

In Vitro and Clinical Data Demonstrating the Ability of Collagen/ORC/Silver to Overcome the Hostile Chronic Wound Environment



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ABSTRACT

Introduction: Chronic wounds can remain unhealed for weeks, months or even years. Persistent and excessive inflammation contribute to this delay in healing. The overproduction of inflammatory cytokines and proteases perpetuates wound chronicity by generating a hostile environment. To neutralise this underlying pathophysiology and help promote healing, advanced wound therapies must address excessive inflammation and protease activity to provide an optimal wound environment.

Methods: In this study we have utilised an *in vitro* model to measure the ability of wound dressings to affect inflammation. We also measured the levels of inflammatory cytokines in wound fluid from venous leg ulcers, pre- and post-treatment with a collagen/ORC/silver dressing. This enabled us to determine the clinical relevance of our *in vitro* findings. In addition, we assessed the ability of collagen/ORC/silver to inactivate proteases, in particular, the inflammatory protease elastase.

Results: Results from our *in vitro* inflammatory model demonstrated that the collagen/ORC/silver dressing reduced the levels of pro-inflammatory cytokines; an effect not observed with similar levels of silver alone. This effect was also observed clinically, where the levels of inflammatory cytokines were reduced in wound fluid from venous leg ulcers post-treatment with collagen/ORC/silver. In contrast, other silver-containing dressings caused significant inflammatory cell death *in vitro*, an effect which may perpetuate the inflammatory process. The ability of collagen/ORC/silver dressings to inactivate elastase was found to be primarily due to the presence of ORC, as collagen/silver dressings had only limited effectiveness against this protease.

Conclusions: This study has demonstrated the ability of collagen/ORC/silver to reduce inflammatory cytokines and proteases both in our *in vitro* model and in the clinical setting. These results suggest that the application of collagen/ORC/silver to chronic wounds will reduce the inflammatory cycle and correct the biochemical imbalance allowing healing to progress.

CONCLUSIONS

The results show that Collagen/ORC/Silver provides many properties which are beneficial to wound healing

- Collagen/ORC/Silver protects inflammatory cell viability
- Collagen/ORC/Silver reduces inflammatory cytokine levels
- Collagen/ORC/Silver reduces inflammatory protease activity

The combination of Collagen/ORC/Silver provides greater functionality over collagen/silver containing dressings

Collagen/ORC/Silver shown to reduce inflammation in patients; both inflammatory protease activity and cytokines were reduced in wound fluid after the wound was treated with Collagen/ORC/Silver

