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

P0568

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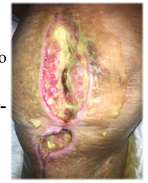
Introduction



Infected total knee arthroplasty

Orthopaedic Devices Related Infections (ODRI) are challenging to treat complications of orthopaedic procedures involving implants. Staphylococci cause **over 50%** of those infections and are known to form **biofilms** on the surfaces of the implants.

The treatment of ODRI relies on **both surgery and antibiotic therapy**. The aim of the surgery is to reduce the bacterial load through debridement and irrigation.

Irrigation is frequently performed using specific **pulsed-washing** devices, that apply pressurised normal saline on the surfaces of the wound and of the implants.

The effect of this irrigation technique on clinically relevant biofilms have been scarcely reported (1,2). Moreover, the combination of irrigation with pertinent antibiotics has not been the object of previous reports.

Our study's objective was to investigate the effects of the **combination** of pulsed-washing and clinically relevant antibiotics, and to compare it to their independent effects, against methicillin-resistant and methicillin-sensitive Staphylococcus aureus (MRSA; MSSA) biofilms grown on Ti6Al4V coupons.

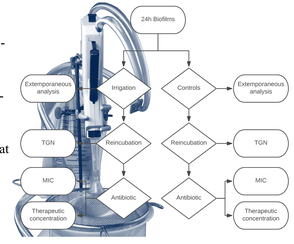
Materials and Methods

Strains: 3 MRSA: ATCC 33591 (reference strain), 749, 676 & 3 MSSA: ATCC 25923 (ref. strain), 578, 611.

Antibiotics: Susceptibilities against oxacillin, flucloxacillin (FLX) and vancomycin (VAN) were tested for all strains following the CLSI guidelines. (Table 1) VAN was used against MRSA biofilms and FLX was used against MSSA biofilms. Concentrations used for reincubation were :MIC in [TSB + 1% Glucose + 2% NaCl] (TGN); therapeutic concentration (ThC): VAN: 20mg/L - target through concentration for bone and joint infections (3); FLX: 20mg/L - concentration observed 3h after administration in a 2g q6h regimen (4).

Biofilm culture: Incubation of bacteria suspended in TGN (initial inoculum: 6,6 $\log_{10}$ CFU/mL) with Ti6Al4V coupons for 24h at 37°C, under continuous agitation (50 rpm).

Treatments: Samples were separated in irrigation and control groups. Irrigation consisted in the application of 50mL of 0,9% NaCl from 5 cm using an Interpulse (Stryker Co., Kalamazoo, MI, USA) pulsed-washing device. Samples were either analysed or reincubated for 24h in TGN +/- antibiotics (see flowchart).



Analysis: a) CFU counts: CFU were harvested by a combination of vortex and sonication before serial dilutions and TSA plating; b) Biomass assays: staining of the samples with crystal violet (CV), removal of the excess dye, resolubilisation of CV in acetic acid and absorbency reading at 570 nm; c) Fluorescence microscopy: Live/Dead staining (ThermoFisher, Waltham, MA, USA), Z-stack acquisition at 20x, post-processing using FIJI.

Statistical analysis: 2-way ANOVA followed by Holm-Sidàk post-hoc test.

All experiments were repeated four times.

Results

MRSA

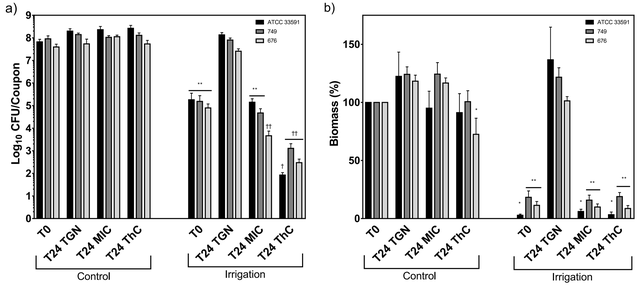


Fig a) $\log_{10}$ CFU counts; fig b) Biomass assays normalized as percentage of T0 control; fig c) Maximal Intensity Projection (MIP) views at 20x after Live/Dead staining. Control: Control samples; Irrigation: samples irrigated with 50 mL of 0,9% NaCl; T0: extemporaneously analysed; T24 TGN: 24h reincubation in TGN; T24 MIC: 24h reincubation in TGN with VAN at MIC; T24 ThC: 24h reincubation in TGN with VAN at 20mg/L. *:p<0,05 when compared to T0 control; **:p<0,001 when compared to T0 control; †:p<0,05 when compared to T0 irrigation; ††:p<0,001 when compared to T0 irrigation. 2-way ANOVA followed by Holm-Sidàk test. n=4 for all conditions.

