Activated Carbon Cloth Dressing Use Associated with Reduction in Protease Levels and Healing of Majority of Chronic, Full-Thickness Lower Extremity Wounds within Four Weeks

Preliminary Results of a Randomized Pilot Study

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INTRODUCTION

Matrix metalloproteinases (MMPs) play an essential role in normal wound healing by remodeling the early matrix.[1,2,6,11,13] Wounds fail to heal when they enter a pathophysiology cascade stimulated by repeated tissue injury, ischemia, and elevated bioburden (Figure 1).[1] Prolonged inflammation leads to elevated levels of protease activity that, in turn, disrupts the balance between tissue breakdown and repair.[1,4,5,9,12,14] Thus, in non-healing or chronic wounds, there is an imbalance between increased levels of MMPs and decreased levels of tissue inhibitors of MMPs (TIMPs).[1,2,4,7,13,16] The elevated levels of proteases degrade the extracellular matrix (ECM), growth factors, and cellular receptors, all of which impair healing.[1,2,6,9,12,13,15] Therefore, reducing excessive MMP levels in a non-healing wound may convert the wound to a healing state.[1,2,11,13]

Targeting one or a combination of MMPs has been proposed as an effective strategy for promoting healing of chronic wounds.[1,2,11,13] Previous clinical studies involving the use of a novel activated carbon cloth dressing (Zorflex®, Chemviron Carbon Cloth Carbon, Tyne and Wear, United Kingdom; a division of Calgon Carbon Corporation, Pittsburgh, PA) (Figure 2) have reported favorable outcomes, including reduction of odor and high rates of wound closure.[8,10,17] A prior in vitro study demonstrated that this dressing can sequester and retain MMP-1, 2, 8, and 9 within 24 hours, with no detectable concentrations following the 24-hour incubation period (Figure 3).[3] Because wound dressings typically remain in situ between 24 and 72 hours, 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.

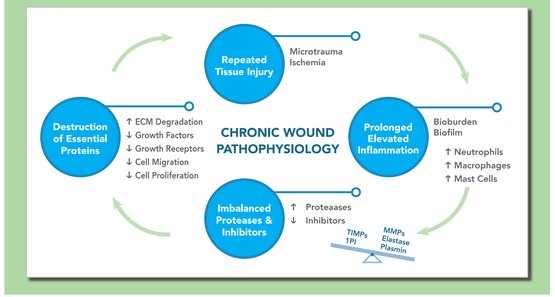
These favorable clinical and in vitro findings were the catalysts for this randomized, prospective study, which evaluates the possible effect of this particular activated carbon cloth dressing on MMP-9 activity within the wound bed and on healing of chronic, full-thickness wounds of the lower extremity compared to a standard non-antimicrobial foam dressing as a control.

MATERIALS AND METHODS

- A randomized, prospective pilot clinical trial was conducted at two sites by a single investigator to evaluate possible effects of this activated carbon cloth dressing on MMP activity levels 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 were randomized with equal probability to one of two study groups (12 subjects per group), designated as the experimental and control groups.
    - Subjects in the experimental group were treated using an activated carbon cloth dressing (Zorflex®) for wet wounds or with saline and the activated carbon cloth dressing for dry wounds (Figure 2).
    - Subjects in the control group were treated using a non-antimicrobial foam dressing, with or without hydrogel, depending on the moisture level of the wound.
    - Compression dressings were used, if needed, for edema management.
  - Subjects must have had full-thickness lower extremity diabetic or venous wounds that were not yet extending to the bone or tendon, not currently being treated with antimicrobial products, and that were present for at least 4 weeks, but no longer than one year.
  - No antimicrobial products or treatments were utilized in either group for the duration of the study.
  - Dressings were changed three times per week, unless otherwise documented by the investigator.
  - Wound photographs and measurements were obtained using the eKare inSight™ 3D wound imaging system (eKare, Inc., Fairfax, VA).
  - Subjects were followed for four weeks or time to complete wound closure, whichever occurs first.
- MMP-9 Activity Level Analysis
  - To ensure sufficient wound fluid for sample collection, only wounds that were not yet healed were included in this analysis.
  - Wound fluid samples were collected weekly with a rayon swab and immediately frozen at -20°C until processing by an independent laboratory (University of Florida, Gainesville, FL).
  - The MMP-9 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
  - Wound closure, defined as 100% epithelialization, was recorded by the investigator or clinical staff.