PROMOGRAN® and PROMOGRAN PRISMA® are the only Collagen/ORC dressings available

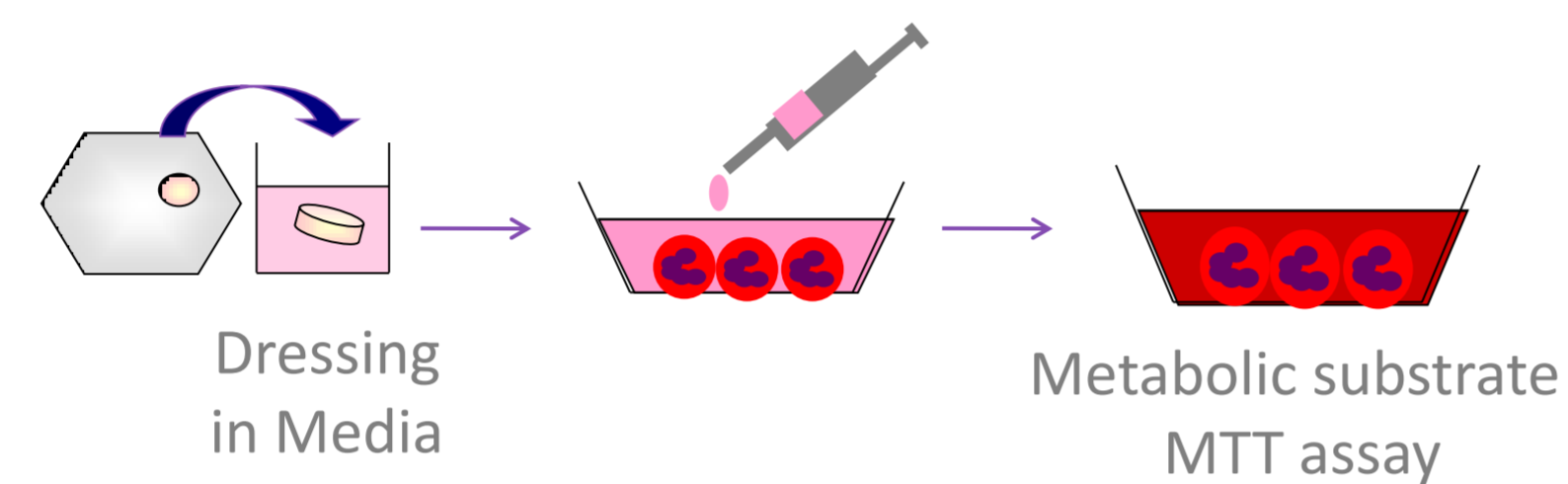
OBJECTIVES

- To demonstrate the ability of Collagen/ORC/Silver to reduce inflammation
- To examine the effect of Collagen/ORC/Silver & other Collagen /silver products on inflammatory cell viability, inflammatory cytokines & inflammatory protease activity

Assay for Inflammatory Cell Viability

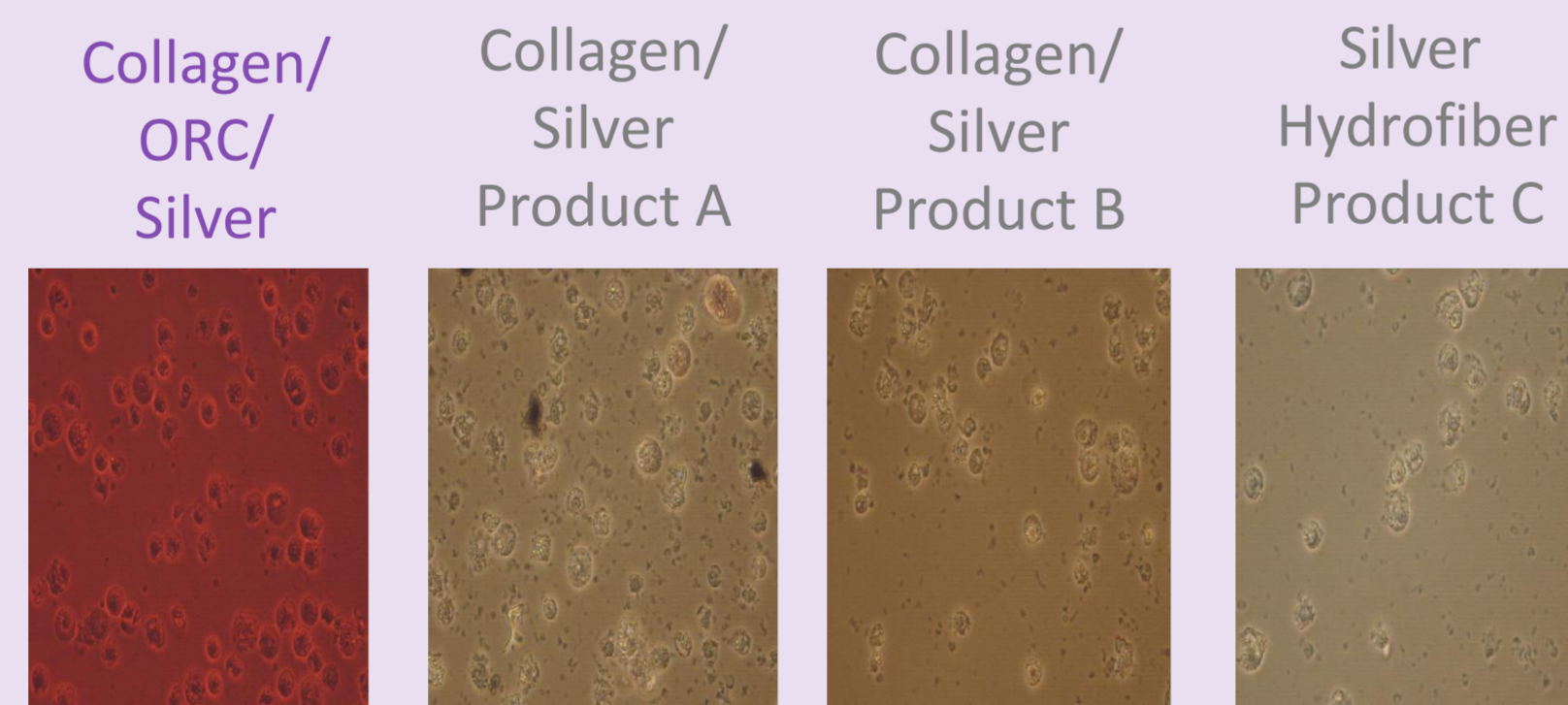
Effect of dressing materials on inflammatory cell viability

