SAAP-148 protects 3D epithelial skin and airway models against colonization by antimicrobial resistant bacteria

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Background

Antimicrobial resistance is an increasing burden on global health systems and bolstering our antimicrobial toolkit with new, synergistic agents is essential to combat this imminent threat. Cationic host defense peptides (HDPs), and their synthetic derivatives, are alluring candidates due to their unique properties. HDPs are amphiphilic peptides expressed by host cells that combine good, broad-spectrum antimicrobial capabilities with the ability to modulate host immune defense and tissue repair.

Cathelicidin and its active peptide, LL-37, is a vital HDP for preventing bacterial and viral infections, and is largely conserved among vertebrate species. A library of synthetic antibacterial and anti-biofilm peptides (SAAPs) was developed from the LL-37 consensus sequence. SAAP-148 has emerged as a leading candidate due to its enhanced microbicidal and anti-biofilm activity, particularly against multidrug-resistant (MDR) bacteria.

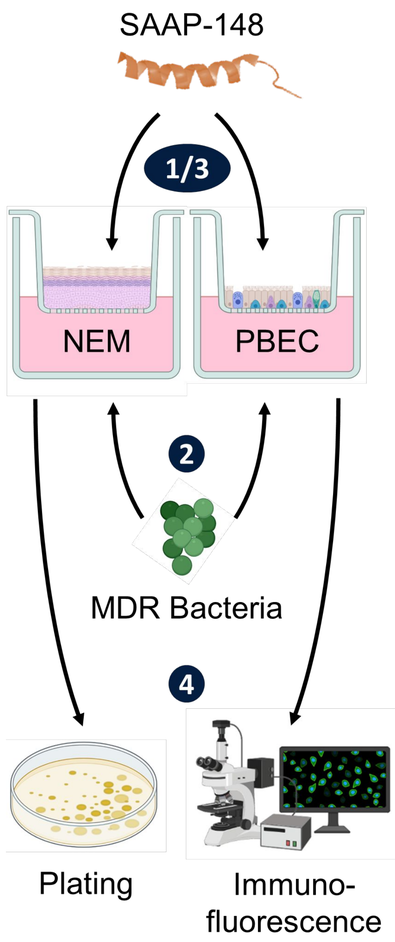
3D tissue models offer a better recapitulation of infection dynamics than suspension or monolayer cultures. Parallel models of skin and respiratory tissue can be constructed as N/TERT-1 epidermal models (NEMs) and primary bronchial epithelial cells (PBECs), respectively. Both NEMs and PBECs have been used extensively to model infection, wounding, inflammation and tissue repair.

Here, we assessed the efficacy of SAAP-148 against MDR infections in parallel 3D epithelial models and the synergistic potential of SAAP-148 with the repurposed agent, halicin. We hypothesised that SAAP-148 would demonstrate both direct and indirect antibacterial activities and synergise with halicin against MDR bacteria.

Methods

NEMs and PBECs, differentiated at the air-liquid interface, were infected with MDR strains of methicillin-resistant *Staphylococcus aureus* (MRSA, LUH14616) or *Pseudomonas aeruginosa* (LUH15103) [2]. Models were treated with SAAP-148 before or after infection [1/3]. Models were then either broken down and bacterial survival and adhesion measured, or stained and their immunofluorescence analysed by confocal microscopy [4]. Apical surfaces of the models were washed with PBS and planktonic bacteria were diluted, plated, and counted, while models were bead-beat to obtain the adherent bacteria then diluted, plated, and counted from the suspensions.

To investigate the synergy between SAAP-148 and halicin, a checkerboard assay of the two agents was performed. Models were pre-treated with a range of SAAP-148 concentrations for 1 hour, infected with MRSA (LUH14616), and then treated with a range of halicin concentrations for 3 hours. The decrease in minimum eradication concentration (MEC) of one agent when paired with any concentration of the other contributes to the fractional index, an indicator of synergy.