FIGURE 1

Wounds fail to heal when they enter a pathophysiology cascade stimulated by repeated tissue injury, ischemia, and elevated bioburden. Prolonged inflammation ensues, which leads to elevated proteases that destroy essential proteins, such as growth factors, and the extracellular matrix. This chronic wound pathophysiology cycle must be broken to achieve healing. Reprinted with permission from Bohn GA, Schultz GS, Liden BA, et al. Proactive and Early Aggressive Wound Management: A Shift in Strategy Developed by a Consensus Panel Examining the Current Science, Prevention, and Management of Acute and Chronic Wounds. Wounds 2017; 29:S37-42.



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. Mann-Whitney Rank Sum Test was used to analyze continuous variables that were not normally distributed. Chi-Square and Fisher's Exact Test were used to determine statistical differences between categorical variables. For repeated variables, one-way and two-way repeated measures analysis of variance (ANOVA) were used to determine statistical differences between weekly intervals and treatments. 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).

FIGURE 2

Activated carbon cloth dressing (Zorflex®, Chemviron Carbon Cloth Carbon, West Midlands, United Kingdom; a division of Calgon Carbon Corporation, Pittsburgh, PA) (**A**) evaluated in this study. This low-adherent, 100% pure activated carbon cloth dressing (**B**) highly conforms to body contours to maintain contact with the wound surface. It may be used either dry or moistened with sterilized water over dry or discharing, partial and full thickness wounds.[18]

RESULTS

This analysis includes the results of 17 subjects (8 activated carbon cloth dressing; 9 non-antimicrobial foam dressing) who received at least one study dressing treatment and who had at least one efficacy assessment after administration of the study dressing treatment.

Patient Demographics

- Four (50.0%) females and four (50.0%) males were enrolled in the activated carbon cloth dressing group. Seven (77.8%) males and 2 (22.2%) females were enrolled into the non-antimicrobial foam dressing group.
- The mean patient ages for the activated carbon cloth and non-antimicrobial foam dressing groups were 70.8 years (median = 68.2; SD = 12.1; range, 54.0 – 86.0 years) and 62.8 years (median = 62.5; SD = 14.4; range, 43.9 – 89.3 years), respectively.
- All patients in the activated carbon cloth dressing group had body mass indices greater than 30 and, therefore, were considered obese. Seven (77.8%) patients in the non-antimicrobial foam dressing group were considered obese.
- Concomitant conditions for the activated carbon cloth dressing group were 7 (87.5%) diabetes; 7 (87.5%) neuropathy; 6 (75.0%) peripheral vascular disease and associated conditions; 4 (50.0%) cardiovascular conditions; 2 (25.0%) lymphedema; 1 (12.5%) chronic obstructive pulmonary disease; and 1 (12.5%) complex regional pain syndrome. Concomitant conditions for the non-antimicrobial foam dressing group included 6 (66.7%) neuropathy; 5 (55.6%) diabetes; 5 (55.6%) cardiovascular conditions; 3 (33.3%) peripheral vascular disease and associated conditions; and 2 (22.2%) lymphedema.
- No statistically significant differences in patient demographics were calculated between study groups.

Wound History

- The mean wound ages for the activated carbon cloth and non-antimicrobial foam dressing groups were 10.1 weeks (median = 4.1; SD = 11.7; range, 4.0 – 36.2 weeks) and 6.3 weeks (median = 4.4; SD = 4.6; range, 4.0 – 18.0 weeks), respectively.
- Wound types for the activated carbon cloth dressing group were 4 (50.0%) diabetic and 4 (50.0%) venous. Wound types for the non-antimicrobial foam dressing group were 4 (44.4%) venous, 3 (33.3%) diabetic, and 2 (22.2%) diabetic/venous mixture.
- Wound location for the activated carbon cloth dressing group included 4 (50.0%) located on the lower extremity; 3 (37.5%) located on the foot, but not involving the heel; and 1 (12.5%) heel. Wound locations for the non-antimicrobial foam dressing group were 3 (33.3%) lower extremity; 2 (22.2%) foot, non-heel; 2 (22.2%) heel; and 2 (22.2%) ankle.
- The mean baseline wound areas (cm^2) for the activated carbon cloth and non-antimicrobial foam dressing groups were 3.4 cm^2 (median = 2.1; SD = 2.5; range, 1.6 – 8.1 cm^2) and 6.8 cm^2 (median = 2.3; SD = 9.5; range, 0.6 – 24.0 cm^2), respectively.
- No statistically significant differences in wound history parameters were calculated between the two study groups.