- Incubate dressing with cell media – Inc 24hrs 37°C
- Add dressing extract to inflammatory cells (THP-1 cells)
- Incubate 24hrs, 37°C, 5% CO₂
- Assess cell viability using metabolic substrate MTT and histologically using trypan blue stain



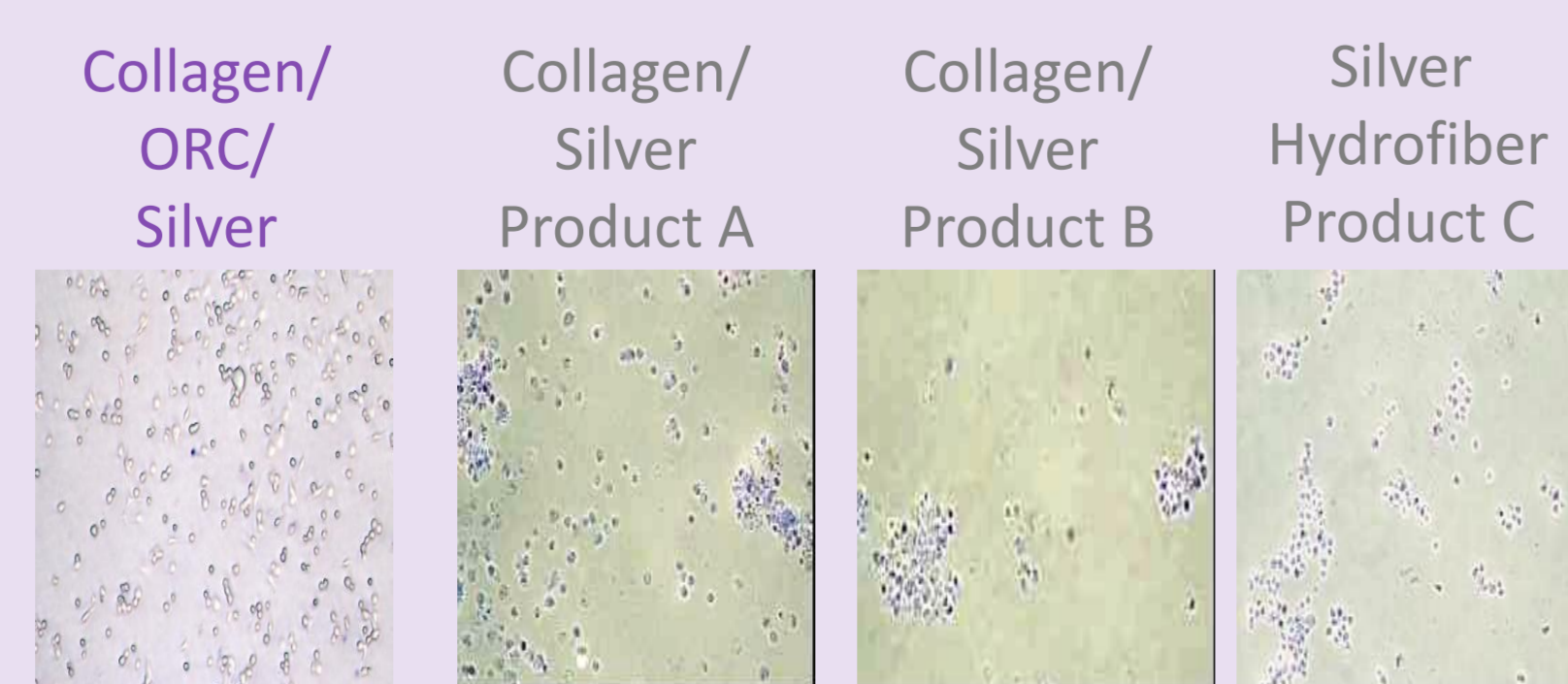
Effect on Inflammatory Cell Viability

Metabolic substrate
If cells are viable they will activate the metabolic substrate causing it to change colour
Initial colour yellow/brown once activated it turns red



