A Chelation Approach to the Biofilm and Metalloprotease Problem in Chronic Wounds

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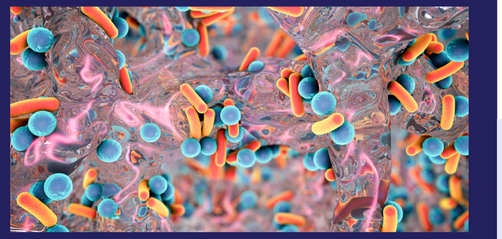
KANE BIOTECH

coactiv+™
ANTIMICROBIAL WOUND GEL

INTRODUCTION

Chronic wounds have a prolonged inflammatory phase which hinders the normal wound healing process. These wounds are often colonized by biofilm forming bacteria that can trigger the inflammatory process and elevate levels of matrix metalloproteases. These enzymes often cause tissue damage[1-3]. The coactiv+™ Antimicrobial Wound Gel (CAWG) has been formulated with metal chelators (EDTA/citric acid), an antimicrobial agent (PHMB), and a non-ionic surfactant (Poloxamer 407) to disrupt the extra polymeric matrix of biofilm and inhibit metalloprotease activity. The objectives of this study were to evaluate prolonged antibiofilm and antimicrobial activity of the CAWG and to assess its anti-metalloprotease activity.



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[1] Wolcott, Hanson, J.D. Rees, E.J. Koenig, L.D. Phillips, C.D., Wolcott, R.A., Cox, S.B., White, J.S. (2016) Wound Rep. Reg. 24: 163-174. [2] Bjarnsholt, T. 2013. APMIS 121:1-51. [3] Trengove, N.J, Stacey, M.C., Fracs, D.S., Macauley, S., Bennett, Gibson, J., Burslem, F, Murphy, G., Schultz, G. (1999) Wound Rep. Reg. 7: 442-452. [4] Hammond, A.A., Miller, K.G, Kruczek, C.J., Dertien, J., Colmer-Hamood, J.A., Grisworld, J.A, Horswill, A.R., Hamood, A.N. (2011) Burns. 37:312-321.

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PROLONGED ANTIMICROBIAL ACTIVITY

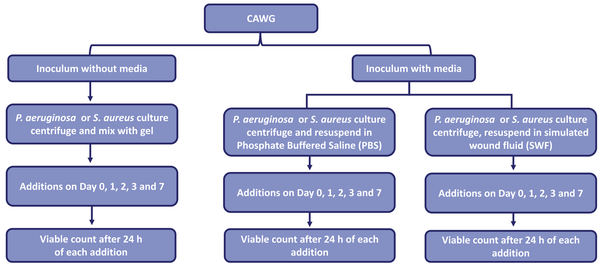


Table 1: Assessing antimicrobial activity of CAWG after repeated exposure to *S. aureus*

Treatment option	Day 0		Day 1		Day 2		Day 3		Day 7	
	Inoculum/mL	After 24 h	Inoculum/mL	After 24 h	Inoculum/mL	After 24 h	Inoculum/mL	After 72 h	Inoculum/mL	After 24 h
SWF	7.12±0.08	<1.0	6.74±0.19	<1.0	6.68±0.15	<1.0	7.25±0.14	<1.0	6.97±0.08	<1.0
PBS		<1.0		<1.0		<1.0		<1.0		<1.0
Adding pellet	7.23±0.03	<1.0	6.59±0.10	<1.0	6.91±0.09	<1.0	6.98±0.06	<1.0	7.07±0.01	<1.0

Table 2: Assessing antimicrobial activity of CAWG after repeated exposure to *P. aeruginosa*

Treatment option	Day 0		Day 1		Day 2		Day 3		Day 7	
	Inoculum/mL	After 24 h	Inoculum/mL	After 24 h	Inoculum/mL	After 24 h	Inoculum/mL	After 72 h	Inoculum/mL	After 24 h
SWF	7.00±0.09	<1.00	6.62±0.07	<1.00	6.57±0.41	<1.0	7.02±0.20	<1.0	7.03±0.05	<1.0
PBS		<1.00		<1.00		<1.0		<1.0		<1.0
Adding pellet	6.86±0.07	<1.00	6.45±0.17	<1.00	6.89±0.10	<1.0	7.00±0.31	<1.0	6.98±0.16	<1.0

The CAWG was effective at killing bacteria even after repeated exposure to high bacterial counts (≥10^6 CFU/mL) over the course of 7 days (Table 1 & 2)

PROLONGED ANTIBIOFILM ACTIVITY

Overnight cultures of test organisms were diluted to 10^7 CFU/mL. Ten µL diluted culture was added onto nitrocellulose membrane which was placed on an appropriate agar surface[4]. Biofilms were grown for 72h on the nitrocellulose membranes. Treatment was incubated for 1, 2, 3, 4, and 7 days at 37°C for viable count. Initial viable count also obtained from day 0, before application of gel and initial count of these organisms were similar to viable counts of no treatments recorded at each sampling day.