MMP-9 Activity Levels

- MMP-9 activity levels measured at weekly follow-up intervals were compared between the activated carbon cloth and non-antimicrobial foam dressing groups (Table 1).
  - No statistically significant differences in mean MMP-9 concentrations between the activated carbon cloth and the non-antimicrobial foam dressing groups were calculated at any follow-up interval.
  - No statistically significant differences within either the activated carbon cloth or non-antimicrobial foam dressing groups between any follow-up intervals were calculated.
- The mean MMP-9 activity levels over the four-week follow-up period were compared between the activated carbon cloth and non-antimicrobial foam dressing groups (Figure 3).
  - A reduction in mean MMP-9 activity upon dressing application is observed in the activated carbon cloth dressing group and is maintained throughout the study duration.

Wound Status at Final 4-Week Follow-up Evaluation

- Wound healing occurred in 4 (50.0%) of 8 subjects in the activated carbon cloth dressing group during the four-week follow-up period.
- Conversely, wound healing was observed in 2 (22.0%) of 9 subjects in the non-antimicrobial foam dressing group prior to the final follow-up evaluation.

Adverse Events

- One (12.5%) diabetic wound on the heel of a 60.4-year-old male in the activated carbon cloth dressing group failed at 2.0 weeks due to worsening infection. The patient had underlying osteomyelitis that was not diagnosed until after the wound failed.
- No additional adverse events occurred in either study group.

DISCUSSION

Activity levels of MMP-9 were measured at weekly follow-up intervals for those wounds that were not yet healed (Table 1). This particular protease was selected for analysis because it is one of the prime proteases responsible for ECM degradation.[11] The levels of MMP-9 have been shown to directly correlate with wound severity.[11] Examining mean MMP-9 concentrations at each weekly follow-up interval, no statistically significant differences were calculated within each study group or between the two study groups.

However, surveying the mean MMP-9 activity levels over the four-week follow-up period between the activated carbon cloth and non-antimicrobial foam dressing groups illustrates a reduction in mean MMP-9 activity upon dressing application in the activated carbon cloth dressing group that is then maintained throughout the study duration (Figure 3). Such a pattern was not observed in the non-antimicrobial foam dressing group.

Adsorption and electrostatic attractive forces are responsible for the odor management and antimicrobial capabilities of activated carbon cloth.[10] Adsorption allows activated carbon to reduce malodor by attracting and binding volatile molecules to the microscopic pores on the surface area of the dressing. The double-jersey textile design unique to the activated carbon cloth dressing evaluated in this study results in an interconnection of fibers that further enhances adsorption (Figure 2).[10]

Naturally occurring van der Waal's electrostatic forces are the other means by which activated carbon cloth dressing physically binds molecules and other materials. Microorganisms also may be attracted to the activated carbon cloth dressing, but they are too large to enter the micropores and instead become trapped on the dressing surface (Figure 4).[10] When the resulting electrostatic tension that builds up in the trapped microorganisms overcomes the tensile strength of the cell walls, the cell walls rupture, killing the microbes. Any endotoxins released in the process are drawn into the micropores of the dressing and also become trapped.

It is theorized that these structural and electrostatic properties of activated carbon cloth dressing also may confer the ability of this dressing to modulate MMP activity in wounds. If the activated carbon cloth dressing 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 (Figure 1).[1,2,11,13]

Given the small sample size of this clinical trial, any definitive conclusions regarding the effects of this dressing on MMP activity levels and wound healing should be reserved until a more thorough analysis is conducted. Other weaknesses of this initial in vivo study are the examination of only one specific MMP and the limitation of fluid sample collection from only non-healed wounds. A recent Cochrane review identified very low validity of evidence demonstrating an association between protease activity and venous leg ulcer healing and urged caution in claiming protease activity as an independent prognostic factor of healing.[14] Most previous studies examining protease activity are small, poorly designed, and fail to account for impact of confounding factors, such as age, infection, and treatment.[14] Using the recommendations from Westby et al[14] and the results from this study, a subsequent extensive clinical trial to evaluate this particular activated carbon cloth dressing is being designed encompassing a larger sample size and analysis of a range of biomarkers for wound healing.