⇒Reincubation in TGN with VAN at either MIC or ThC did not affect the control samples

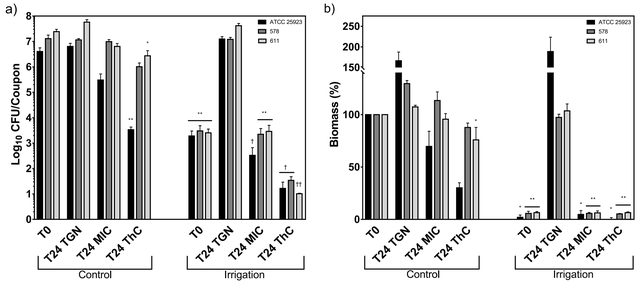
⇒Irrigation removed ~3 log CFU, and over 90% of the biomass for all strains

⇒Reincubation in TGN of irrigated samples restored the cells numbers and the biomass to control levels

⇒Reincubation in TGN with VAN at MIC inhibited the restoration of the biofilms

⇒Reincubation in TGN with VAN at ThC further reduced the CFU counts by ~3 log CFU. No further reduction of the biomass was observed

MSSA



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⇒Reincubation in TGN FLX at both MIC and ThC reduced CFU counts and biomass of strains ATCC 25923 and 611, albeit more modestly

⇒Irrigation removed 3 to 4 log CFU, and over 90% of the biomass for all strains

⇒Reincubation of irrigated samples in TGN restored the cells numbers and the biomass to control levels

⇒Reincubation of irrigated samples in TGN with FLX at MIC inhibited the restoration of the biofilms of strains 578 and 611, and caused a 1 log reduction of CFU counts for strain ATCC 25923

⇒Reincubation of irrigated samples in TGN with FLX at ThC further reduced the CFU counts by ~2 log CFU. No further reduction of the biomass was observed

Table 1. MIC (mg/L) values for the tested strains

	Strains	Oxacillin	Flucloxacillin		Vancomycin	
		CA-MHB	CA-MHB	TGN	CA-MHB	TGN
MRSA	ATCC 33591	>64	>64	>64	1	4
	749	>64	>64	>64	1	8
	676	>64	64	64	1	8
MSSA	ATCC 25923	0.25	0.125	0.0625	1	8
	578	0.25	0.25	0.125	2	8
	611	0.25	0.25	0.0625	1	8

CLSI breakpoints values (in CA-MHB): Flucloxacillin: N/A; Oxacillin: S≤2, R≥4; Vancomycin: S≤2, R≥16.

Conclusion

- Irrigation has a **synergistic effect** with vancomycin and flucloxacillin against MRSA and MSSA biofilms grown on metallic substrates.
- Irrigation alone reduces CFU from biofilms in a more important way than previously reported (2-4) but only **transiently**.
- Vancomycin had **no effect** on MRSA biofilms, even at therapeutic concentrations.
- Flucloxacillin had a **strain-dependent effect** on MSSA biofilms.

Those results are in accordance with the hypothesis that a lowered bacterial density in biofilms improves the susceptibility to antibiotics.

This support the assumption that a thorough debridement is needed to successfully treat patients suffering from ODRI.

However, The in-vivo effectiveness of pulsed lavage 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.

References

1. Schwechter EM et al. Optimal irrigation and debridement of infected joint implants: an in vitro methicillin-resistant Staphylococcus aureus biofilm model. J Arthroplasty. 2011. 2. Urish KL et al. Pulse lavage is inadequate at removal of biofilm from the surface of total knee arthroplasty materials. J Arthroplasty. 2014. 3. Liu C et al. Clinical Practice Guidelines by the Infectious Diseases Society of America for the Treatment of Methicillin-Resistant Staphylococcus aureus Infections in Adults and Children. Clinical Infectious Diseases. 2011. 4. FAMHP. Floxapen. 2017Rev.nr.1711 – EMA/PRAC/610988/2017 + BE517777.

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