

Bactericidal effect of fluoride-TiO₂ nanotubular anodic layers on Ti6Al4V loaded with gentamicin and vancomycin

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Background

Prosthetic joint infections (PJI) occur infrequently, but they represent the most devastating complication with high morbidity and substantial cost. *Staphylococcus aureus* and coagulase-negative *S. epidermidis* are the most common infecting agents associated with PJI.

The use of F-TiO₂ nanotubular (F-T NT) surfaces as drug delivery platform for local antibiotic therapy is an interesting approach to prevent surface colonization during the early moments after surgery. Here we describe the bactericidal effect of F-T NT loaded with gentamicin (Gm, Figure 2.A) and vancomycin (Vm, Figure 2.B).



Figure 1. Nanoscale micrograph of FT-NT layers on Ti6Al4V alloy.

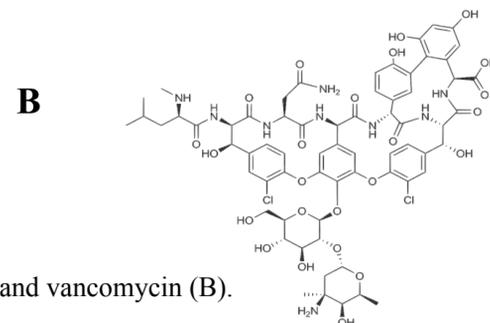
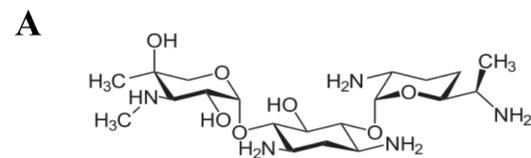


Figure 2. Chemical structures of gentamicin (A) and vancomycin (B).

Material/methods

F-T NT layers on Ti6Al4V alloy were produced as described previously by Arenas *et al.* (*Colloids Surf B Biointerfaces*. 2013. 1:105:106-12).

Gm, Vm and a mixture were loaded in the F-T NT samples by the method described by Doadrio *et al.* (*International Journal of Pharmaceutics*. 2015. 492: 207–12). F-T NT samples without antibiotic were used as control.

The *S. aureus* ATCC 15981 and *S. epidermidis* ATCC 35984 strains adherence study was performed using the protocol described by Kinnari *et al.* (*J Biomed Mater Res A*. 2008; 86(3):760-8) with a 24 h incubation. After incubation, the samples were stained with LIVE/DEAD BacLight Bacterial Viability Kit. Proportion of live and dead bacteria was calculated and studied by using ImageJ software. The experiments were performed in triplicate.

The statistical data were analyzed by nonparametric Kruskal-Wallis test and by pairwise comparisons using the nonparametric Mann-Whitney test with a level of statistical significance of $p < 0.05$. Values are cited and represented as medians.

Results

Our results are shown in the Figures 3 and 4 and Table 1.

