Chapter 2

FUTURE STRUCTURE AND PROPERTIES OF MECHANISM-BASED WOUND DRESSINGS

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Abstract

The research and development of chronic wound dressings, which possess a mechanism-based mode of action, has entered a new level of understanding in recent years based on improved definition of the biochemical events associated with pathogenesis of the chronic wound. Recently, the molecular modes of action have been investigated for skin substitutes, interactive biomaterials, and some traditional material designs as balancing the biochemical events of inflammation in the chronic wound to improve healing. The interactive wound dressings have activities including up-regulation of growth factors and cytokines and down-regulation of destructive proteolysis. Carbohydrate-based wound dressings have received increased attention for their molecular interactive properties with chronic and burn wounds. Traditionally, the use of carbohydrate-based wound dressings including cotton, xerogels, charcoal cloth, alginates, chitosan, and hydrogels have afforded properties such as absorbency, ease of application and removal, bacterial protection, fluid balance, occlusion, and elasticity. Recent efforts in our lab have been underway to design carbohydrate dressings that are interactive cotton dressings as an approach to regulating destructive proteolysis in the non-healing wound. Elastase is a serine protease that has been associated with a variety of inflammatory diseases and has been implicated as a destructive protease that impedes wound healing. The presence of elevated levels of elastase in non-healing wounds has been associated with the degradation of important growth factors and fibronectin necessary for wound healing. Focus will be given to the design, preparation, and assessment of a type of cotton-based interactive wound dressing designed to intervene in the pathophysiology of the chronic wound through protease sequestration.

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2.1 Historical characteristics of wound dressing fibers and wound healing

Through the ages, both vegetable and animal fibers have been applied to human wounds to stop bleeding, absorb exudate, alleviate pain and provide a protective barrier for the formation of new tissue. Some milestones of wound dressing development down through the ages are summarized in Figure 2.1. Early humankind employed many different materials from the natural surroundings including resin-treated cloth, leaves, and wool-based materials with a variety of substances including eggs and honey. Some of these ancient remedies were probably more than palliative treatments. For example, the antibacterial activity of honey in the treatment of wounds has been established [1], and honey is now being reconsidered as a dressing when antibiotic-resistant strains prevent successful antibiotic therapy. Recent studies suggest that honey may promote wound healing through stimulation of inflammatory cytokines from monocytic cells [2]. Leaves of *chromolaena odorata*, a weed found in crops in countries of the Southern Hemisphere, have been found to exert potent antioxidant effects

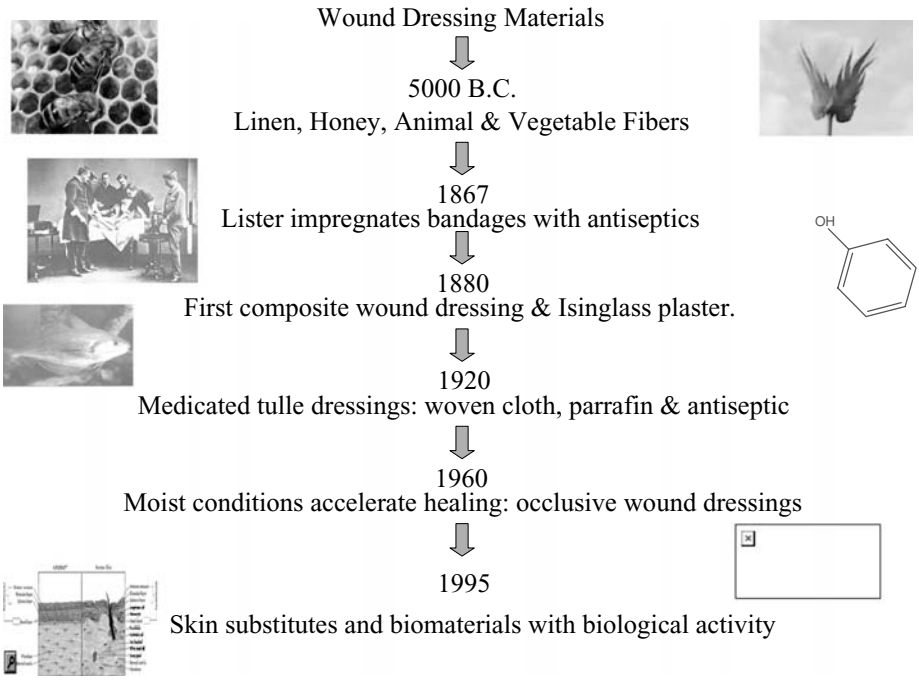

Figure 2.1. Timeline overview for historical developments in wound dressing materials. See *Wound Management and Dressings by Stephen Thomas, Pharmaceutical Press, London 1999* for an in-depth treatment of the development and history of wound dressings

that enable enhanced proliferation of human dermal fibroblasts and epidermal keratinocytes [3]. Wool-based dressings are also being rediscovered for their unique properties applicable to burn and chronic wounds [4]. Many ancient remedies for wound healing were contaminated with microorganisms, which increased the likelihood of infection. However, with the work of Lister in 1867, who impregnated bandages with carbolic acid, antiseptic treatment arose, and shortly thereafter Joseph Gamgee produced the first composite wound dressing as a cotton or viscose fiber medicated with iodine [5]. The first film dressings composed of Isinglassplaster were also introduced in the 18th century and were reported to be used with some improved success after skin grafting at that time. The first generation of medicated tulle dressings (see Contact Layer Dressings in Table 2.1) were introduced in the 1920s for treatment of burn wounds and were composed of an open weave cloth with soft paraffin and antiseptic. The finding that moist wounds heal faster than when desiccated and that collagen at the interface of the scab and dermis impedes epidermal cell movement prompted the development of occlusive dressings for wound management [6, 7]. In 1975, Rheinwald and Green [8] developed a method that made it possible to cultivate human keratinocytes so that a 1–2 cm^2 of keratinocyte cultured grafts in about 3 weeks. This work paved the way for the eventual development of skin substitutes and biomaterials with wound interactive properties and biological activity which has progressed from the mid 1990s through the present.

