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RESEARCH AND DEVELOPMENT OF ACTIVE COTTON WOUND DRESSINGS FOR CHRONIC WOUND HEALING

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Abstract

The research and development of cotton-based wound dressings based on a molecular mechanism of action for development of an active chronic wound dressing is presented. The research prototype for sequestration of destructive protease from chronic wound fluid is based on a peptide conjugate of cellulose consisting of an enzyme active site recognition sequence (Val-Pro-Val that possesses affinity for the active site of human neutrophil elastase. This prototype cellulose conjugate demonstrated elastase lowering activity, and prompted development of a cotton-based dressing that was constructed with molecular design of the fiber using aqueous finishing techniques that can be economically adapted for the textile mill production of the active wound dressing. During the course of the development of the dressing issues concerning the selective activity of the dressing in the presence of the wound proteins prompted alternative design considerations. This report constitutes an update on the progress towards commercialization of the active wound dressings.

Introduction

The design and preparation of active chronic wound dressing has been become increasingly important to addressing the critical worldwide health crisis of the growing number of chronic wound patients. In the United States alone there are over two million patients a year who suffer from chronic wound due to the formation of decubitus bed sores brought on in the elderly nursing home patient or spinal chord paralysis patient. Recent efforts to develop improved wound dressings that do more than simply offer a moist wound environment for better healing has prompted most major wound dressing companies to develop active chronic wound dressings (Cullen et al., 2002). An advantage of employing a cotton-based wound dressing would be the improved value of cotton bandages with mechanistic activity targeted to the biochemical imbalance of the chronic wound. In the chronic wound the onset of destructive neutrophil proteases in high concentration prompts breakdown of important growth factors and fibronectin needed for normal healing. Through the research and development of a cotton-based wound dressing directed to sequestration of these proteases it is thought that improved healing will occur. This paper discusses the design, preparation and assay of active cotton-based wound dressings for chronic wound (Edwards et al., 2001). Issues that have been turning points in the research and development of a cotton product for this critical need area will be stressed.

Prototype Design

The protease human neutrophil elastase found in high concentration in the chronic wound creates considerable protein destruction and prevents the wound from healing. The design of wound dressings that selectively sequester proteases from the chronic wound is couched in the concept that molecular features and properties of the protease can be used to tailor the molecular design of the cotton fiber needed for selective sequestration of the protease. Thus, the enzyme size, over all charge, and active site mechanism for binding substrate may be employed to create the appropriate fiber design that might best bind the enzyme selectively. To approach this problem a molecular model of a cellulose-conjugate containing an active site recognition sequence was docked to the active site of the human neutrophil elastase as shown in Figure 1. The subsites of enzyme active site interaction consist of the sequence conjugate H-Val-Pro-Glycine-O-ester-Cellulose.

