Use of Modified Superabsorbent Polymer Dressings for Protease Modulation in Improved Chronic Wound Care

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Abstract: With more than 6 million patients affected with them in the United States, chronic ulcers represent one of the greatest problems in wound care. High levels of corrosive proteases, particularly matrix metalloproteinases (MMPs), within the wound environment are thought to contribute to the persistence of these wounds through denaturation of connective tissue proteins crucial to healing progression. Therefore, there is considerable interest in protease modulation using wound dressings to promote healing in chronic wounds. Such modulation could be achieved by direct absorption of proteases, by depleting co-factors within the wound, or by release of protease inhibitors. Method. The aim of this study is to examine protease modulation of a range of dressings with different chemistries, particularly those having demonstrated efficacy in chronic wound healing. Results. XTRASORB® HCS (dressing A) and XTRASORB® Foam (dressing B) were able to modulate proteases by both direct absorption of MMPs and depleting metal ion co-factors, and resulted in complete elimination of protease activity in the assay used. Duoderm® (dressing C) was able to modulate proteases by direct absorption only, and not by co-factor depletion. Promogran® (dressing D) was able to reduce MMP activity, but this was shown to be pH dependant, with any protease modulation being lost at neutral pH. Neither Allevyn® (dressing E) nor Vigilon® (dressing F) were able to modulate proteases by any mechanism. None of the protease modulating dressings acted through the release of protease inhibitors. Conclusion. Of the dressings studied, dressing A and dressing B were the most effective protease modulators due to their acting through 2 separate mechanisms.

Chronic ulcers are one of the greatest problems in wound management, with more than 6 million patients affected in the United States, and an annual cost in excess of $25 billion. Despite advances in wound care this situation continues to deteriorate, due to an aging population and the increase in prevalence of conditions linked to chronic ulcers, such as obesity, diabetes, and peripheral vascular disease. It has long been recognized that chronic ulcers are associated with increased activity of proteases in wound fluids and tissues, with proteases such
as matrix metalloproteinases (MMPs), correlating with clinical status and poor healing potential.2 It is thought that protease-mediated destruction of important components of dermal connective tissues, such as collagen and fibronectin, is causal in the persistent nature of chronic wounds.3 In particular, MMP-9, which is an essential element in normal inflammation and tissue repair, is present at greatly elevated levels in ulcers, and this may contribute to the corrosive nature of the chronic wound environment. This has led to considerable interest in the possibility of protease modifying treatments in managing chronic ulcers.4,5

Activity of MMPs in the wound environment may be reduced either by their direct removal, by reducing cofactors essential for their function, such as metal ions, or by releasing components which inhibit their activity.4 Removal of proteases or their cofactors from the immediate wound environment are the preferred options, as release of inhibitory components may have detrimental effects beyond the target area and hence presents both practical and regulatory difficulties.

There has been considerable interest in the use of protease-modulating dressings that aim to reduce the levels or activity of corrosive proteases by removal of components within wound exudates. These include cellulose/collagen,9 phosphorylated cotton,10 and sulfonated hydrogels.11

Gel materials with very high fluid-handling capabilities are now commercially available as wound dressings. These can be of natural origin, such as hyaluronic or alginic acid, or be entirely synthetic, such as polycrylamide. Gel-based devices have found many other medical applications due to their inherent biocompatibility, including contact lenses, drug delivery devices, and electrodes for heart monitoring. Commercially available superabsorbent gel-based polymer dressings comprising crosslinked sodium polycrylamide acid have also been shown to modulate proteases.3,12 Wound dressings based on sulfonated hydrogels comprising cross-linked sulfonated copolymers, such as XTRASORB® HCS (dressing A) and XTRASORB® Foam (dressing B), have been reported to have successful outcomes on wounds,10,21 though the precise mechanism of this efficacy is as yet not clear. The purpose of this study is to investigate protease modulation of these sulfonated superabsorbent polymers as a potential mechanism for the beneficial effects on wound care.

