 1A) and the QAC-treated biofilm (fig. 1B). The cell walls of the treated bacteria are clearly lysed in several cases, and the majority of QAC-treated bacteria have lost significant amounts of intracellular material. These observations are reinforced by the reconstructed 3-D images of figure 2. While gentamicin is effective at killing bacteria there appears to be much less obvious cellular damage (fig. 2 left) than in the case of the QAC-treated biofilm (fig. 2 right). Work is now on-going to explore such specimens in the unfixed, unstained, frozen-hydrated state using cryo-SEM techniques. Figure 3 shows confocal images of the variously treated biofilms after exposure to live/dead stain. The untreated control forms a robust and viable biofilm. Gentamicin treatment leads to some killing, but substantial viable biofilm remains. In contrast, the QAC treatments are clearly very effective at both killing bacteria and inhibiting their growth.

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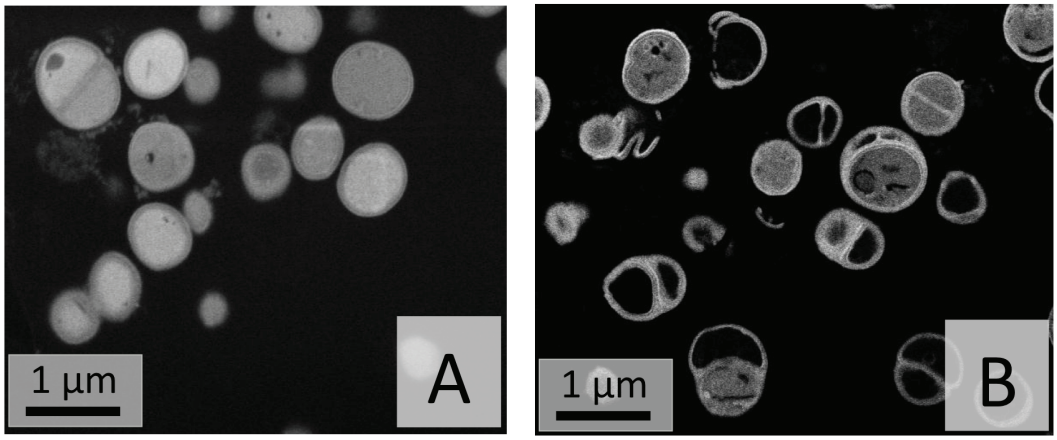

Fig. 1: Backscattered SEM Images of *S. epidermidis* biofilms without (A) and with (B) exposure to 1xMBC of QAC during growth.

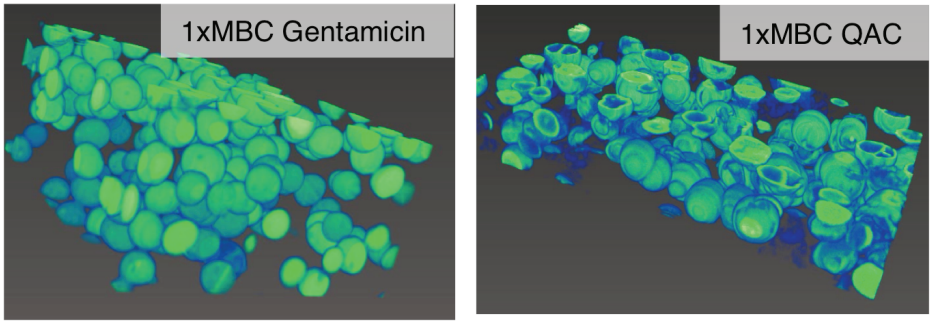

Fig. 2: 3D reconstruction of *S. epidermidis* biofilms after exposure to: (left) 1xMBC Gentamicin and (right) 1xMBC QAC.

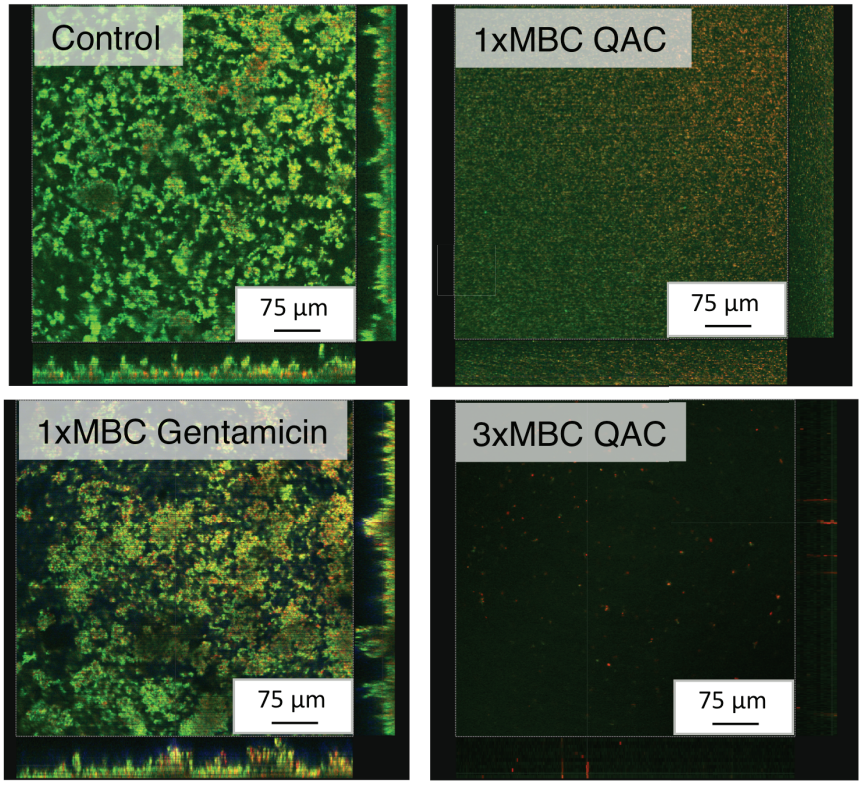

Fig. 3: Confocal imaging of live (green)/dead (red) stained *S. epidermidis* biofilms shows that QAC exposure at 3xMBC leads to complete biofilm destruction. Gentamicin and 1xMBC QAC treatments inhibit but do not fully eradicate *S. epidermidis* biofilm formation. (1xMBC QACs = 210µg/mL)