In Vitro Testing of Protease and Oxidant Inhibition by a Novel Collagen Dressing

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Introduction

- Chronic wounds are thought to be non-healing due partly to persistent inflammation.
- Wound bed preparation and the TIMERS concept aim to restore balance to the wound bed by removing barriers to healing including infection, inflammation and moisture imbalance.[1]
- In vitro studies have shown that collagen/ORC (oxidized, regenerated cellulose) dressings (ORC) are able to reduce certain protease activity.[2]

Purpose

- The purpose of the current study was to assess the in vitro ability of a novel collagen/ORC dressing with antimicrobial (COA) to manage common markers of wound inflammation including elastase, collagenase and oxidants and compares results to those for collagen/ORC dressings*.

Methods

- Fluorescent, kinetic enzyme activity assays were run in vitro for collagenase and neutrophil elastase.
- 6 mm COA samples were incubated for 1 hour with either a collagenase + CaCl2 solution (1.1 mg/mL collagenase; Sigma C5138; pH 7.5) or neutrophil elastase solution (1.48 uL/100 uL elastase; Abcam Ab280938; pH 8.0).
- After incubation, supernatants were mixed with fluorescent substrate (Millipore SCP0193 for collagenase or Abcam Ab142178 for neutrophil elastase).
- Vmax rates were calculated from the linear portions of the spectrograms and values from positive controls were compared to enzyme samples incubated with COA or ORC.
- Antioxidant capacity of the COA dressings was assessed using a spectrophotometric end point assay.

Figures

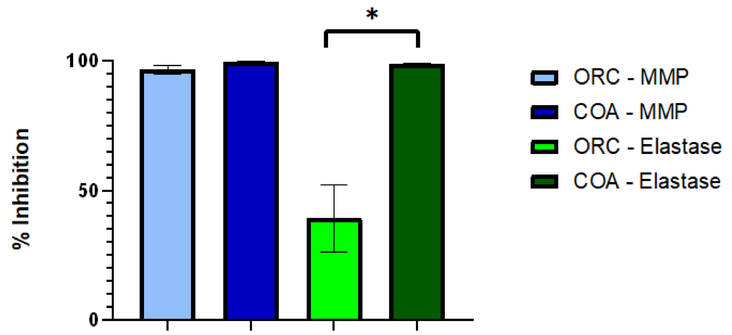

Figure 1. The percent MMP1 inhibition for ORC was 96.7 ± 1.7 and for COA 99.6 ± 0.1 (p=0.160). The percent neutrophil elastase inhibition for ORC was 39.1 ± 13 and for COA 98.5 ± 0.6. Asterisk indicates these values are statistically different (p=0.011). Values are presented as average ± s.e.

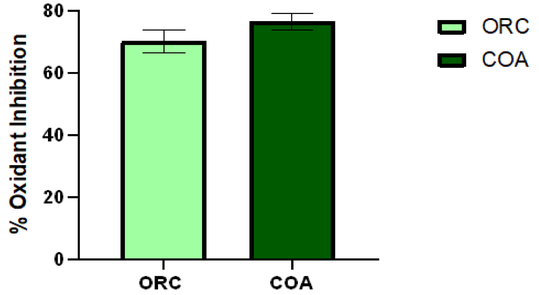

Figure 2. The percent oxidant inhibition for ORC was 70.3 ± 3.6 and for COA 76.6 ± 2.6 (p=0.220). Values are presented as average ± s.e.

Methods (Cont'd)

- Inhibition of the oxidation of a substrate (KP1003 Sigma) by a known chromogen (Metmyoglobin; KP1002 Sigma) was assessed by incubating the chromogen with COA and comparing the resulting OD values to negative controls without antioxidant.

Results

- Neutrophil elastase activity decreased by 98.5% when incubated with COA and by 39.1% when incubated with ORC ($p<0.05$; **Figure 1**).
- Collagenase activity (MMP1) decreased 96.7% when incubated with ORC and by 99.6% when incubated with COA ($p>0.05$).
- When the chromogen in the antioxidant capacity test was incubated with COA, oxidation of substrate was reduced by over 76.6 and by 70.3% ($p>0.05$) when the chromogen was incubated with ORC (**Figure 2**).

Conclusions

- In vitro testing of a novel COA dressing indicated its ability to modulate known contributors to inflammation in chronic wounds; namely collagenase activity (MMP1), neutrophil elastase activity and substrate oxidation by oxidants.
- MMP1 inhibition for both COA and ORC was over 96% and not statistically different. This high percentage of inhibition was to be expected for collagen based dressings.
- The novel COA dressing had high levels of oxidant inhibition, which was similar between the two dressings.
- Additionally, it was significantly better at neutrophil elastase inhibition.

References

1. Atkin L, Bućko Z, Conde Montero E, et al. *J Wound Care*. 2019;28(Suppl 3a):S1–S49.
2. Cullen et al. 2002. *Int J Biochem Cell Biol* 34(12): 1544-1556.

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***3M™ Promogran Prisma™ Collagen Matrix (Solventum Corporation, Maplewood, MN, USA)**

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