A Novel Antimicrobial Wound Gel* to Target Wound Related Antibiotic Tolerant Biofilms

Jeyachchandran Visvalingam, PhD, Nandadeva Yakandawala, PhD, Miloslav Sailer, PhD, Anna Muzaleva, Greg Schultz, PhD

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Abstract

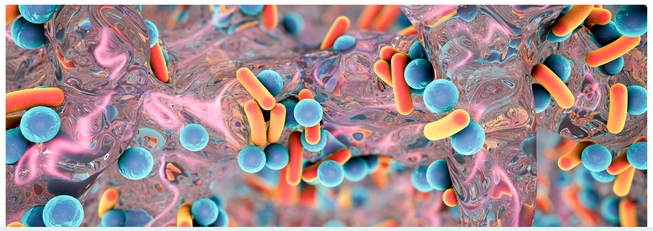
Chronic wounds are often colonized by biofilm forming bacteria and one of the key characteristics of chronic biofilm-based infections are extreme tolerance to antibiotics and many other conventional antimicrobial agents[1]. Thus, any wound care treatment targeting chronic wound infections need to be able to inactivate antibiotic tolerant biofilm. A Novel Antimicrobial Wound Gel (NAWG) was tested for its effectiveness against antibiotic tolerant biofilms using colony biofilm and porcine skin explant biofilm models. In both models, NAWG effectively reduced viable numbers of antibiotic tolerant biofilm of *S. aureus* and *P. aeruginosa*.

Introduction

Chronic wounds are often colonized by biofilm forming bacteria and one of the key characteristics of chronic biofilm-based infections are extreme tolerance to antibiotics and many other conventional antimicrobial agents[1]. Thus, any wound care treatment targeting chronic wound infections need to be able to inactivate antibiotic tolerant biofilm. A Novel Antimicrobial Wound Gel (NAWG) has been formulated with metal chelators, an antimicrobial agent, and a non-ionic surfactant to disrupt extra polymeric matrix of biofilm and to enhance inactivation of biofilm embedded microorganisms. In this study, ability of NAWG to control antibiotic tolerant biofilms was evaluated using conventional colony biofilm and pig skin explant biofilm model.

Materials and Methods

Colony Biofilm[2]: Mature biofilms of *Staphylococcus* aureus and *Pseudomonas aeruginosa* were grown on nitrocellulose membrane for 72 h at 37°C on Tryptic Soy Agar (TSA). *S. aureus* and *P. aeruginosa* Biofilms were washed twice in PBS to remove loosely attached and planktonic cells and treated for 24 h at 37°C in 50x of minimal inhibitory concentration (MIC) of Oxacillin (3.125 µg/mL) and Gentamicin (312.5 µg/mL), respectively. Antibiotic treated biofilms were washed twice in PBS and treated for 24 h at 37°C with NAWG. Viable numbers of biofilm embedded organisms were determined before antibiotic treatment, after antibiotic treatment and after NAWG treatment. Experiments were performed in triplicate and at least two independent experiments were performed (n=6).



Porcine Skin Explant Biofilm[3]: Mature biofilms of *S. aureus* and *P. aeruginosa* were grown on 13 mm diameter porcine skin explant containing 2-3 mm partial thickness wound placed on to 0.5% TSA for 24 h at 37°C and 48 h at 37°C on 0.5% TSA containing 1.5625 µg/mL Oxacillin and 62.5 µg/mL Gentamicin, respectively. *S. aureus* and *P. aeruginosa* biofilms were treated for 24 h at 37°C in 1.5625 µg/mL of MIC of Oxacillin and 62.5 µg/mL Gentamicin, respectively. Antibiotic treated biofilms were washed three times in PBS and treated for 72 h (3 d) at 37°C with NAWG to mimic clinical dressing change interval. Viable numbers of biofilm embedded organisms were determined before antibiotic treatment, after an tibiotic treatment and after NAWG treatment. Porcine explants containing biofilm were treated with NAWG without antibiotic pretreatment to determine its effect on total biofilm.

