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Optimization of fluorimetric and confocal microscopy techniques to unravel vancomycin activity against Staphylococcus aureus biofilms

Sandra Pinto*[1], Dalila Mil-Homens[2], Miguel Castanho[3], Manuel Prieto[4], Ana Coutinho[5], Ana Salomé Veiga[3]

[1]*Instituto Superior Técnico; Cqfm, Centro de Quimica-Fisica Molecular Complexo Interdisciplinar*

[2]*Instituto Superior Técnico; Ibb-Institute for Bioengineering and Biosciences*

[3]*Instituto de Medicina Molecular; Faculty of Medicine, University of Lisbon*

[4]*Instituto Superior Técnico*

[5]*Instituto Superior Técnico and Faculdade de Ciências*

Background: The treatment of bacterial biofilm-related infections within hospital environments is facing serious threats with increasing numbers of antibiotic resistant bacteria. Moreover, the low numbers of novel compounds or strategies under development to fight bacteria biofilm infections can strongly impact the future of worldwide economies. In this study, we aim to analyse the mechanisms responsible for the anti-biofilm activities of clinically relevant antimicrobial agents and for that we used the antibiotic vancomycin. Understanding the mechanisms behind antibiotic anti-biofilm action is crucial for: i) select adequate treatments, ii) minimize antibiotic resistance, and iii) develop new compounds with an effective anti-biofilm action.

Material/methods: The model organism chosen to perform our studies was *Staphylococcus aureus* ATCC 6538. *S. aureus* is a Gram-positive bacteria pathogen responsible for acute bacterial infection. The main methodologies implemented to characterize biofilms produced by *S. aureus* were: (i) crystal violet assay, to evaluate the total biomass present in the biofilm; (ii) AlamarBlue reduction assay, to assess changes in the metabolically active cells present in the biofilm; (iii) colony-count assay, to evaluate the density of viable and culturable cells within the biofilm; (iv) confocal microscopy techniques, to characterize the architecture and diffusion barrier properties of biofilms.

Results: The results showed that although vancomycin is very active against young biofilms, in the case of mature biofilms the antibiotic reduced bacterial viability and biofilm biomass only moderately. Using the fluorescently-labeled vancomycin and confocal microscopy techniques we evaluated the factors responsible for the ineffectiveness of the antibiotic in removing all biofilm. Overall, the results showed that Vanc-BODIPY diffuses rapidly through all biofilm and interacts strongly with bacteria in the deepest layers of the biofilm.

Conclusions: Altogether our findings illustrate that biofilm maturity strongly influences antibiotic activity and the importance of an early and correct diagnostic. Importantly, we show that vancomycin within mature *S. aureus* biofilms does not display reduced diffusion, suggesting that for this model organism resistance to antibiotics is not achieved through diffusion barrier properties of the biofilm. Moreover, we observed that the antibiotic interacts preferentially with bacteria found in inner layers of mature *S. aureus* biofilm. Since these bacteria could display different metabolic properties than bacteria in other regions of the biofilm, it is likely that biofilm phenotype heterogeneities can play a role in defining the efficiency of therapeutic agents. In this way, antibiotic resistance within biofilms can be associated with a reduction in antibiotic bioavailability due to the sequestering of antibiotic to specific biofilm regions (Figure 1). Finally, the implementation/optimization of biofilm-quantitative techniques are currently been used by us to identify promising antimicrobial agents with anti-biofilm activity.

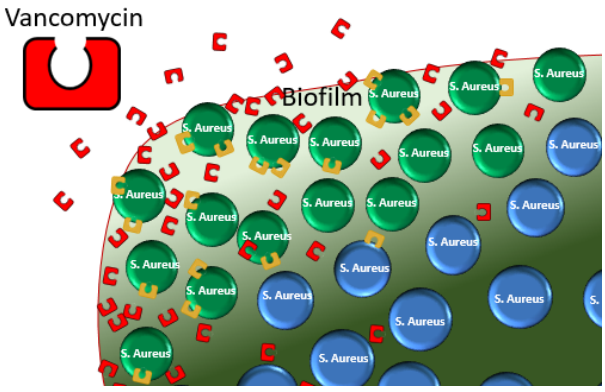

Figure 1: Vancomycin bioavailability inside *S. aureus* biofilm. Vancomycin unbound fraction is represented in red and bound fraction is represented in yellow.