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Further evidence on the bacterial-killing mechanism and biofilm inhibition of high-density cationic surfaces

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Background: Numerous *in vitro* studies showed the interest of biocidal polymers grafted on plastics or metals in the medical field. Quaternary-ammonium polymers (QAPs) are easily obtained and grafted on various surfaces to prevent bacterial adhesion and proliferation on surfaces. These high-density cationic surfaces are extensively described in the literature while data concerning their non-selective bacterial killing remains poorly detailed. A QAP was synthesized and grafted on titanium surfaces to assess its bactericidal activity and visualize its killing mechanism and anti-biofilm activity.

Material/methods: *in vitro* antibacterial activity: a MRSA strain isolated from a patient with a prosthetic joint infection was cultured in Brain Heart Infusion (BHI) at 37°C overnight. According to a modification of the 22196:2011 ISO norm, a 10^7CFU/mL bacterial suspension of 20 µL in rich medium (BHI) was simultaneously applied on pure titanium 1cm^2 plates (control *vs* grafted with a QAP, mono-layer or thick coating) at 37°C. Cultures were sequentially stopped after 1h (bacterial killing) and 24h (growth inhibition), diluted in 0,9% saline and vortexed for detachment of live bacteria and bacterial counting. ***In vitro* anti-biofilm activity:** an *in vitro* biofilm was created on similar titanium plates with a 10^6 CFU/mL bacterial suspension of 500µL in BHI in each well. Cultures at 37°C were then stopped sequentially after 6h, 12h, 24h, 72h, and 7 days (medium was replaced every 24h). Plates were rinsed three times with PBS 5% and fixed in a 2,5% glutaraldehyde solution. AFM (Atomic Force Microscopy)

of the biofilm after 3 hours and SEM-FEG (Scanning Electron-Microscopy with Field Emission Gun) from 6h to 7 days were obtained.

Results: A 1,6 log_{10} reduction of bacteria occurred on mono-layer coated surfaces in 1 hour, respectively 6,96 log_{10} UFC/mL *vs* 8,56 log_{10} UFC/mL, and 1,1 log_{10} in 24 hours, respectively 9,20 log_{10} UFC/mL *vs* 10,17 log_{10} UFC/mL, p<0,0001. Surfaces with thick coatings were sterilized, respectively 6,96 log_{10} UFC/mL *vs* 1 log_{10} UFC/mL, p<0,0001. An anti-biofilm effect was visualized on SEM-FEG up to 7 days. A specific effect on the 3D structure of killed bacteria was characterized using AFM: bacterial shrinkage and volume reduction as well as perforation were visualized.

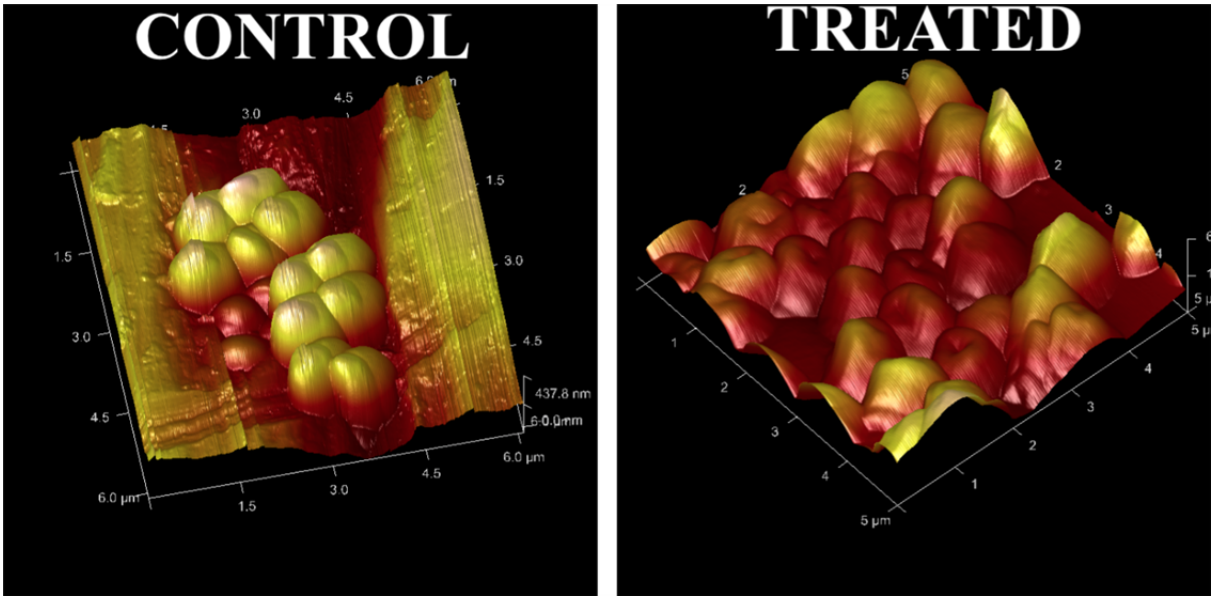

Figure 1. Bacterial shrinkage and perforation (red) after a 3-hour-contact on QAP-coated titanium (AFM)

Conclusions: The present study confirms the current body of evidence that bacterial membrane perforation is the primary mechanism by which bacteria are killed by QAPs. Such molecules could become promising candidates for coating on biomaterial implants provided further assessment using relevant in vivo models is performed.