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Comparative Description of In Vitro and In Vivo MRSA Biofilms on Titanium Surfaces: Why Animal Models Still Matter

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Background: *In vitro* biofilm studies on controlled surfaces demonstrate the advantages of facilitating imaging studies and allowing extensive bacteriological studies while being easily accessible and cheap. However, the *in vivo* role of the environment is completely occulted. An *in vitro* and *in vivo* (rabbit prosthetic joint infection (PJI) model) assessment of a quaternary ammonium polymer (QAP) antibacterial coating was performed on titanium against methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, *in vitro* and *in vivo* characteristics of biofilm growth patterns and organization were described.

Material/methods: In vitro antibacterial activity (AB): a MRSA strain isolated from a patient with a PJI was cultured in Brain Heart Infusion (BHI) at 37°C overnight. A 10^7 CFU/mL (20 µL) bacterial suspension in BHI was simultaneously applied on 1cm^2 titanium plates (control *vs* coated with QAP) at 37°C. Cultures were sequentially stopped after 1h and 24h, diluted in 0,9% saline and vortexed for detachment of live bacteria and bacterial counting. ***In vivo:*** forty-two New-Zealand White female rabbits (2,8kg) underwent a unipolar knee joint replacement (TiAl6V4 tibial implant, 24 controls *vs* 18 grafted). A $7x10^6$ inoculum was injected following wound closure. Implants were removed at day 14, rinsed with PBS, vortexed for adherent bacterial count. Proximal-third tibias were resected, crushed and cultured. **Anti-biofilm activity *in vitro:*** a 10^6 CFU/mL bacterial suspension in 500 µL BHI was simultaneously deposited on 10 plates (control *vs* coated). Cultures at 37°C were sequentially stopped after 6h, 12h, 24h, 72h, and 7 days (medium was replaced every 24h). Plates were rinsed with PBS

5% and fixed in PBS/2,5% glutaraldehyde. SEM-FEG (Scanning Electron-Microscopy with Field Emission Gun) biofilm images were obtained**. Comparison of biofilm morphology *in vitro/vivo***: implants for 3 controls rabbits were removed at day 3, 7, and 14, fixed and compared with *in vitro* control plates (SEM-FEG).

Results: AB: a 1,6 log_{10} reduction of bacteria occurred on coated surfaces *vs* control in 1 hour (6,96 log_{10} UFC/mL *vs* 8,56 log_{10} UFC/mL), and a 1,1 log_{10} reduction in 24 hours (9,20 log_{10} UFC/mL *vs* 10,17 log_{10} UFC/mL) (p<0,0001). ***In vivo*****,** bone bacterial density did not differ between treated and control rabbits (4,5±1,5 log_{10} *vs* 5,2±1,4 log_{10}, p=0,07); neither did bacterial adherence (respectively 3,6±1,9 log_{10} *vs* 3,8 ±1,8 log_{10}, p=0,36). **Anti-biofilm activity *in vitro*:** anti-biofilm activity of QAP against controls was visible up to 7 days. **Comparison *in vitro/vivo*:** while *in vitro* MRSA on controls showed a high bacterial proliferation with a rare biofilm matrix, *in vivo* MRSA on controls produced profuse polysaccharide complex filaments with rarely visible bacteria.

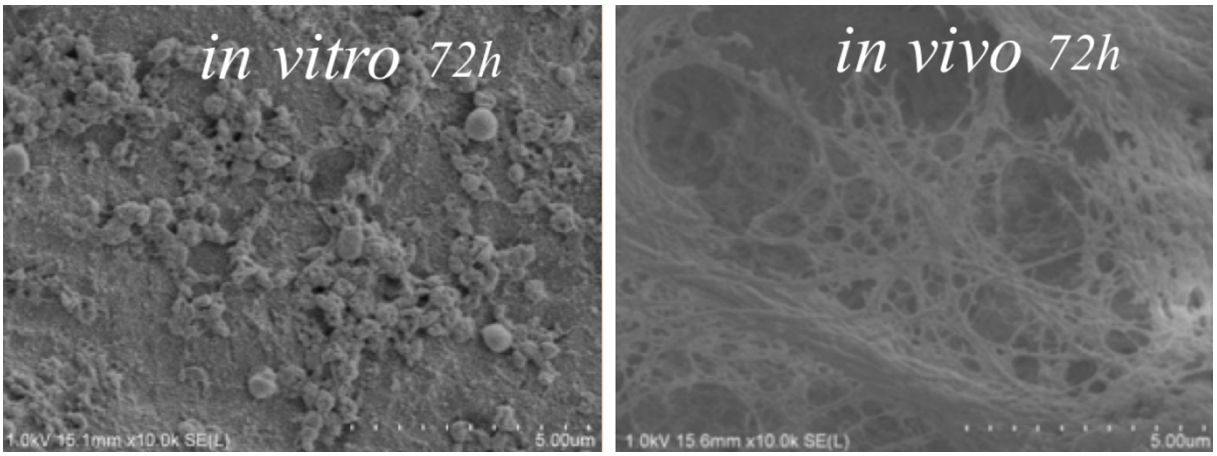

Figure 1. In vitro/vivo MRSA biofilm

Conclusions: *in vitro* anti-biofilm activity of QAP-coated surfaces did not predict *in vivo* outcome. Animal models are crucial as biofilm morphology and growth considerably differed from *in vitro.*