Anti-biofilm and Anti-metalloprotease Activity of a Novel Wound Gel*

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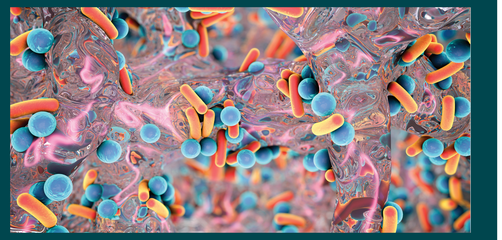
INTRODUCTION

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]. A novel wound hydrogel (coactiv+™ Antimicrobial Wound Gel) has been formulated with metal chelators, an antimicrobial agent, and a non-ionic surfactant to disrupt the extra polymeric matrix of biofilm and to enhance inactivation of biofilm embedded microorganisms as well as to inhibit metalloprotease activity. The objectives of this study were to evaluate anti-biofilm activity of coactiv+™ Antimicrobial Wound Gel, assess anti-metalloprotease activity, compatibility with a variety of dressings, and to assess safety.



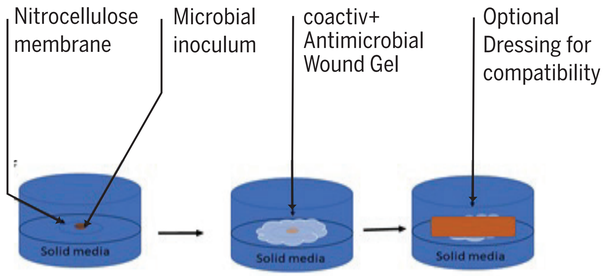
REFERENCES

(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.

* This product is not currently approved for sale in the USA.

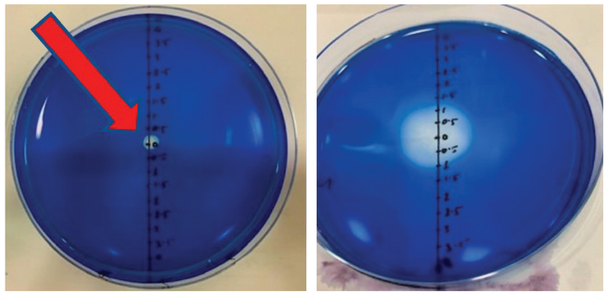
SAFETY AND EFFICACY

Biofilm in-vitro testing: 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]. Treatment regimen was applied after inoculation for inhibition or after 24 hours of incubation time at 37°C for eradication. Treatment was incubated for 24 hours at 37°C for single application, while for multiple application, treatment was removed at the 24h interval and re-applied on a new agar plate. Then viable numbers were enumerated. When compatibility with wound dressings were assessed, the wound dressing was placed onto coactiv+.



Protease inhibition: coactiv+ without antimicrobial and surfactant significantly inhibited MMP-9 activity, see image below. It also showed inhibition of TACE and complete inhibition of Elastase. When formulated into the coactiv+ wound gel, collagenase and TACE activity were completely inhibited.

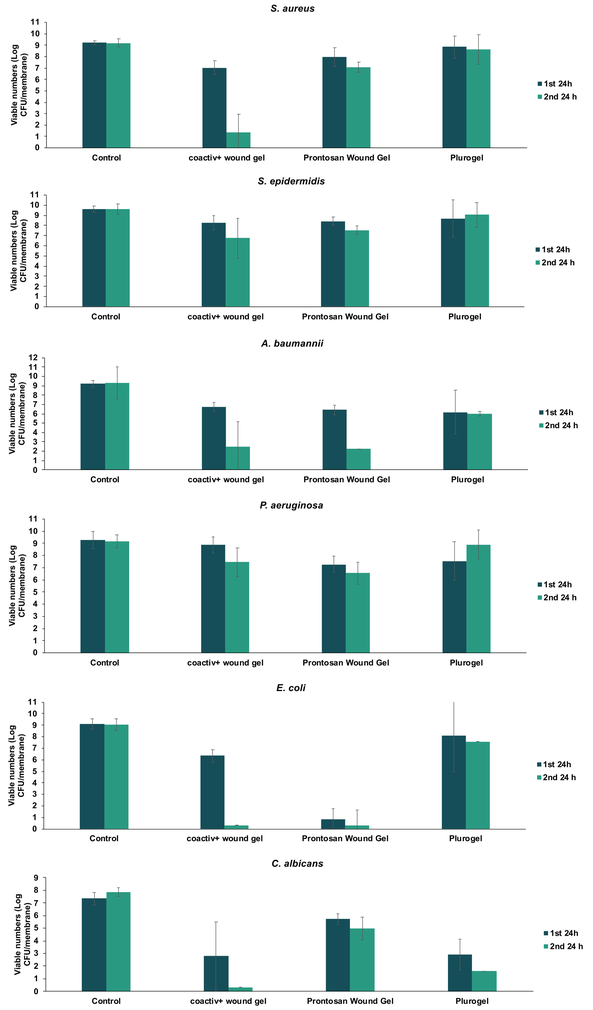
Agar (0.5%) + Gelatin (0.5%) gel in 50 mM Tris-HCl in Petri dish 10 µl 50 µg/mL MMP-9 or 10 µl 50 µg/mL MMP-9 + 10 µl 5x coactiv+ loaded at the center Stained with Coomassie blue after overnight incubation at 37˚C. The white circle at the center is due to Gelatin degraded by MMP-9.



