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Eradication of coagulase negative staphylococcal biofilms by P128 alone and synergistic inhibition in combination with antibiotics

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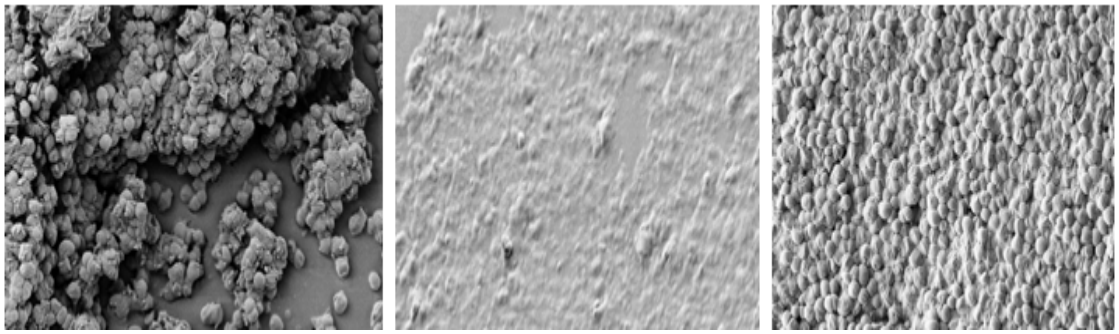
Background: Biofilms are highly recalcitrant to the action of antibiotics and thus infections involving biofilms are known to be difficult to treat and to have high relapse rates. S. epidermidis, a coagulase negative Staphylococcus (CoNS) species is a prolific biofilm producer and is commonly associated with device associated infections in humans. P128, a chimeric recombinant ectolysin (phage lysin involved in cleaving the peptidoglycan from outside the bacterium during DNA injection) has previously demonstrated efficacy on MRSA biofilms. We have now tested the anti-biofilm activity of anti-staphylococcal protein P128 on multiple CoNS biofilm models in vitro alone and in combination with standard-of-care (SoC) antibiotics.

Material/methods: Minimum biofilm inhibitory concentration (MBIC) was determined in 72 hour grown biofilm in microtitre plates using MTT dye. A similar method was followed for determination of synergy in biofilms with SoC drugs using a checkerboard protocol. Eradication of biofilm biomass by P128 was investigated by crystal violet staining in microtitre plates and by safranin staining in biofilms grown on catheters. Scanning electron microscopy was used to confirm the eradication of biofilms by P128. Bactericidal activity of P128 on CoNS biofilms was measured by CFU reduction assay in biofilms grown on catheters.

Results: MBIC values of P128 on biofilms of 3 clinical strains of S. epidermidis ranged from 16 to 32 µg/ml, only 2 to 4-fold higher than their MICs. The SoC antibiotics showed much higher MBIC values, ranging from 16-250 µg/ml, a 10 to 100-fold increase over their MICs. In checkerboard assays no antagonism was seen with any of the drugs used in combination with P128. Low FIC index values seen in combination with SoC antibiotics meant that P128 inhibits CoNS growth in biofilms in a synergistic manner. P128 could destroy the CoNS biofilms in microtitre plates and on catheters in less

than two hours when used at 1X MIC, while SoC antibiotics even at > 10X MIC showed poor activity.. Enumeration of biofilm CFU in catheters after treatment with 1 X MIC of P128 for 6 hours showed a reduction of> 99% in cellular viability, suggesting a strong bactericidal effect of P128 on CoNS

Conclusions: P128 shows potent inhibition of bacterial growth in all CoNS biofilms.Lower FIC index values obtained in MBIC assays using antibiotics in combination with P128 suggests a strong synergistic inhibitory effect of P128 and antibiotics. P128 at low concentrations showed rapid eradication of biofilm mass in microtitre plates and catheters, which was confirmed by absence of biofilm structure and cells in P128 treated samples by electron microscopy. Potent anti-biofilm activity of P128 on CoNS biofilms makes it a candidate for clinical development for treating device associated infections.



Cell Control P128 (1X MIC, 8 μg/ml) Vancomycin (15X MIC, 30 μg/ml)

Fig: SEM images of *S. epidermidis* biofilm treated with P128 and Vancomycin