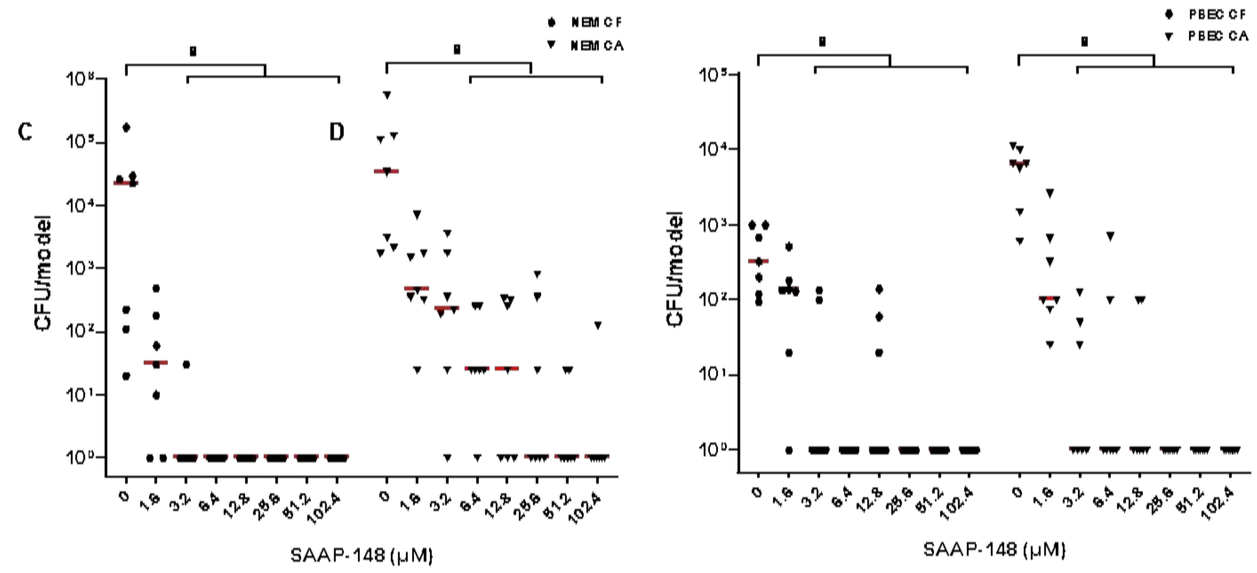


Figure 1. Survival and adherence of MRSA in NEM and PBEC models after infection and SAAP-148 treatment.

Results

Effect of SAAP-148 on bacterial survival and adherence in models post- and pre-infection – Bacteria are eradicated from the cell-free (CF) and –associated (CA) fractions of NEMs and PBECs (Figure 1). Models treated with SAAP-148 for 24, 6 or 1 hour(s) protected models from MRSA colonisation (Figure 2).

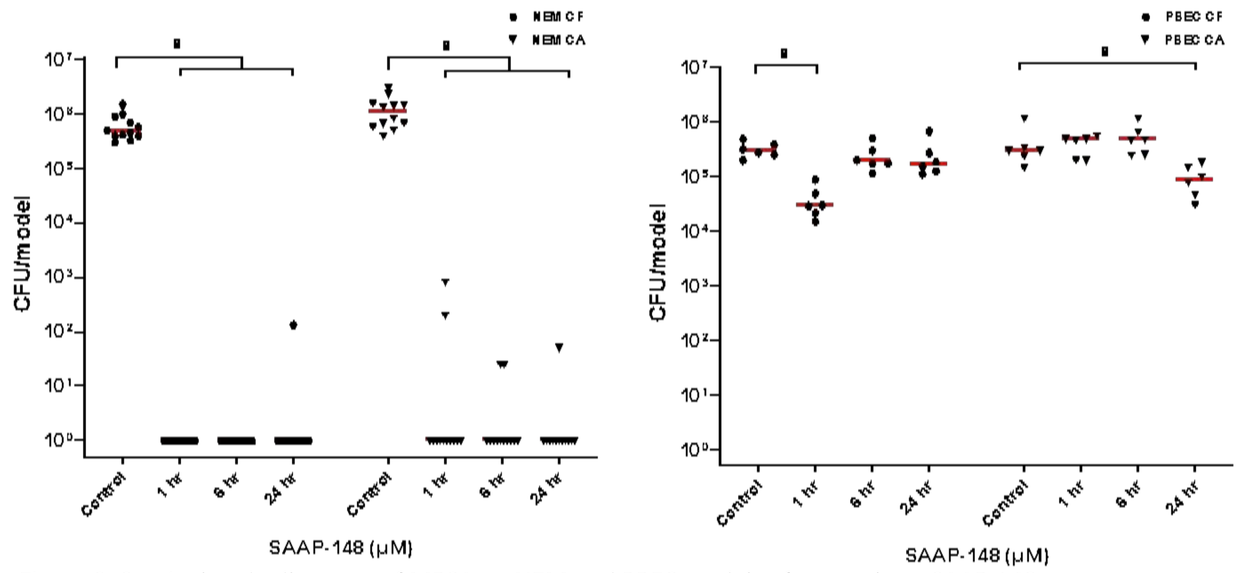


Figure 2. Survival and adherence of MRSA on NEM and PBEC models after one hour SAAP-148 pre-treatment.

Effect of SAAP-148 pre-treatment against Gram–negative MDR bacteria – In NEMs, P. aeruginosa was eradicated from both fractions at 51.2 μM, and neither of the PBEC fractions (Figure 3).