MMP-9 + coactiv+

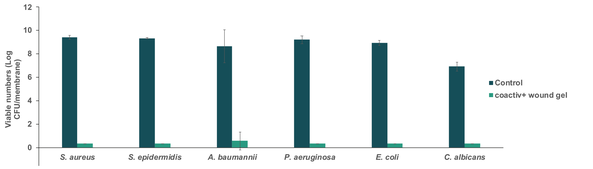
MMP-9 alone

BIOFILM ERADICATION ASSAY

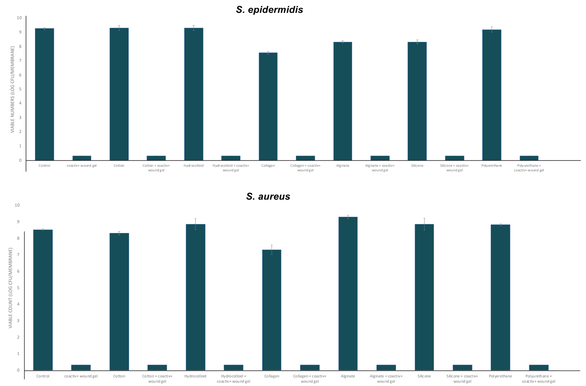


coactiv+ Antimicrobial Wound Gel completely inhibited the formation of biofilms in all organisms tested. After 2, 24h treatments biofilms consisting of *S. aureus*, *A. baumannii*, *E. coli*, and *C. albicans* were completely eradicated and 99% of bacteria were eradicated from biofilms consisting of *S. epidermidis* and *P. aeruginosa*. Overall, coactiv+ Antimicrobial Wound Gel performed equivalent or better than commercial competitor products in the biofilm assays.

BIOFILM INHIBITION ASSAY



COMPATIBILITY WITH DRESSING (BIOFILM INHIBITION)



coactiv+™ Antimicrobial Wound Gel completely inhibited the formation of biofilms in the presence of all common dressing types.

Pig Wound Healing Study: No irritation or inflammation were observed as demonstrated by lack of clinical signs of erythema. This was confirmed histologically by assessing the degree of inflammatory cells, e.g. macrophages and giant cells. No signs of tissue fibrosis as confirmed by absence of mast cells. When compared to commercial competitor, wound healing was considered equivalent or better.

Biocompatibility/safety: Through GLP testing it was shown that the coactiv+ Antimicrobial Wound Gel was non-genotoxic, non-irritating, non-sensitizing, non-pyrogenic and showed no evidence of acute systemic toxicity.

CONCLUSIONS

The dressing compatible coactiv+™ Antimicrobial Wound Gel developed by Kane Biotech approaches the biofilm problem with a triple threat; inhibition of metalloproteases and biofilm formation through chelation of divalent ions, a strong antimicrobial preservative, and a gentle non-ionic surfactant. coactiv+™ Antimicrobial Wound Gel has passed ISO 10993 biocompatibility testing and was equivalent or better than the commercial competitor when it comes to wound healing, which is afforded by providing a clean moist wound environment conducive to wound healing.