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In Vitro Protease Activity of Activated Carbon Cloth Dressing Versus a Standard Knitted Viscose Gauze Dressing

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INTRODUCTION

Matrix metalloproteinases (MMPs) play an essential role in normal wound healing.[1,2,5,6] Wounds with highly elevated MMPs are associated with delayed healing.[1,2,5,6] Reducing excessive MMP levels in a non-healing wound may convert the wound to a healing state.[1,2,5,6]

The activated carbon cloth dressing evaluated in this study is Zorflex® (Chemviron Carbon Cloth Carbon, West Midlands, United Kingdom; a division of Calgon Carbon Corporation, Pittsburgh, PA). According to the Instructions for Use for the product, it is a low-adherent, 100% pure activated carbon cloth dressing that highly conforms to body contours and maintains contact with the wound surface.[8] It provides an effective antimicrobial barrier up to 7 days per dressing, protecting the wound from invasive microorganisms, while exhibiting an antimicrobial effect against microorganisms already present in the wound. The dressing may be used either dry or moistened with sterilized water. This activated carbon cloth dressing is indicated as an antimicrobial dressing over dry or discharing, partial and full thickness wounds.

Several studies, primarily case series, have reported favorable early results with the use of this activated carbon cloth dressing as an antimicrobial dressing for wound management.[3,4,7] In a case series involving four patients with recalcitrant venous leg ulcers that were prone to recurrent infection, treatment with this particular activated carbon cloth dressing resulted in a reduction in clinical signs of infection, such as exudate and pain levels, and improvement in wound bed appearance after 7 days.[4] A retrospective study evaluating the use of this activated carbon cloth dressing for the management of 18 chronic wounds demonstrated 90.7% wound closure at 5 weeks.[7] Finally, another case series examining this product's use in chronic lower extremity and foot wounds demonstrated a reduction in odor control and progression of healing with the use of the dressing.[3]

The purpose of this in vitro study was to evaluate protease activity following 24-hour incubation with an activated carbon cloth dressing compared to a standard knitted viscose gauze dressing. A preliminary analysis of MMP-9 activity levels and wound healing from a randomized, prospective pilot clinical trial that currently is being conducted to evaluate how this activated carbon dressing affects the total bacterial load and biofilm in a wound bed in wounds of the lower extremity and foot also is presented.

MATERIALS AND METHODS

In Vitro MMP Modulation Capability Analysis

- To assess MMP modulation capabilities of an activated carbon cloth dressing, MMP sequestration studies were performed (Perfectus Biomed, Cheshire, England).
- Working solutions of MMP-1, 2, 8, and 9 were prepared at a concentration of approximately 2 $ngml^{-1}$.
- Dressing samples (1 cm^2) were placed into 24-well plates and 1 ml of protease was added to each sample.
  - An activated carbon cloth dressing (Zorflex®) was used as the study dressing.
  - N-A® Knitted Viscose Primary Dressing (gauze) was used as a negative control dressing.
  - Untreated samples were used as a control.
- Plates were sealed and incubated at 37° C ± 2° C and 50 rpm ± 5 rpm for 24 hours.
- Following incubation, supernatants were collected and immediately tested or were immediately frozen at -20° C ± 2° C until processing.
- Concentrations of MMPs remaining in each supernatant were determined using the specific ELISA from R&D Systems, following manufacturer's instructions.
- All dressings were tested in triplicate.

Preliminary Analyses of MMP-9 Activity Levels and Wound Healing from a Randomized, Controlled Pilot Clinical Trial