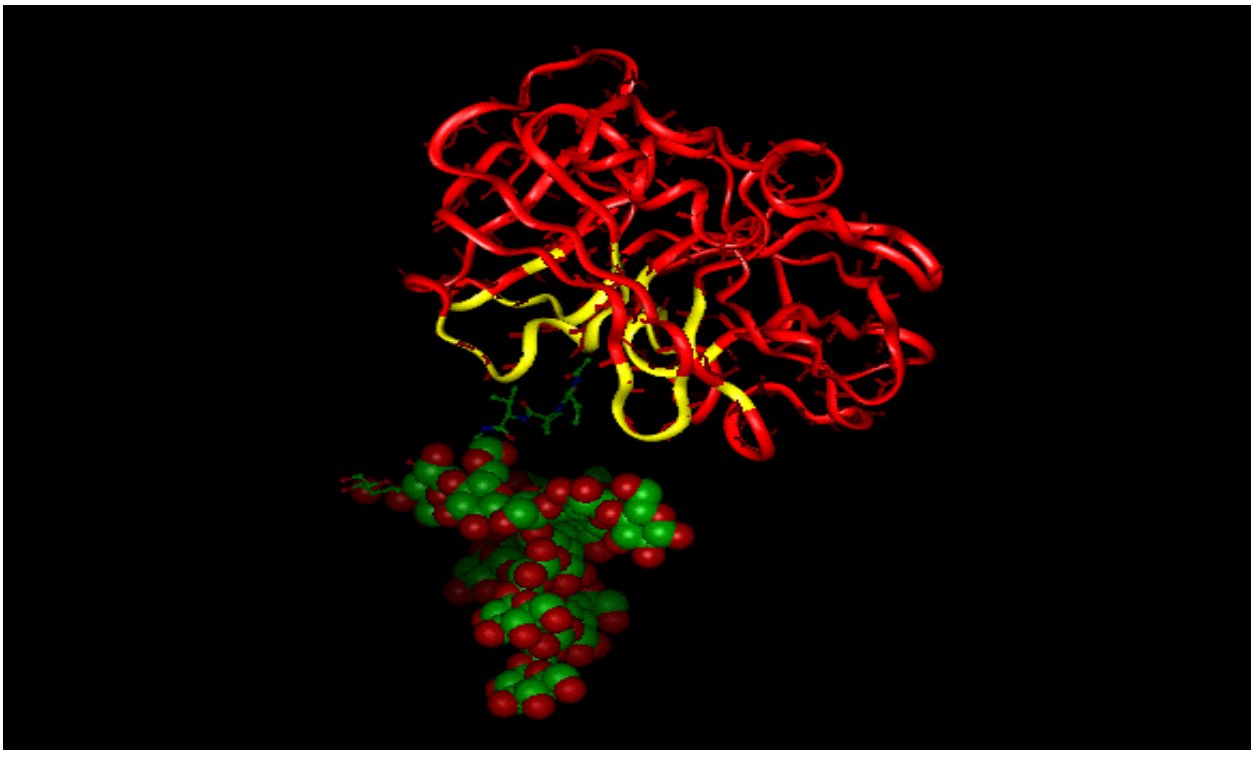

Figure 1. Computational chemistry model of a cellulose peptide docked in the active site of elastase. The model demonstrates the concept of using a cotton derivative to bind proteases found in the chronic wound.

Preparation and Assay of the Prototype Cotton Dressing

The preparation of the prototype cotton wound dressing required synthesis of a tripeptide sequence on the cotton fiber. This peptide sequence was linked to the cellulose of the cotton fiber at both ends of the peptide sequence and tested for activity to sequester human neutrophil elastase. Assay of the peptide conjugate on cotton was completed by incubating the cotton wound dressing in a solution of elastase for one hour and assessing the sequestration activity of the wound dressing fiber. Determination of the amount of enzyme taken up by the fiber was based on the kinetic profile of the reaction progress curve of enzyme remaining in solution and its reaction with substrate as shown in Figure2. The elastase substrate is employed as a putative protein associated with healing. Thus a smaller amount of substrate left in solution and a slower reaction progress curve is associated higher levels of activity bound to the dressing and a more active wound-dressing fiber. It is noteworthy in this regard that the activity of the peptide conjugate fiber is dose dependent.

