

Study of the effect of pulsed washing on antibiotic susceptibility of *Staphylococcus aureus* biofilms

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Background:

Pulsed washing (PW) is a major step during debridement surgery in the context of orthopaedic device-related infections (ODRI), but its effects on biofilms are poorly characterized.

The present study aims at evaluating the effect of PW alone or followed by antibiotics on MSSA and MRSA biofilms grown on titanium Ti6Al4V coupons in-vitro.

Materials/methods:

Strains: 1 reference (MSSA: ATCC25923; MRSA: ATCC33591) and 2 clinical MSSA and MRSA isolated from ODRI.

Biofilm culture: Coupons were incubated during 24h at 37°C with bacteria (starting inoculum ~6.6Log₁₀CFU/mL in TGN [TSB + 1% glucose + 2% NaCl]), under shaking at 50rpm.

Treatment: Half of the coupons received PW with 50mL physiological serum using a Stryker Interpulse from a distance of 5cm; the coupons were then either analysed (24h control and PW) or reincubated for 24h in TGN or TGN + flucloxacillin (MSSA) or vancomycin (MRSA) at two concentrations (MIC and 20mg/L).

Analysis: Coupons were rinsed twice with PBS. Biomass was determined by crystal violet (CV) staining. CFUs were counted after recovering bacteria from coupons using sonication and TSA plating.

Results: See Figure.

Antibiotics alone: Flucloxacillin significantly reduced both CFU and biomass for ATCC25923 but not for clinical isolates of MSSA. Vancomycin had no statistically significant effects on CFUs for all MRSA and only a weak effect on biomass for 1 clinical isolate.

PW alone markedly reduced CFUs and biomass for all strains but had no persistent effect after 24h reincubation in TGN.

PW + antibiotics: Antibiotics prevented bacterial regrowth after PW when used at their MIC and further decreased CFUs when used at 20 mg/L. They also impaired biomass recovery after PW at both concentrations.

Conclusions:

PW alone only has a transient effect on coupon colonisation by *S. aureus* biofilms. Vancomycin alone at therapeutic concentrations is ineffective and flucloxacillin has a strain-dependent effect.

Yet, in combination, these treatments show synergistic effects, indicating the importance of PW followed by high antibiotic doses for ODRI.

However, PW efficacy in-vivo is limited by poor access to parts of the implants due to anatomical constraints, providing an argument for the development of alternative strategies to disrupt biofilms.