Materials and Methods

Dressings. The sulfonated copolymer dressings A and B (Derma Sciences, Princeton, NJ); a hydrocolloid consisting of carboxymethyl cellulose, gelatin, and pectin in a hydrophobic polymer Duoderm® (dressing C) (Convatec, Uxbridge, UK); a cellulose-collagen mix Promogran® (dressing D), (Systagenix, Graftgrave, UK); the polyurethane foam Allevyn® (dressing E) (Smith & Nephew, Andover, MA); and the polyethylene oxide hydrogel dressing Vigilon® (dressing F) (Bard Medical, Coviington, GA) were used in this study. The dressings were selected on the basis of previously reported efficacy in protease modulation or wound healing progression, or as representative of sulfonated copolymer chemistry.

Direct absorption of MMP-9 by dressings. MMP-9 solution of 22ng/ml MMP-9 (Calbiochem) was prepared in MMP buffer (5mM TRIS/HCl [pH7.5], 5mM CaCl2 and 50mM NaCl). Ten portions of approximately 40mg of each dressing were accurately weighed out, and MMP-9 solution added at 30μl per mg dressing and incubated at room temperature for 4 hours with gentle agitation. Residual buffer was removed and 10μl mixed with 10μl non-reducing SDS-PAGE sample buffer, and run onto gelatin zymogram SDS-PAGE gels at 10μl per lane. The gels were washed in 2.5% Triton X100, and incubated in proteolysis buffer (50mM TRIS/HCL [pH7.5], 50mM CaCl2 and 0.5M NaCl) for 18 hours at 37°C.

Release of inhibitory elements from dressings. To determine whether any protease modification detected was the result of release of inhibitory components from the dressings, dressings were incubated in MMP buffer as above, but without MMP-9. The residues were used to make up MMP-9 solution at 22ng/ml, which was analyzed by gelatin zymogram SDS-PAGE as above.

Cofactor depletion. The aim was to determine whether dressings could attenuate MMP activity in the wound environment by reducing levels of the essential metal ion cofactors of MMPs. Five portions of each test dressing, approximately 1g, were accurately weighed and incubated with 35 fold (w/v) 50mM TRIS-HCl [pH7.5] with ion concentrations of 6.25mM NaCl/0.625mM CaCl2 (high ionic
strength) and 3.125 mM NaCl/0.3125 mM CaCl₂ (low ionic strength) for 4 hours at room temperature with gentle agitation. High and low ionic strength proteolysis buffers were used in order to distinguish a broad range of depletion capabilities. Gelatin zymography was performed using MMP-2 and MMP-9 (Calbiochem), and the gels washed in 2.5% Triton X100. Gels were incubated in buffers pre-absorbed with the dressings, or with untreated buffers as control, for 18 hours at 37°C. Each gel was stained and scanned as described above.

**Key Points**

- The dressings were selected on the basis of previously reported efficacy in protease modulation or wound healing progression, or as representative of sulfonated copolymer chemistry.
- To determine whether any protease modification detected was the result of release of inhibitory components from the dressings, dressings were incubated in MMP buffer as above, but without MMP-9.
- The aim was to determine whether dressings could attenuate MMP activity in the wound environment by reducing levels of the essential metal ion co-factors of MMPs.

**Statistical Analysis**

Data is expressed as mean ± standard error of the mean (SEM) and normalized to control dressing. One way ANOVA was used to assess overall treatment effects, with a Bonferroni’s multiple comparison post hoc test to compare individual dressings.

**Results**

**Direct absorption of MMP-9 by dressings.** In order to demonstrate that dressing trials were a reflection of the nature of the dressing rather than simply the presence of any dressing, the authors established the use of a non-protease modifying dressing as a control. In a series of 26 determinations, dressing F was found to sustain protease activity for 96.2% (± 7.6) of untreated controls with pro-MMP-9 and 103.7% (± 5.4) of untreated controls with activated MMP-9. Therefore dressing F was adopted throughout as the control dressing.