FIGURE 3

Comparison of MMP-9 activity (µg/mL) in exudates of wounds not yet healed in patients with chronic full-thickness diabetic or venous wounds between the activated carbon cloth and non-antimicrobial foam dressing groups over the course of study.

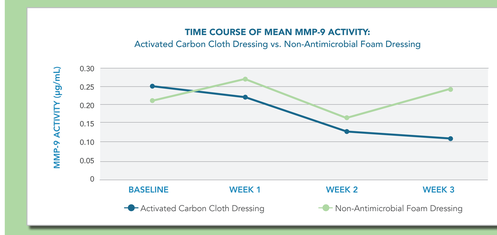


TABLE 1

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

MMP-9 ACTIVITY (µg/mL)	BASELINE	WEEK 1	WEEK 2	WEEK 3
Activated Carbon Cloth Dressing Group	N = 7	N = 6	N = 3	N = 3
Mean ± SD*	0.25 ± 0.22	0.22 ± 0.27	0.13 ± 0.09	0.11 ± 0.11
Median	0.18	0.11	0.15	0.06
Range (min–max)	0.04 – 0.65	0.01 – 0.35	0.03 – 0.21	0.03 – 0.23
Non-Antimicrobial Foam Dressing Group[a]	N = 8	N = 8	N = 7	N = 6
Mean ± SD*	0.20 ± 0.07	0.27 ± 0.21	0.15 ± 0.07	0.25 ± 0.14
Median	0.18	0.19	0.16	0.21
Range (min–max)	0.15 –0.36	0.17 – 0.79	0.02 – 0.27	0.16 – 0.52

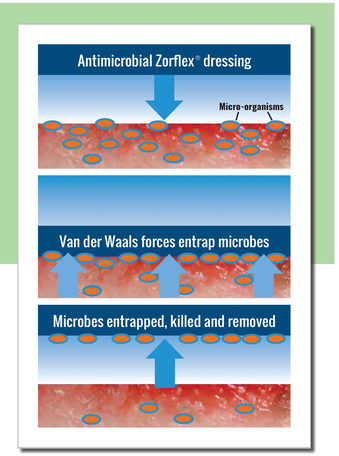
*SD = Standard Deviation [a]Laboratory error occurred in two patients (one in the activated carbon cloth dressing group and one in the non-anti microbial foam dressing group) and were excluded from the statistical analysis of the laboratory data.

CONCLUSIONS

- A wound healing rate within four weeks of 50.0% (4 of 8 subjects) in the activated carbon cloth dressing group versus 22.0% (2 of 9 subjects) in the non-antimicrobial foam dressing group suggests that the activated carbon cloth dressing is functioning at least as well as a high-performing standard-of-care control dressing in terms of safety and efficacy in healing chronic wounds of the foot and lower extremity in a patient population with high incidences of obesity, diabetes, and neuropathy.
- A reduction in mean MMP-9 activity upon dressing application is observed in the activated carbon cloth dressing group and is maintained throughout the study duration. This pattern suggests that the activated carbon cloth dressing may be capable of an immediate reduction of MMP-9 activity in wound beds upon dressing application and that reduced concentrations may be maintained throughout duration of use.
- The adsorption and electrostatic attractive forces exhibited by this particular activated carbon cloth dressing may be responsible for its potential protease-modulating capability suggested by this clinical trial.
- The small sample size of this clinical trial may be influencing the lack of statistical significance in the healing trends and MMP-9 activity levels between study groups.
- A future clinical trial including a larger patient population and analysis of various biomarkers of healing is being designed to further examine the effects of this dressing on wound healing.
- The possible modulation of a key MMP in the wound bed and the favorable healing rate observed within four weeks with the use of this particular activated carbon cloth dressing in chronic, full-thickness wounds of the lower extremity suggest that it has the potential to become a valuable addition to the wound management armamentarium.

FIGURE 4

Theoretical mechanism 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;25:S38-44.



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