Histological staining of cells:
Trypan blue exclusion assay

If cells are dead they leak, the blue dye penetrate the cell & it will stain the cell nucleus
If the cells are viable, they will not absorb the dye

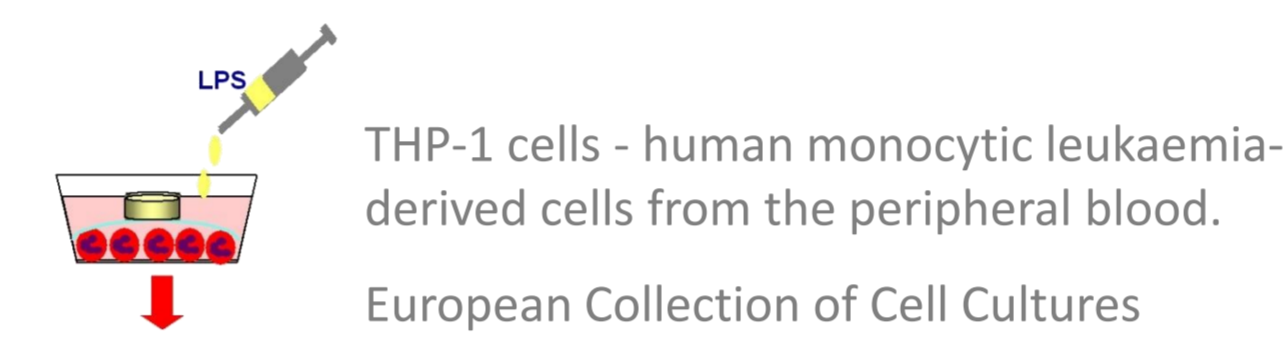


Only Collagen/ORC/Silver treated cells remain viable, other collagen-silver & silver products cause cell death

Effect on Inflammatory Cytokines

Modulation of inflammatory cytokines *in vitro*

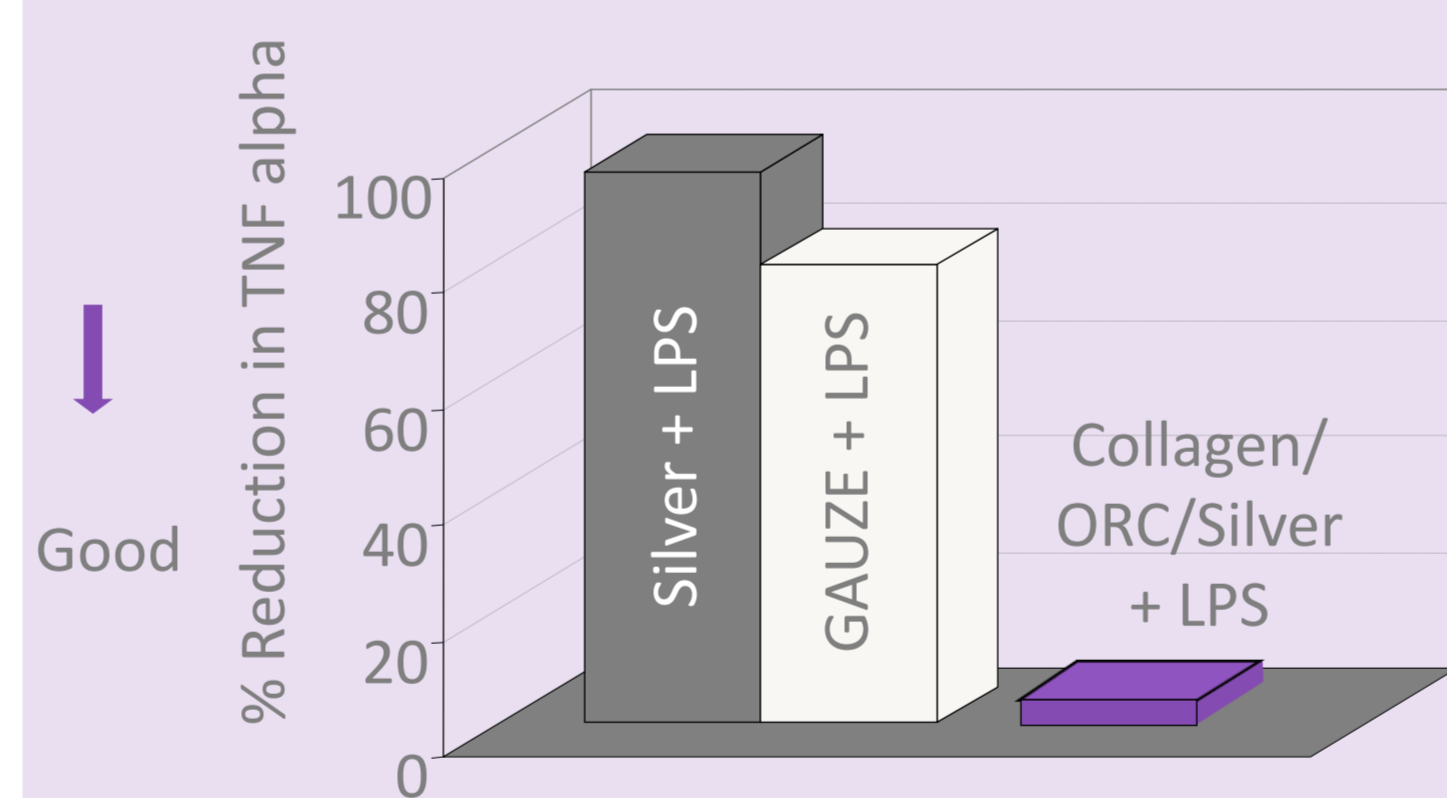
- Effect on inflammatory cytokine production was measured *in vitro*.
- The ability of a dressing to affect the inflammatory response was evaluated by adding the dressing in combination with LPS (Lipopolysaccharide) a bacterial endotoxin to the inflammatory cells and measuring the resultant cytokine levels.