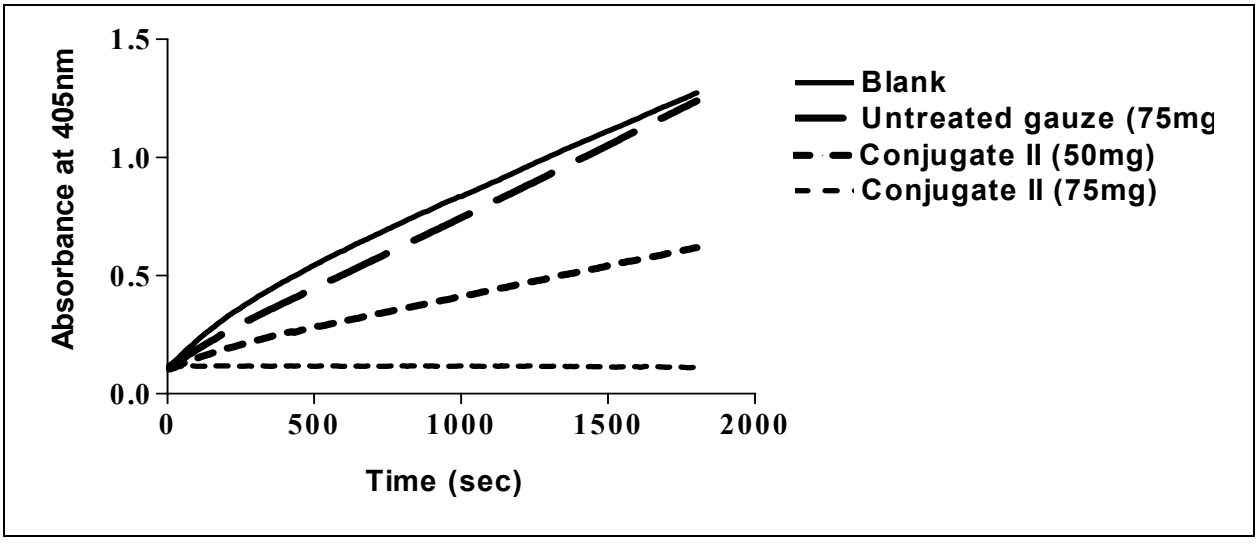

Figure 2. Kinetic profile of enzyme kinetic reaction progress curves as an approach to determine the degree of sequestraton achieved by the cellulose-peptide conjugate shown in Figure 1. Conjugate II is a Val-Pro-Val-Gly-Cellulose conjugate. Its sequestration of elastase is shown to be effective compared with untreated cotton.

Design of Cotton Dressings Based on Cotton Aqueous Finishing Chemistries

The design of the chronic wound dressing requires a simple, economically feasible modification that is imparted to the cotton fiber in a one or two-step aqueous finishing technique. For this purpose four functional modification have been constructed as shown in Figure 3 that are based on sequestration of the protease though active site interaction or charge pairing with the enzyme. For example an active site sequestratant would be based on the potential for the modified fiber to interact analogous to an enzyme inhibitor or substrate as shown in the previous peptide conjugate of cellulose example. Whereas, a charge sequestrant is based on binding of the enzyme to the cotton fiber through ion pairing: elastase is positively charged, thus a negatively charged fiber would ion-pair with the enzyme. The structures of modified cellulose shown in Figure 3 consist of a dialdehyde, carboxymethylated, phosphorylated and polycarboxylate crosslinked modification. The active site sequestrant is the dialdehyde functional group, and the negatively charged modifications are the two forms of carboxylated and phosphorylated functionalities. The preparation of these functionally finished cotton wound dressings has been previously reported. The proposed mechanism of action of the dialdehyde cotton wound dressing is shown in Figure 4. The proposed mechanism for sequestration is thought to occur by formation of a hemiacetal through attack of the Ser-195 within the active site of residue with assistance from Histidine-57 and Aspartate-102. The concerted interaction of these residues termed the catalytic triad of the serine protease leads to cleavage of a peptide bond when proteolytic activity occurs, but in this model the interaction is more similar to inhibitor binding of the enzyme. To show that the dialdehyde cotton may function to sequester the elastase via this molecular mechanism the enzyme has been assayed with a soluble form of dialdehyde starch which best approximates properties of cotton as a carbohydrate in solution.

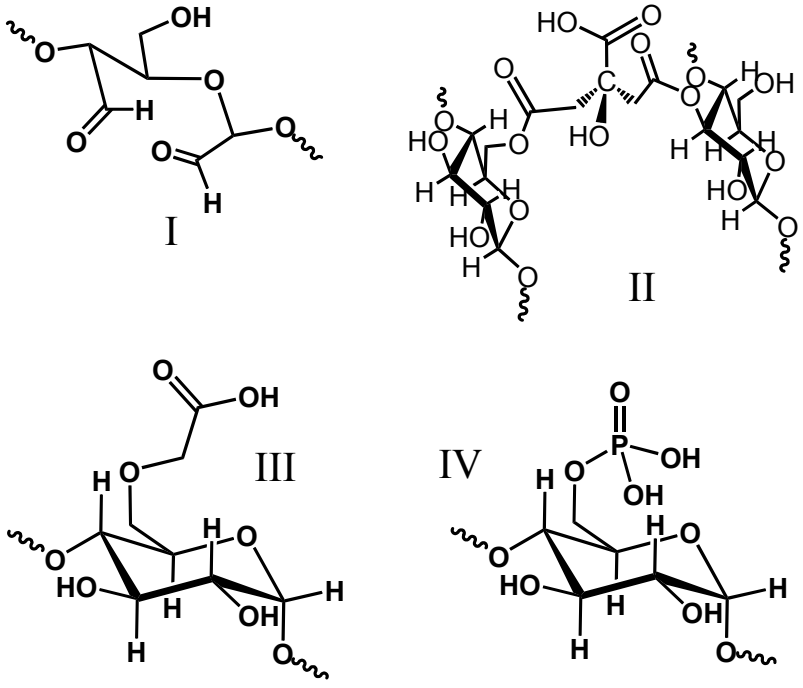

Figure 3. Structure of cotton derivatives designed for their structural features to bind elastase either at the active site or through formation of salt bridges.