- A randomized, prospective pilot clinical trial is being conducted at two sites by a single investigator to evaluate how this activated carbon dressing affects the total bacterial load and biofilm in a wound bed in wounds of the lower extremity and foot.
  - The study design was approved by an institutional review board and all patients signed an informed consent form (ClinicalTrial.gov Registration Identifier: NCT03461783).
  - Twenty-four subjects will be randomized with equal probability to one of two groups (12 subjects per group), designated as the experimental and control groups.
    - Subjects in the experimental group will be treated using an activated carbon cloth dressing (Zorflex®) for wet wounds or with saline and the activated carbon cloth dressing for dry wounds.
    - Subjects in the control group will be treated using foam, calcium alginate or compressive dressings for wet wounds or with hydrogel and compressive dressings for dry wounds.
  - Subjects must have full-thickness lower extremity diabetic or venous wounds that are not yet extending to the bone or tendon, not currently being treated with antimicrobial products, and that have been present for at least 4 weeks, but no longer than one year.
  - No antimicrobial products or treatments will be utilized in either group for the duration of the study.
  - Dressings will be changed two times per week. If more frequent dressing changes are required, the investigator will make note of reason(s).
  - Subjects will be followed for four weeks or time to complete wound closure, whichever occurs first.
  - This preliminary analysis examined two secondary objectives of the clinical trial—MMP-9 activity levels and wound healing.
- MMP-9 Activity Level Analysis
  - Wound fluid samples were collected weekly with a rayon swab and were immediately frozen at -20°C until processing by an independent laboratory (University of Florida, Gainesville, FL).
  - Preliminary analysis of MMP-9 activity levels were conducted on samples received by March 19, 2018.
  - The MMP activity level was measured with a FRET peptide using a plate reader and the activity levels were compared to a recombinant-active human MMP-9 standard curve.
- Wound Healing
  - Subjects who healed or who completed the 4-week follow-up period by April 6, 2018, were included in the preliminary analysis.
  - Wound closure, defined as 100% epithelization, was recorded by the investigator or clinical staff.

STATISTICAL ANALYSIS

- Descriptive statistics were used to summarize all study variables.
- Student's t-test was employed to analyze continuous variables that were normally distributed. Fisher's Exact Test was used to determine statistical differences between categorical variables. For variables that were based on repeated observations, one-way repeated measures analysis of variance (ANOVA) was utilized to detect statistical differences in mean values. Differences were considered statistically significant when the p-value was less than 0.05 with a power of at least 0.8.
- Statistical analysis was performed using SigmaPlot™ (version 13.0, Systat Software, Inc., San Jose, CA).

TABLE 1 Comparison of MMP-9 activity levels measured at weekly follow-up intervals between a non-antimicrobial foam dressing and the activated carbon cloth dressing.

MMP-9 ACTIVITY (µg/mL)	WEEK 1	WEEK 2	WEEK 3	WEEK 4
Non-Antimicrobial Foam Dressing Group*	N = 2	N = 2	N = 2	N = 2
Mean ± SD*	0.18 ± 0.25	0.40 ± 0.56	0.01 ± 0.01	0.26 ± 0.37
Median	0.18	0.40	0.01	0.26
Range (min – max)	0.00 – 0.36	0.00 – 0.79	0.00 – 0.02	0.00 – 0.52
Activated Carbon Cloth Dressing Group	N = 5	N = 5	N = 3	N = 2
Mean ± SD*	0.20 ± 0.27	0.05 ± 0.06	0.06 ± 0.08	0.05 ± 0.02
Median	0.06	0.04	0.03	0.05
Range (min – max)	0.00 – 0.65	0.00 – 0.16	0.00 – 0.15	0.03 – 0.06

* SD = Standard Deviation

* Two of four subjects with clinical data had available MMP samples by the shipment deadline for this preliminary analysis. The remaining follow-up samples missed the 03/19/18 shipment deadline to the laboratory, but will be included in the final MMP analysis upon completion of the clinical study.

RESULTS

In Vitro MMP Modulation Capability Analysis

- Protease activity of MMP-1 and MMP-8 (collagenases) following 24-hour incubation are compared between a standard knitted viscose gauze dressing and the activated carbon cloth dressing (**Figure 1**).
  - After 24 hours of incubation, the concentrations of MMP-1 and MMP-8 recovered from supernatant in contact with the standard knitted viscose gauze dressing were 0.42 ± 0.06 $ngml^{-1}$ and 0.25 ± 0.13 $ngml^{-1}$, respectively.
  - No levels of MMP-1 and MMP-8 were detected from supernatant in contact with the activated carbon cloth dressing.
  - These results equate to 100% reductions in MMP-1 and MMP-8 levels by the activated carbon cloth dressing compared to the standard knitted viscose gauze dressing ($p < 0.001$ and $p < 0.05$, respectively).
  - Untreated control samples demonstrated that MMP-1 remained stable, with 2.12 ± 0.04 $ngml^{-1}$ detected after 24 hours of incubation. This finding suggests that the reduction in MMP-1 concentration observed in the dressing samples were due to the binding of the MMPs to the dressings.
  - Untreated control samples demonstrated that MMP-8 was not stable over the 24-hour period, with less concentration detected in the untreated samples than in the treated samples. This observation indicates that the dressings afforded MMP-8 some stability over the testing period.
- Protease activity of MMP-2 (gelatinase A) and MMP-9 (gelatinase B) following 24-hour incubation are compared between a standard knitted viscose gauze dressing and the activated carbon cloth dressing (**Figure 2**).
  - After 24 hours of incubation, the concentrations of MMP-2 and MMP-9 recovered from supernatant in contact with the standard knitted gauze supernatant were 3.02 ± 0.04 $ngml^{-1}$ and 2.66 ± 0.05 $ngml^{-1}$, respectively.
  - No levels of MMP-2 and MMP-9 were detected from supernatant incubated with the activated carbon cloth.
  - These results equate to 100% reductions in MMP-2 and MMP-9 levels by the activated carbon cloth dressing compared to the standard knitted viscose gauze dressing ($p < 0.001$ for both).