Modulation of inflammatory cytokines in patients

- Effect on inflammatory cytokines was also measured clinically.
- Wound fluid samples were collected from patients before and after treatment with Collagen/ORC/Silver (PROMOGRAN PRISMA®)
- Levels of TNF-alpha (an inflammatory mediator) measured by ELISA

Reduction of Inflammatory Cytokines In Vitro

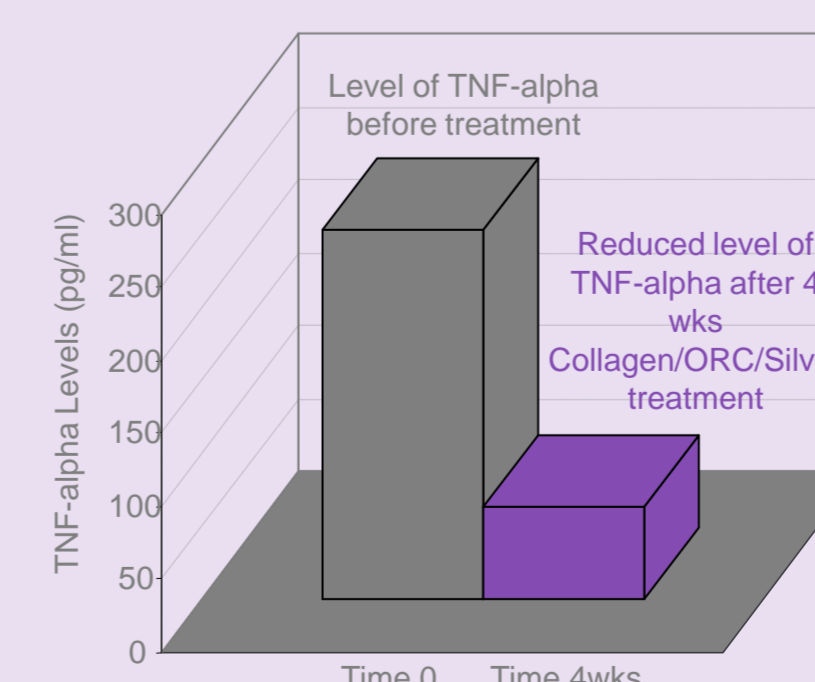
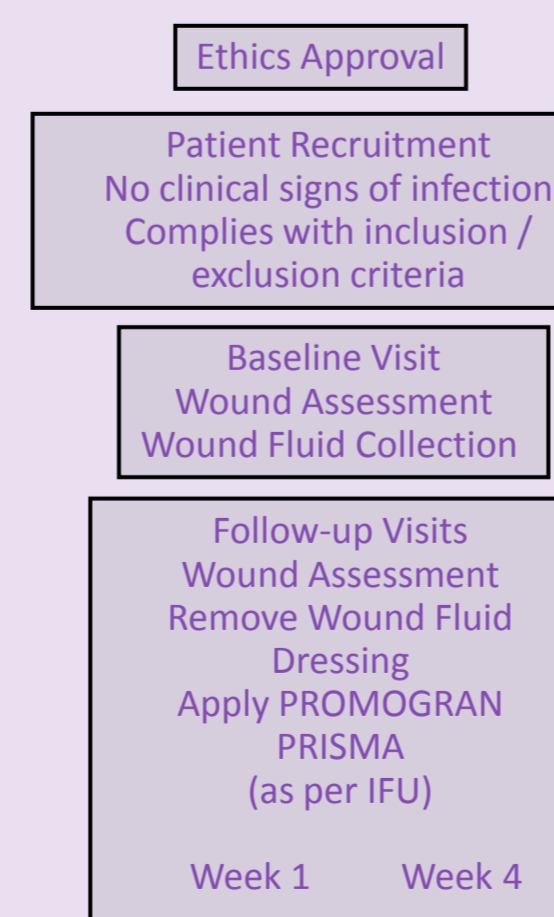


