A PROTEASE ACTIVITY MODEL TO EVALUATE WOUND THERAPIES IN VITRO

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ABSTRACT

Objective: To compare the ability of Collagen/ORC/Silver with other collagen & Collagen/Silver products to reduce inflammatory proteases.

Methods: Samples of Collagen/ORC (oxidised regenerated cellulose), Collagen/ORC/Silver, collagen-only, collagen-silver or NOSF dressings were incubated at 37°C with solutions containing EPA. The level of activity present was clinically relevant based on previous clinical analysis of wound fluid. Using fluorometric assays, neutralophil-elastase derived (VHE) and matrix metalloproteinases (MMPs) activity was measured over time in the presence of each dressing. To confirm results wound fluid containing elevated protease activity (EPA) were also tested.

Results: Results indicate differences between each dressing type in their ability to reduce EPA, specifically in regard to neutrophil-derived elastase and MMPs. The Collagen/ORC/Silver and Collagen/ORC dressings were more effective at reducing EPA than other dressings tested. This was primarily due to the presence of ORC which provides unique functionality that cannot be achieved with either silver or collagen alone.

Conclusions: This study supports previous clinical studies where the combination of Collagen/ORC (V- Silver) was most effective at reducing EPA. A reduction in EPA helps rebalance the wound environment which in turn can help facilitate healing. This study also demonstrates our ability to measure EPA and compare the relative efficacy of different wound therapies, using an in vitro model.

Inflammatory Proteases – Measurement Methods

The effect of Collagen/ORC/Silver compared to Collagen and Silver products on protease levels was evaluated using protease activity assays. Clinically relevant levels of proteases were used in the in vitro assay; chronic wound fluid was used to assess effect in a wound with EPA.

Neutrophil-derived elastase was measured using a specific substrate and buffer system

Buffer: 0.1M Hapes, pH 7.5, 0.5M NaCl, 10% DMSO

Substrate: MαOsc/Ala-Ala-Pro-Val-7-amino-4-methylcoumarin

Total MMP activity was measured using a specific substrate and buffer system

Buffer: 50mM Tris, 10mM Calcium chloride dehydrate, 100mM Sodium chioride, 50mM Zinc chloride, 0.025% Brij 35, 0.09% Sodium Azide.


MMP levels were measured using ELISA based activity assays (Anspec).

Case Study – Clinical and Biochemical effect of Collagen/ORC/Silver treatment on a Diabetic foot ulcer

A 74 year old male with type 2 diabetes presented with a diabetic foot ulcer on the right foot. The patient had previously undergone transtibial amputation on this foot. At baseline the duration of the wound was 7 months with an area of 2.5 cm² and a maximum depth of 0.4 cm. Over 14 weeks treatment with Collagen/ORC/Silver there was a reduction in protease activity which was coupled with a reduction in wound area.

CONCLUSIONS

The results show that Collagen/ORC/Silver has properties which are beneficial to wound healing.

The results indicate differences between each dressing type in their ability to reduce the level of inflammatory proteases.

The unique combination of Collagen/ORC/Silver provides the greatest reduction of inflammatory protease activity over collagen/silver containing dressings both in vitro and in wound fluid.

Reducing inflammatory protease activity will rebalance the biochemical environment of the wound facilitating healing.