Preliminary Analyses of MMP-9 Activity Levels and Wound Healing from a Randomized, Controlled Pilot Clinical Trial

- MMP-9 activity levels measured at weekly follow-up intervals are compared between a non-antimicrobial foam dressing and the activated carbon cloth dressing (Table 1).
  - No statistically significant differences in mean MMP-9 concentrations between the activated carbon cloth dressing and the non-antimicrobial foam dressing groups were calculated at any follow-up interval.
  - No statistically significant differences were ascertained within either the non-antimicrobial foam dressing group or the activated carbon cloth group between any follow-up intervals.
- The time course of mean MMP-9 activity levels over the four-week follow-up period are compared between a non-antimicrobial foam dressing and the activated carbon cloth dressing (**Figure 3**).
  - Although a rapid reduction in mean MMP-9 activity upon dressing application is observed in the activated carbon dressing group and is maintained throughout the study duration, no statistically significant differences in mean MMP-9 activity levels were calculated between the two dressing groups.
- Wound healing occurred in 3 (60.0%) of 5 subjects in the activated carbon cloth dressing study group who healed during the four-week follow-up period. Conversely, wound healing was observed in 1 (25%) of 4 subjects in the non-antimicrobial foam dressing group prior to the final follow-up.
  - No statistically significant difference in wound healing was determined between the non-antimicrobial foam and the activated carbon cloth dressing groups.

FIGURE 1 Comparison aof recovered MMP-1 and MMP-8 after 24-hour incubation between a standard knitted viscose gauze dressing and an activated carbon cloth dressing.

MMP-1 and MMP-8 Recovered After 24-Hour Incubation:
Standard Knitted Viscose Gauze Dressing vs. Activated Carbon Cloth Dressing

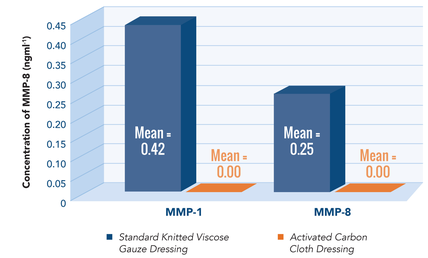


FIGURE 2 Comparison of recovered MMP-2 and MMP-9 after 24-hour incubation between a standard knitted viscose gauze dressing and an activated carbon cloth dressing.

MMP-2 and MMP-9 Recovered After 24-Hour Incubation:
Standard Knitted Viscose Gauze Dressing vs. Activated Carbon Cloth Dressing

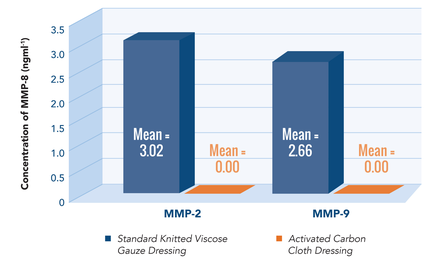
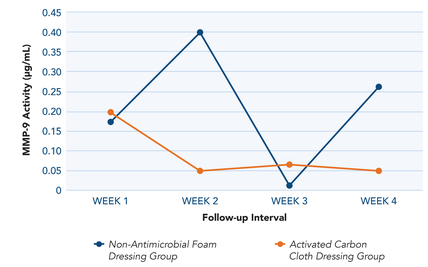


FIGURE 3 Comparison of the time course of mean MMP-9 activity levels over the four-week follow-up period between a non-antimicrobial foam dressing and the activated carbon cloth dressing.