Other Collagen/Silver & Silver-containing dressings caused significant cell death therefore it was not possible to measure effect on TNF-a

Only Collagen/ORC/Silver reduced the levels of TNF-alpha; An affect not observed with Silver alone or Control gauze dressings

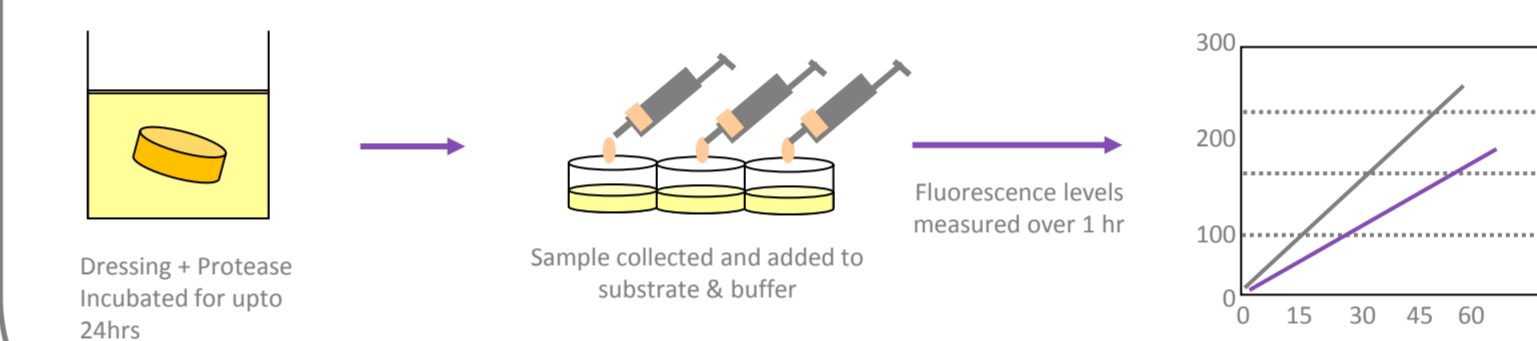
Reduction of Inflammatory Cytokines In Patients