The sulfonated copolymer dressings (dressings A and B) each eliminated any residual MMP-9 activity (P < 0.001) (Figure 1). Dressing D also virtually eliminated residual MMP-9 activity (P < 0.001). However, it was noted that incubation with this dressing substantially reduced the pH of the supernatant (the other dressings had no effect). When it was neutralized, the capacity to reduce the activity of residual MMP-9 was abolished. Dressing E showed no reduction in residual MMP-9 activity, indeed this polyurethane dressing appeared to facilitate the activity of MMP-9 by comparison with control. Dressing C demonstrated substantial and significant reduction in MMP-9 activity (P < 0.001).

**Release of inhibitory elements from dressings.** For those dressings that demonstrated reduced MMP-9 activity in neutral residual fluids, a potential mechanism for attenuation of protease activity is release of inhibitory components from the dressings. A comparison was made between activities of fluids where MMP-9 was added before or after incubation with dressings. If inhibitory component release from the dressings was the mechanism of MMP attenuation, depletion of activity would be the same irrespective of the timing of adding the protease, (ie, pre- or post-incubation with the dressings). There was substantial activity with the post-incubation (compared with pre-incubation) addition of MMP-9 (P < 0.0001), for dressing A (P < 0.05), dressing B (P < 0.001), and dressing C (P < 0.001), demonstrating that release of inhibitory components was not the mechanism of reduction or elimination of protease activity by the dressings (Figure 2).
Figure 2. Direct absorption of MMP-9 by dressings. Dressing A and dressing B entirely eliminated MMP-9 in residual fluids. Dressing C virtually eliminated activity, as did dressing D at low pH (L), but not at neutral pH (H). *** P < 0.001.

Figure 3. Protease modulating dressings did not release inhibitory components. Comparing the attenuation of MMP-9 activity by absorption (pale bars) and inhibition (dark bars) demonstrated that the modulation seen in Figure 1 was not due to release of inhibitory components from the dressings for dressing B (p < 0.05), dressing A (p < 0.001) or dressing C (p < 0.001).

Figure 4. Protease modulating dressings did not release inhibitory components. Comparing the attenuation of MMP-9 activity by absorption (pale bars) and inhibition (dark bars) demonstrated that the modulation seen in Figure 1 was not due to release of inhibitory components from the dressings for dressing B (p < 0.05), dressing A (p < 0.001) or dressing C (p < 0.001).

Cofactor depletion by wound dressings. In a series of 10 determinations of activity following depletion of metal ions, dressing F was found to maintain 91.6% (± 14.0) of untreated fluid for pro-MMP-9, 75.6% (± 11.7) of untreated fluid for pro-MMP-2, and 97.3% (± 10.8) of untreated fluid for activated MMP-2. Therefore this was used throughout as the control dressing.

The sulfonated copolymer dressings (dressings A and B) demonstrated marked and significant depletion of metal ions, virtually eliminating the activities of pro-

MMP-9 (Figure 4A), Pro-MMP-2 (Figure 4B), and activated MMP-2 (Figure 4C) in incubations at both low and high ionic strength buffers (p < 0.001) (Figure 4). Dressing C showed no inhibition of pro-MMP-9 and activated MMP-2 activity by ion depletion in the high ionic strength buffers. There was, however, evidence of some ion depletion for pro-MMP-2 (high and low ionic strength) and activated MMP-2 (low ionic strength only) by this dressing, though activity was not entirely eliminated as was seen for the dressings A and B. There was no ion depletion de-
Table 1. Composition of dressings used in the study.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Material Type</th>
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<tbody>
<tr>
<td>Dressing A (XTRASORB® HCS)</td>
<td>Superabsorbent sulfonated copolymer plus carboxymethylcellulose</td>
</tr>
<tr>
<td>Dressing B (XTRASORB® Foam)</td>
<td>Superabsorbent sulfonated copolymer backed by polyurethane foam</td>
</tr>
<tr>
<td>Dressing C (Duoderm®)</td>
<td>Hydrocolloid of gelatin, carboxymethylcellulose, and pectin</td>
</tr>
<tr>
<td>Dressing D (Promogran®)</td>
<td>Oxidised regenerated cellulose and Bovine collagen</td>
</tr>
<tr>
<td>Dressing E (Alleyn®)</td>
<td>Polyurethane foam</td>
</tr>
<tr>
<td>Dressing F (Vigilon®)</td>
<td>Polyethylene oxide</td>
</tr>
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tected for the dressing E; there was evidence of enhanced activity in the assay, suggesting the release of components capable of enhancing MMP activity.