Results

Colony Biofilm: Treatment with 50x MIC of Oxacillin and Gentamicin treatment caused significant ≥2 and ≥4 log reduction ($P \leq 0.05$) in viable numbers of *S. aureus* and *P. aeruginosa* biofilm, respectively. Proportion of antibiotic tolerant *S. aureus* and *P. aeruginosa* in biofilms were ≥7 and ≥6 log CFU, respectively. NAWG reduced viable numbers of both organisms below detection after 24 h treatment at 37°C (Fig 1).

Porcine Skin Explant Biofilm: Treatment with NAWG was performed for 3 days to mimic clinical dressing change interval. NAWG treatment significantly ($P<0.05$) reduced viable numbers of *S. aureus* and *P. aeruginosa* by 6.3 and 1.3 log CFU, respectively from total biofilm (Fig 2A). After 24 h antibiotic treatment of *S. aureus* and *P. aeruginosa* biofilms, viable numbers did not change and remained ≥ 7 log CFU. After 3 days of NAWG treatment, viable numbers of *S. aureus* reduced to below detection, causing 7 log CFU reduction compared to control, while, numbers of *P. aeruginosa* was 2.4 log CFU lower than control and similar to initial viable numbers.

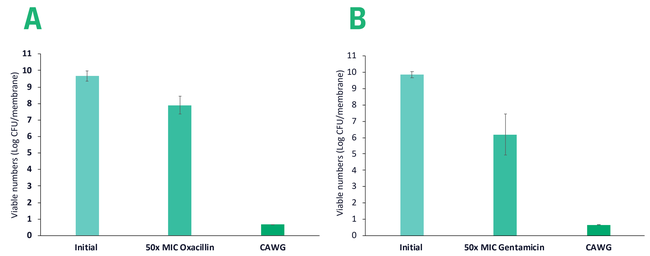


Figure 1: Effect of NAWG on antibiotic tolerant biofilm of A) *S. aureus* and B) *P. aeruginosa* using colony biofilm model. Horizontal line indicates detection limit.

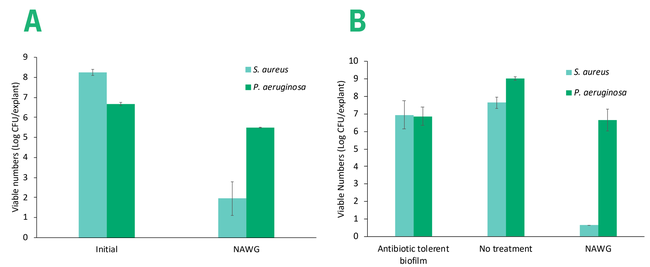


Figure 2: Effect of NAWG on A) total and B) antibiotic tolerant biofilm of *S. aureus* and *P. aeruginosa* using porcine skin explant biofilm model.

Discussion

Tolerance of biofilm embedded bacteria to antibiotics has been attributed to restricted penetration of the antibiotics, restricted growth at low-oxygen tension, expression of biofilm-specific genes and the presence of persister cells. Presence of antibiotic tolerant cells in biofilm related chronic wound infections may lead to treatment failures and prolonged infection or other complications. Results from this study demonstrated that ≥ 6 log CFU of biofilm embedded cells of *S. aureus* and *P. aeruginosa* were tolerant to treatment Oxacillin and Gentamicin, respectively. Testing against antibiotic tolerant biofilms using in vitro and ex vivo models showed that NAWG was effective at reducing viable numbers of antibiotic tolerant *S. aureus* and *P. aeruginosa* in biofilms.

References

[1]Bjarnsholt T. 2013. APMIS Suppl. 136:1-51. [2]Hammond, A.A., Miller, K.G, Kruczek, C.J., Dertien, J., Colmer-Hamood, J.A., Griswold, J.A, Horswill, A.R., Hamood, A.N. (2011) Burns. 37:312-321. [3]Yang Q, Phillips PL, Sampson EM, Progulske-Fox A, Jin S, Antonelli P, Schultz GS. Wound Repair Regen. (2013) 21:704-714.

*Commercial names of Novel Antimicrobial Wound Gel are coactiv+™ Antimicrobial Wound Gel and revyve™ Antimicrobial Wound Gel

Kane Biotech Inc, Winnipeg, Manitoba, Canada | **Contact email: msailer@kanebiotech.com**