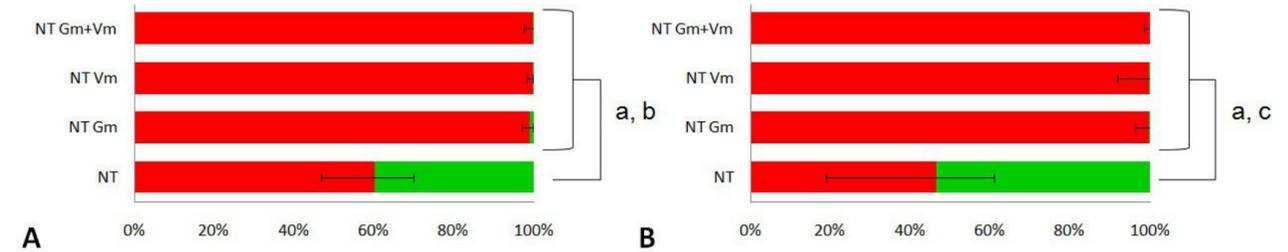


Figure 3. Proportion of live (green) and dead (red) bacteria of tested strains: *S. aureus* ATCC 15981 (A) y *S. epidermidis* ATCC 35984 (B). The bar represents interquartile range. a: p -value <0.05 of Kruskal-Wallis test between NT and the rest and p -value <0.05 of Mann-Whitney test between NT and the rest. b: p -value >0.05 of Kruskal-Wallis test between NT Gm, NT Vm and NT Gm+Vm. c: p -value <0.05 of Mann-Whitney test between NT Gm and NT Gm+Vm.

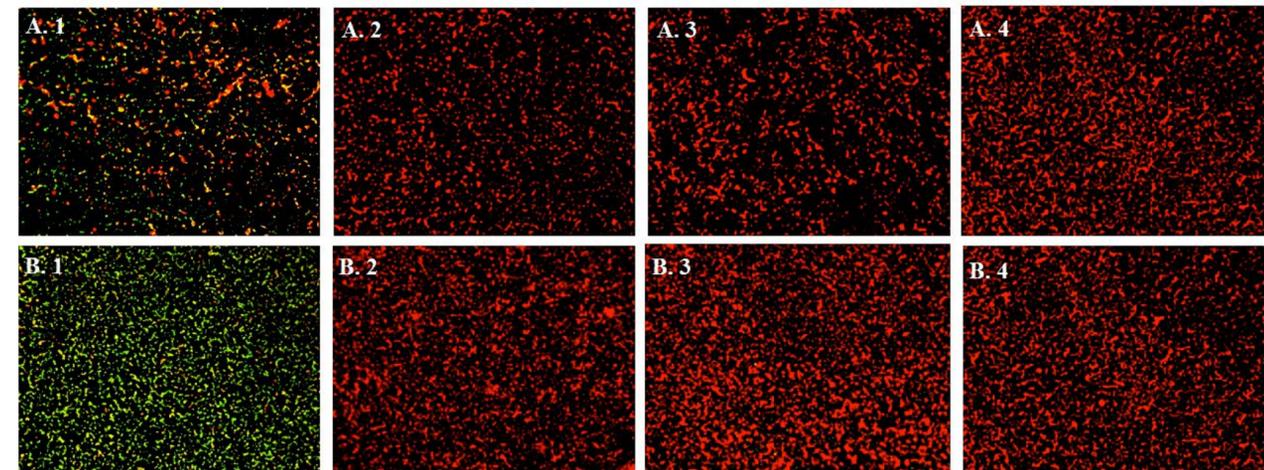


Figure 4. Micrographs of adhered bacteria of tested strains *S. aureus* ATCC 15981 (A) and *S. epidermidis* ATCC 35984 (B) on F-T NT layers on Ti6Al4V without antibiotic (1) and loaded with Gm (2), Vm (3) and Gm+Vm (4).

F-T NT layer	<i>S. aureus</i> ATCC 15981		<i>S. epidermidis</i> ATCC 35984	
	L (%)	AED (%)	L (%)	AED (%)
NT	39.66	0	53.48	0
NT Gm	0.82	97.93	0.25	99.53
NT Vm	0.23	99.42	0.04	99.93
NT Gm+Vm	0.07	99.82	0	100

Table 1. Live (L) and antibiotic-effect dead (AED) bacteria on each type of F-T NT layer.

Conclusions

F-T NT layers on Ti6Al4V loaded with antibiotic have an important bactericidal effect against two common bacterial species. Gm+Vm mixture was able to reduce more than 99.8 % of adherent bacteria. Therefore, they can be considered as promising and preventing approach against PJI.

Acknowledgement

This work was funded by the following grants from Spanish MINECO (MAT2013-48224-C2-1-R and MAT2013-48224-C2-2-R) and MICINN (CS02010-11384-E, MAT2012-35556).