Clinical Protocol



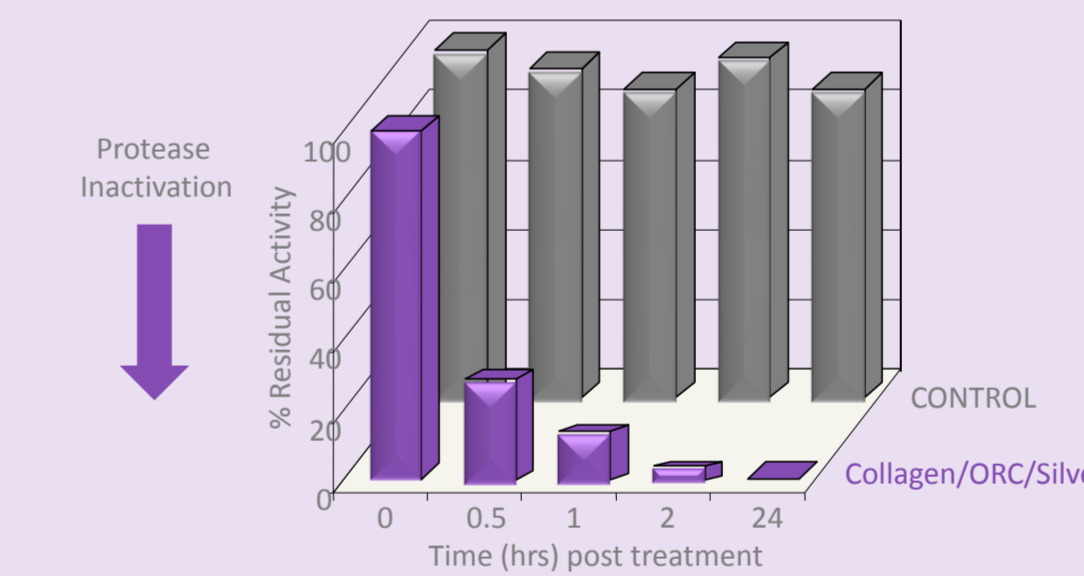
Effect on Inflammatory Proteases

- Protease levels were evaluated using protease activity assays.
- Clinically relevant levels of elastase was used in the assay
- Peptide substrate with a fluorescent reporter groups (AMC) was used for the activity assays in combination with an appropriate buffer system
- Neutrophil-derived elastase measured using a specific substrate and buffer system
- 0.1M HEPES, pH 7.5, 0.5M NaCl, 10% DMSO