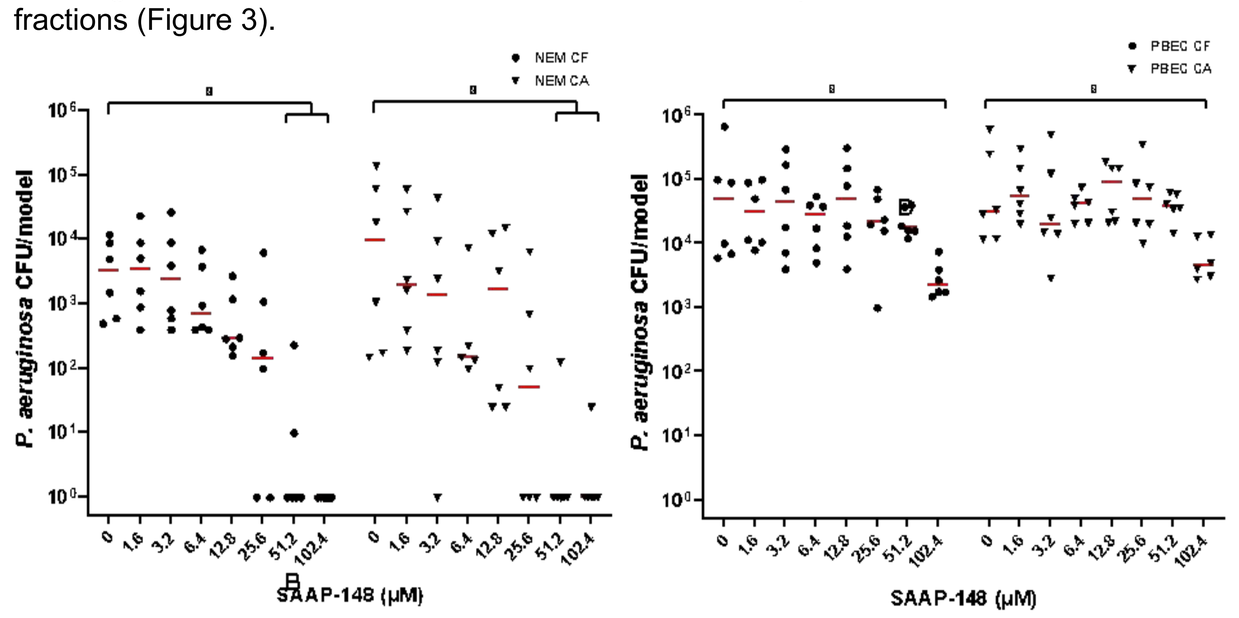


Figure 3. Survival and adherence of MDR P. aeruginosa *on NEM and PBEC models after one hour SAAP-148 pre-treatment.*

Retention of SAAP-148 in models – Treatment of models with SAAP-148-FITC for up to 24 hours revealed the retention of SAAP-148 in the stratum corneum equivalent of NEMs and nuclear aggregates, as opposed to no retention in PBEC models (Figure 3).

Effect of combined SAAP-148 and halicin treatment on bacterial survival and adherence in models – MRSA were eradicated from NEMs with 4-8 fold lower concentrations of either agent, while 2-64 fold lower concentrations were required for PBECs (Table 1). The fractional eradication scores for NEMs were <0.5 indicating synergism and <1.0 for PBECs, indicating an additive effect.

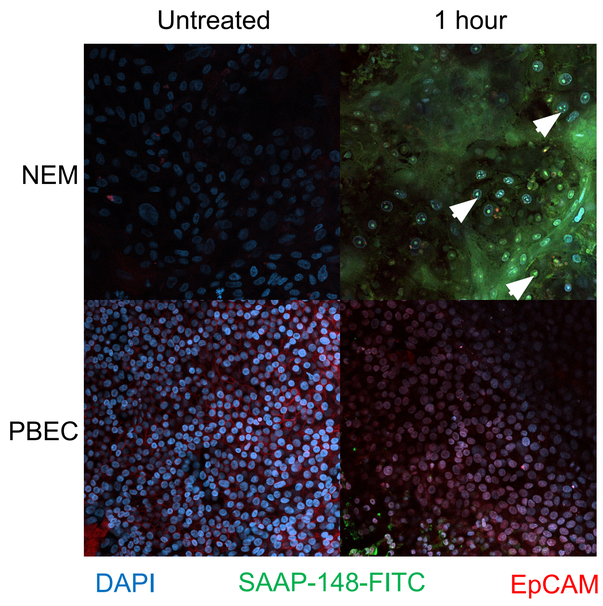


Figure 3. Models exposed to FITC-SAAP-148 and stained for nucleus (DAPI) and membrane (EpCAM). White arrows indicate nuclear aggregates.

Table 1. MEC table of checkerboard assay between SAAP-148 and halicin, and their fractional indices

		Cell Free		Cell Associated			
Model	**Agent**	**Individual MEC (μM)**	**Combined MEC (μM)**	**Individual MBEC (μM)**	**Combined MBEC (μM)**	**FEC (MEC)**	**FEC (MBEC)**
NEMs	Halicin	102.4	12.8	102.4	12.8	0.25	0.37
	SAAP-148	51.2	6.4	51.2	12.8		
PBECs	Halicin	204.8	3.2	204.8	51.2	0.52	0.75
	SAAP-148	102.4	51.2	102.4	51.2		

Summary

- SAAP-148 prevents bacterial colonisation up to 24 hours before infection in NEMs.
- SAAP-148 pre-treatment deters both Gram-positive and –negative MDR bacteria.
- SAAP-148 is retained in NEMs but not PBECs.
- SAAP-148 pre-treatment synergises with halicin against MDR bacteria in epithelial models of infection.