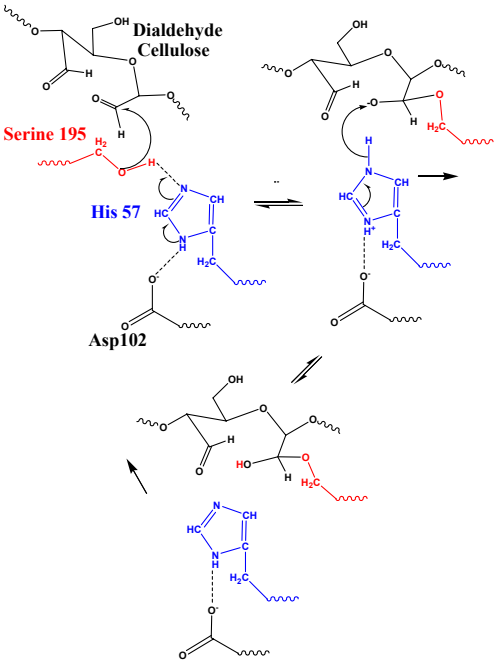

Figure 4. Proposed mechanism of action for binding of elastase to dialdehyde cotton.

Understanding and Predicting How Active Cotton Wound Dressings May Perform in the Chronic Wound

There is some controversy concerning the usefulness of animal models in testing chronic wound dressings for efficacy. Thus it is thought that the best predictor for efficacy of a chronic wound dressing is to test the wound dressing in vitro with chronic wound fluid or proteins that mimic the environment, protein concentration and make-up of chronic wound exudate. During the course of developing a cotton product for commercialization two models for studying the performance of the modified cotton fiber under conditions that mimic chronic wound fluid exudate were made. One model consisted of assaying the modified fiber in diluted chronic wound fluid containing high elastase activity similar to that of the chronic wound. More recently we have developed a model utilizing albumin concentrations that mimic those levels of albumin found in the chronic wound in the presence of elastase. Another purpose in utilizing the albumin model is to better understand how albumin may compete for binding sites on different functional group cottons, and compare capacities for competitive elastase binding. Using these types of models we have begun to study and compare more closely mechanisms for competitive binding through ion pairing between the enzyme and cotton as shown in Figure 5 with the 'inhibitor-active site' motif shown in Figure 4.

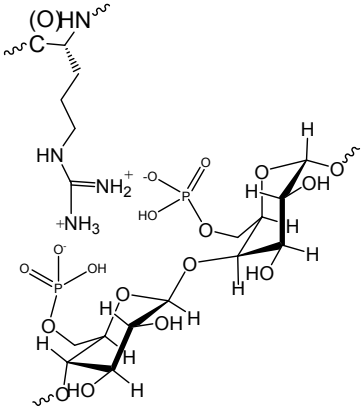

Figure 5. Portrayal of a phosphorylated cellulose derivative developed in the cotton-based wound dressing for its binding of positively charged elastase in the presence of wound fluid proteins such as albumin.

Conclusion

Research and development issues in creating an active chronic wound dressing have been: 1) Identifying the molecular source of chronic wound pathology. 2) Investigating the molecular features of the protease sequestrant that are useful in designing a selective sequestrant of the protease. 3) Developing the in vitro assay for assessment of optimal features needed for efficacy in human wounds. The future challenges to bring cotton bandages into the active wound dressing market will depend on the success of completing these steps. Efforts currently indicate there is a promising future for intelligent cotton-based wound dressings that perform an important function in the wound necessary to healing.

References

Cullen, Breda, Smith, R. McCulloch, Silcock, D., Morrison, L., Mechanism of action of Promogran, a protease modulating matrix for the treatment of diabetic for ulcers. 2002; Wound Rep. Reg. 10: 26-37.

Edwards, J.V., Yager, D.R., Cohen, I.K., Diegelmann, R.F., Montante, S., Bertoniere, N., Bopp, A.F., Modified cotton gauze dressings that selectively absorb neutrophil elastase activity in solution. 2001; Wound Rep. Reg. 2001; 9: 50-58.