Time Course of Mean MMP-9 Activity:
Non-Antimicrobial Foam Dressing vs. Activated Carbon Cloth Dressing



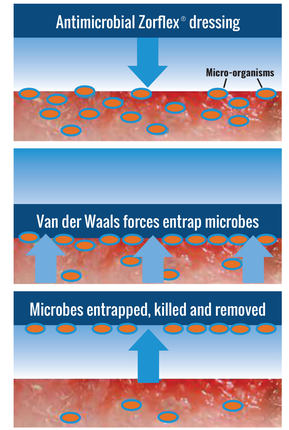
DISCUSSION

The manufacturing process for activated carbon cloth produces a surface area containing numerous microscopic pores, which make the material highly adsorptive to certain molecules. This particular type of activated carbon cloth dressing has a different structure than its counterparts.[4] It is comprised of long staple fibers that are twisted into a yarn, which then is knitted into a double-jersey textile. All fibers of the resulting cloth are interconnected, which enhances adsorption.

Activated carbon reduces malodor through adsorption, whereby the volatile molecules are attracted to and bind to the microscopic pores on the surface area of the dressing. Activated carbon also has naturally occurring van der Waal's electrostatic forces that draw small gas or liquid molecules, including endotoxins and odor molecules, away from the wound into the highly structured micropores of the dressing, where they become trapped (**Figure 4**). Bacteria cells are attracted to activated carbon dressing, but the cells are too big to enter the micropores. Instead, the microorganisms become trapped on the surface, away from the wound bed. Electrostatic tension builds up in the trapped microorganisms until the tension overcomes the tensile strength of the cell walls, at which time the cell walls of the microorganisms rupture, killing the microorganisms. Any endotoxins released in the process are drawn into the micropores and also become trapped.

Adsorption and electrostatic attractive forces are responsible for the odor management and antimicrobial capabilities of activated carbon cloth.[4] It is theorized that these properties of activated carbon cloth dressing also may confer the ability to modulate MMP activity in wounds. If the activated carbon cloth is able to attract and entrap MMPs in a similar fashion, then the reduction of excessive MMP levels in the wound bed may convert a non-healing wound to a healing state.[1,2,5,6] One of the prime proteases responsible for extracellular matrix degradation is MMP-9, which is the most effective MMP at collagen degradation.[5] The levels of MMP-9 have been shown to directly correlate with wound severity.[5] Targeting one or a combination of MMPs, therefore, is proposed as an effective strategy for promoting healing of chronic wounds.[1,2,5,6]

FIGURE 4 Diagram of one of the mechanisms by which activated carbon cloth dressing attracts and sequesters volatile molecules, endotoxins, bacteria, and possibly MMPs away from the wound bed, which may encourage wound healing. Reprinted with permission from Murphy, N. Reducing infection in chronic leg ulcers with an activated carbon cloth dressing. *Br J Nurs. 2016 Jun 23;25(12):S38-44.*



The in vitro study demonstrated that this particular activated carbon cloth dressing can sequester and retain MMP-1, 2, 8, and 9 within 24 hours, with no detectable concentrations following the 24-hour incubation period. Wound dressings typically remain in situ between 24 and 72 hours. Therefore, the findings from this in vitro study suggest that this particular activated carbon cloth dressing may be capable of reducing levels of MMP-1, 2, 8, and 9 within the wound bed.

Based on these promising in vitro results, a randomized, prospective study currently is underway to evaluate how this activated carbon dressing affects bacterial concentrations and MMP activity levels in wound beds of lower extremity and foot chronic wounds. Preliminary analysis suggests that the activated carbon cloth dressing may be capable of an immediate reduction of MMP-9 activity in wound upon dressing application and that reduced concentrations may be maintained throughout duration of use. The majority of wounds treated with this activated carbon cloth dressing healed during the 4-week follow-up period. Given the small sample size, any definitive conclusions regarding the effects of this dressing on MMP activity levels and wound healing should be reserved until enrollment is complete and the final analysis has been conducted.

By controlling odor, reducing bioburden, and possibly modulating production of key MMPs in the wound bed, this particular activated carbon cloth dressing aids in patient comfort and encourages wound healing, thereby establishing it as a valuable addition to the wound care management armamentarium.

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