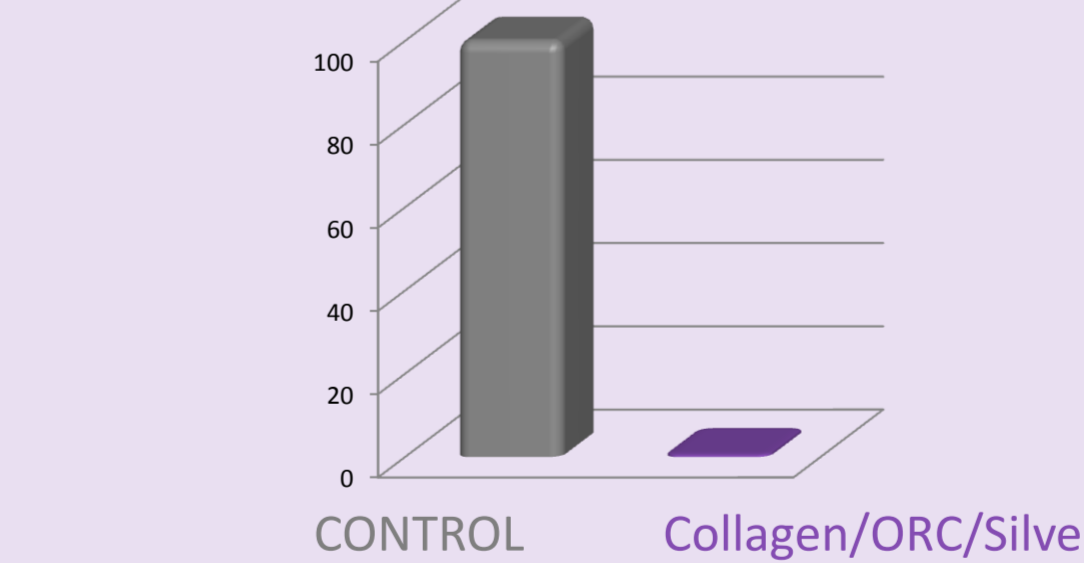


Effect on Inflammatory Protease Activity

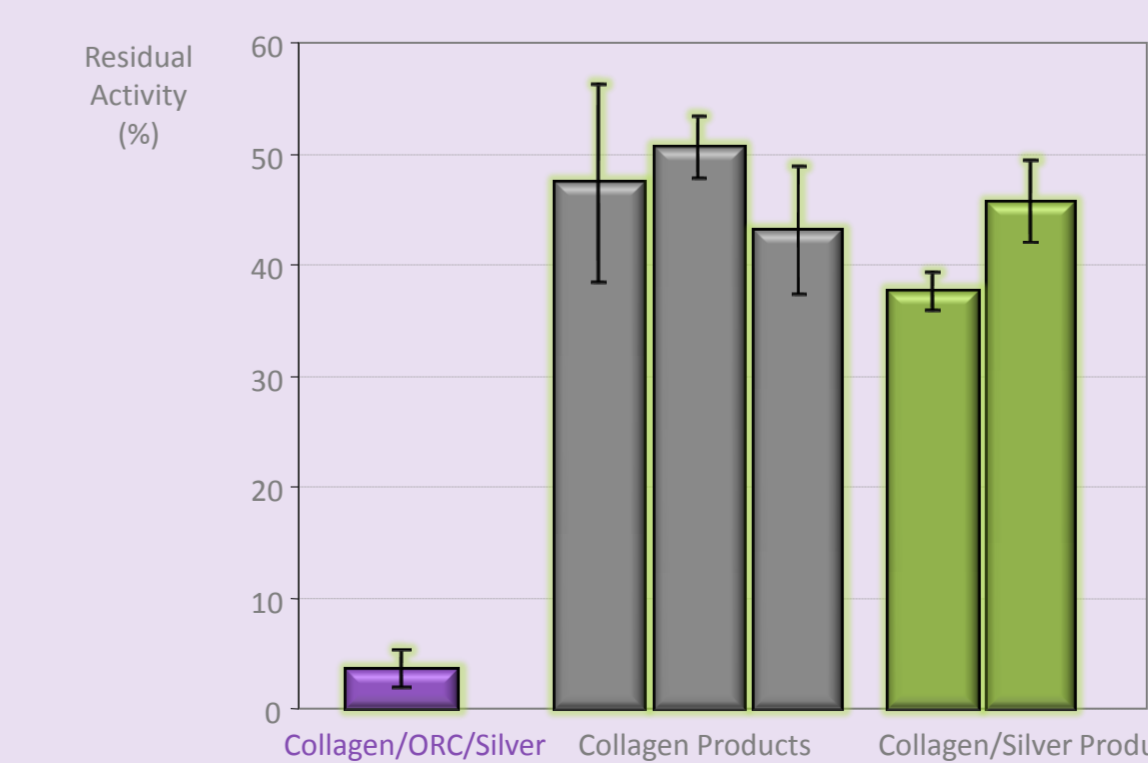
Effect on Elastase Activity



Effect on MMP-9 Activity



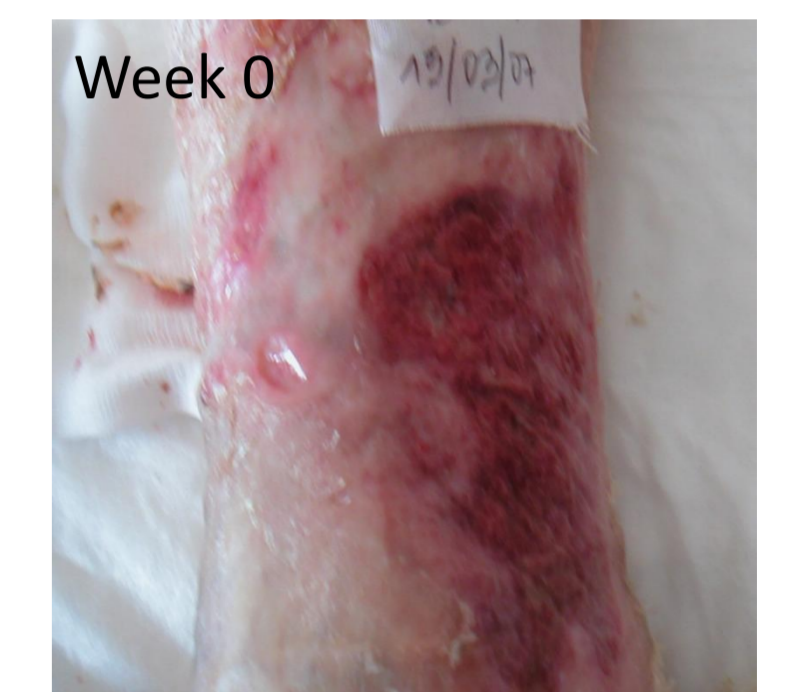
Collagen/ORC/Silver significantly better than Collagen & Collagen/silver dressings on inactivating Elastase Activity



Effect in Patients

Patient Age: 77
Aetiology: recurrent venous leg ulcer
Wound age: 30 days
Wound size: 34cm²

Wound healed after 5wks
PROMOGRAN PRISMA® therapy



Patient Age: 91
Aetiology: recurrent venous leg ulcer
Wound age: 30 days
Wound size: 5.8cm²

Wound healed after 9wks
PROMOGRAN PRISMA® therapy



* This product is a trademark of its owner