Discussion

A major goal of chronic wound health care is the modification of the wound environment to favor healing. Dressings, such as those comprising sulfonated copolymers studied here, are capable of passively modifying the wound environment through their fluid handling properties. The potentially corrosive nature of MMPs in chronic wound exudates, in particularly MMP-9, has made these a particular target for improving chronic wound healing with the aim to shift the balance of protein deposition and degradation in favor of repair. Dressings able to actively influence the wound environment have been developed in recent years, and there is some evidence of efficacy using dressings with a capability for protease modulation. Dressings may reduce protease activity by direct absorption, removal of cofactors, or release of inhibitors. All 3 mechanisms of protease modulation were explored in this study. The dressings used had shown quantifiable benefit in wound care and represent a range of different chemistries, providing some insight into mechanisms of efficacy.

The sulfonated copolymer dressings showed the greatest direct absorption of MMP-9 by comparison with the control dressing, with complete elimination at the concentrations used in the analysis. There was no release of MMP inhibitory components from the sulfonated copolymers. Similarly, the hydrocolloid dressing was able to reduce MMP-9 activity without releasing any inhibitory components. Dressing D also substantially reduced MMP-9 activity in residual fluid. However, in this study, it was seen to markedly lower the pH of the residual fluid, and when this was adjusted to neutrality, all apparent protease modification was lost. Dressing D has previously demonstrated its ability to reduce the activity of MMP-9 when incubated with chronic wound fluid, and this was purported to be due to protease binding. However, the authors’ results in this study suggest that this dressing may function, at least in part, by modifying the pH of the fluid.

Neither of the sulfonated copolymer dressings measurably modified the pH of the fluid. The protease modification of these dressings and of the hydrocolloid dressing were a result of direct sequestration of

Keypoints

- Dressings may reduce protease activity by direct absorption, removal of co-factors, or release of inhibitors. All 3 mechanisms of protease modulation were explored in this study. The dressings used had shown quantifiable benefit in wound care and represent a range of different chemistries, providing some insight into mechanisms of efficacy.
- The sulfonated copolymer dressings showed the greatest direct absorption of MMP-9 by comparison with the control dressing, with complete elimination at the concentrations used in the analysis.
MMP-9. Dressing E demonstrated no direct adsorption or ion depletion, and did not modify protease levels or activity in any of the experiments.

Dressings A and B were both shown to sequester ionic cofactors necessary for the activity of MMPs. At both high and low ionic strengths, these dressings were able to entirely eliminate activity of pro-MMP-9, pro-MMP-2, and activated MMP-2. There was recovery of activity when metal ions were returned to the residual fluids, demonstrating that ion depletion was the mechanism of action (data not shown), and that the attenuation of proteolysis was not due to release of any inhibitory components from the dressings. Dressing C also proved capable of some sequestration, but to a substantially lesser extent than dressings A and B, with little or no ion depletion at the higher ionic strengths (significant for pro-MMP-2 only), and moderate depletion at low ionic strength (significant for MMP-2 only). Again, any depletion was reversed by replenishing the ionic cofactors in the residual fluid. Recovery of activity after replenishment of metal ions confirmed that ion depletion was the mechanism of action here. For dressing A recovered activity was 47.94% of control (versus 0% for nonrecovered [low ionic strength], \( P < 0.0001 \)).

**Conclusion**

In summary, the sulfonated copolymers in dressing A and dressing B were capable of protease modulation via 2 separate mechanisms, and direct absorption of protease and sequestration of cofactors, but did not release inhibitory components capable of reducing MMP activity. Dressing C was capable of direct absorption of protease, but had substantially reduced capacity for cofactor depletion by comparison with dressings A and B. The authors therefore hypothesize the efficacy of these dressings may be, at least in part, due to their protease modulation; but this would need to be confirmed using actual wound fluids and in patient trials.

**References**


10. Edwards JV, Howley PS. Human neutrophil elastase and collagenase sequestration with phosphorylated cotton


