

O1133 Optimisation of confocal microscopy imaging techniques to study the diffusion-controlled action of a cationic antimicrobial peptide along *Staphylococcus* biofilms

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Background: Biofilm-related infections are particularly difficult to treat since microbial cells in biofilms exhibit increased resistance to antibiotics. One promising alternative to conventional antibiotics is the use of cationic and amphipathic peptides with antimicrobial properties. In this work, the ability of pepR (a highly cationic peptide) to inhibit and act on pre-formed *S. aureus* biofilm is evaluated and its mechanism of action at the molecular level is elucidated.

Materials/methods: Biofilm mass, metabolic activity and viability were quantified using conventional techniques, while fluorescence imaging methods, including a real-time calcein-release assay, were employed to investigate the kinetics of pepR activity at different biofilm depths.

Results: We show that pepR is able to prevent staphylococcal biofilm formation due to a fast killing of planktonic bacteria, which in turn resulted from peptide-induced increase permeability of the bacterial membranes. The activity of pepR against pre-formed biofilms was evaluated through the application of a quantitative live-dead confocal laser scanning microscopy (CLSM) assay. The results shown that the bactericidal activity of pepR on pre-formed biofilms is dose and depth-dependent. A CLSM-based assay of calcein release from biofilm-embedded bacteria was further developed to indirectly assess the diffusion and permeabilization properties of pepR throughout the biofilm (Fig 1). We detect that diffusion and activity of the peptide at the deeper layers of the biofilm are negligible. The results of pepR re-dosing suggest that the observed low peptide activity in deep layers of the biofilm is due to an almost irreversible binding of peptide to the biofilm matrix. This mechanism is likely to contribute greatly to bacterial biofilms tolerance to the action of cationic AMPs in general.

Conclusions: The pepR AMP is shown to have limited diffusion and activity on the inner layers of a staphylococcal biofilm, and results are consistent with irreversible binding of the AMP to the biofilm matrix. This confirms that in order to maximize AMP bactericidal potential, strategies must be used to maximize diffusion of these peptides through the biofilm matrix.

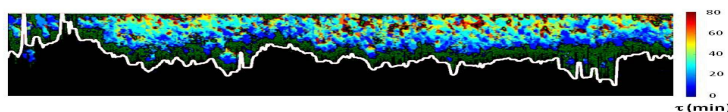


Fig 1: 2D illustrative image of confocal microscopy -based calcein release assay. False-color image of half-time for permeabilization of bacteria within biofilms exposed to pepR.

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