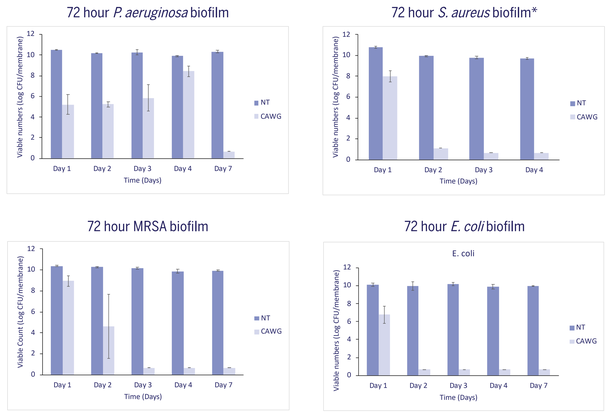
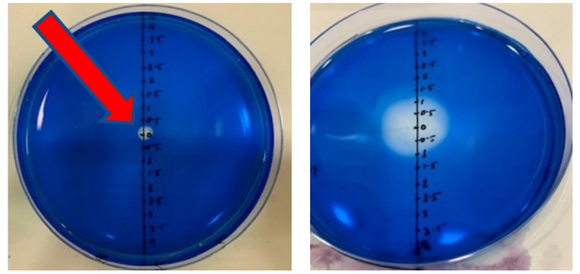


Figure 1: Changes in viable numbers of wound related pathogens in mature biofilm after exposure to CAWG.
* No counts were observed after Days 3 and 4, therefore no count was taken at Day 7

Over the course of 7 days (typically maximum for between dressing change) the CAWG remained active against *P. aeruginosa*, *S. aureus*, MRSA and *E. coli* mature biofilms. The viable counts of these organisms in biofilms were below detection by ≤7 days. (Figure 1)

PROTEASE INHIBITION

The components of the CAWG without antimicrobial and surfactant significantly inhibited MMP9 activity, see image below. It also showed inhibition of TACE and complete inhibition of Elastase. When formulated into the CAWG, collagenase and TACE activity were completely inhibited.



MMP9 + CAWG components | MMP-9 alone

Figure 2: Photograph showing CAWG chelating components inhibiting MMP9 activity. Agar (0.5%) + Gelatin (0.5%) gel in 50 mM Tris-HCl in Petri dish. 10 µl 50 µg/mL MMP9 or 10 µl 50 µg/mL MMP9 + 10 µl 5x CAWG components loaded at the center. Stained with Coomassie blue after overnight incubation at 37°C. The white circle at the center is due to Gelatin degraded by MMP9.

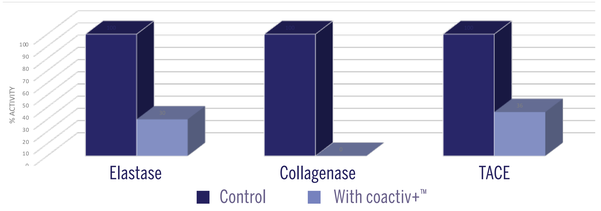


Figure 3: In vitro laboratory data shows reduction in protease activities. Experiment was performed as per commercial testing kit instructions and demonstrated that active ingredients in the CAWG effectively inhibited metalloproteases (matrix metalloprotease and TACE).

ANTIMICROBIAL ACTIVITY TESTING

Testing was performed according to ASTM E235-03 and compared to other wound gel products.

CAWG was effective at reducing viable count of majority of common wound pathogens tested below detection limit by 30 min. CAWG performed similar to premium wound gels on the market against the same organisms. (Table 3)

* PWG1, PWG2, and PWG3 represent the three premium wound gels tested for comparison

Disclaimer (based on time of poster submission): coactiv+™ Antimicrobial Wound Gel is currently being reviewed by the FDA and is not yet cleared for sale in the USA

Table 3: Log CFU reductions observed after treating with different premium wound gels for 30 min

Organism	Log Reduction (Log CFU/mL)			
	CAWG	PWG1	PWG2	PWG3
Staphylococcus aureus	>5	>5	>5	>5
Methicillin Resistant *S. aureus* (MRSA)	>5	>5	>5	>5
Pseudomonas aeruginosa	>6	>6	>6	>6
S. epidermidis	>5	>5	>5	>5
Escherichia coli	>5	>5	>5	>5
Acinetobacter baumannii	>5	>5	>5	>5
Klebsiella pneumoniae	>5	>5	>5	>5
Candida albicans	≥4	≥4	≥4	1.4
Streptococcus pyogenes	>4.5	>4.5	>4.5	>4.5
Cutibacterium acnes	>5	>5	>5	>5

CONCLUSIONS

This study demonstrated that in addition to CAWG having similar antimicrobial activity to many commercially available wound gels, CAWG also maintained its antimicrobial activity for ≥7 days after repeated exposure to ≥10^6 CFU/mL major wound pathogens. Furthermore, CAWG retained its antibiofilm activity for ≥7 days. CAWG active ingredients were also effective at inhibiting activity of common metalloproteases found in chronic wounds. Overall, these results demonstrated that the combination of chelators, antimicrobial agent and surfactants effectively provided prolonged antimicrobial and antibiofilm activity against major wound pathogens.