The science of wound healing has progressed rapidly over the past 30 years. An understanding of the progress of wound healing science, as seen by how the "future" of wound healing was viewed in the late sixties, can be seen from this quote taken from Christopher Textbook of Surgery:

Will the surgeon of 2000 AD encounter the same healing problems as the present-day surgeon? Let us hope not. Prudden's studies with cartilage have shown unquestioned stimulation of the healing process in a number of different healing situations; surely a purification and chemical dissection of this crude product can result in a sterile, more potent compound which can be used parenterally as well as locally. There may be no need to use such a compound in normal patients but it could prove invaluable in patients in whom impaired healing is to be expected [9].

If we fast forward 35 years from this quote it is found that the "potent compound" alluded to is today thought of as a variety of biologically potent protein families that play a central role in stimulating and regulating wound healing. These include growth factors, cytokines, and chemokines. Growth factors are mitogens that stimulate proliferation of wound cells. Growth factors are "messages for cells" with hormone-like potency, and as proteins they bind and activate specific cell receptors. They regulate gene expression, protein

Table 2.1.

Dressing and fiber type	Description	Properties	Indications	Contraindications
Thin films (bioclusive, tegaderm, opsite)	Semipermeable, polyurethane membrane with acrylic adhesive.	Permeable to water and oxygen providing a moist environment.	Minor burns, pressure areas, donor sites, postoperative wounds.	Deep cavity wounds, third-degree burns infected wounds.
Sheet hydrogels (clearsite, nu-gel, vigilon, geliperm, duoderm gel, intrasite gel, geliperm granulate)	Solid, non-adhesive gel sheets that consist of a network of cross-linked, hydrophilic polymers that can absorb large amounts of water without dissolving or losing its structural integrity. Thus they have a fixed shape.	Carrier for topical medications. Absorbs its own weight of wound exudate. Permeable to water vapor and oxygen, but not to water and bacteria. Wound visualization.	Light to moderately exudative wounds. Autolytic debridement of Stages II and III pressure sores.	Heavily exuding wounds.
Hydrocolloids (duoderm, comfeel)	Semipermeable polyurethane film in the form of solid wafers; contain hydroactive particles as sodium carboxymethyl cellulose that swells with exudate or forms a gel.	Impermeable to exudate, microorganisms, and oxygen. Moist conditions produced promote epithelialization.	Shallow or superficial wound with minimal to moderate exudate.	Wounds with dry eschar or very light exudate.

Semipermeable foam (allevyn, lyfoam)	Soft, open cell; hydrophobic, polyurethane foam sheet 6–8 mm thick. Cells of the foam are designed to absorb liquid by capillary action.	Permeable to gases and water vapor but not to aqueous solutions and exudate. Absorbs blood and tissue fluids while the aqueous component evaporates through the dressing. Cellular debris and proteinaceous material are trapped.	Used for leg and decubitus ulcers, sutured wounds, burns, and donor sites.	Not to be applied to wounds covered with a dry scab or hard black necrotic tissue.
Amorphous hydrogel (carrasyn, royl derm, dermassist gel, hyfil, biolex, carraborb M, woundres collagen, duoderm hydroactive gel, dermagran, curasol, restore, stericare, nugel, curafil, spand-gel, intrasite, elta dermal)	Similar in composition to sheet hydrogels in their polymer and water make-up. Amorphous gels are not cross-linked. They usually contain small quantities of added ingredients such as collagen, alginate, cooper ions, peptides, and polysaccharides.	Gels clear, yellowish, or blue from copper ions. Viscosity of the gel varies with body temperature. Available as tubes, foil packets, and impregnated gauze sponges.	Used for full-thickness wounds to maintain hydration. It may be used on infected wound or as wound filler.	Heavily draining wounds. Improper use may lead to periwound maceration.

(*continued*)

Table 2.1. (Continued)

Dressing and fiber type	Description	Properties	Indications	Contraindications
Fillers (kaltostat, debrisan beads)	Calcium alginate that consists of an absorbent fibrous fleece with sodium and calcium salts of alginic acid (ratio 80:20). Dextranomer beads consist of are circular beads, 0.1–0.3 mm in diameter, when dry. The bead is a three-dimensional cross-linked dextran and long chain polysaccharide.	Heavily exudating wounds including chronic wounds as leg ulcers, pressure sores, fungating carcinomas. Wounds containing soft yellow slough, including infected surgical or posttraumatic wounds.	Heavily exudating wounds.	Minimally exudating wounds.
Contact layer dressings (Tulle gauze with petroleum jelly)	Greasy gauzes consisting of Tulle gauze and petroleum jelly. Dressing sheet silicone impregnated which consists of an elastic transparent polyamide net that is impregnated with a medical grade cross-linked silicone.	The dressing that is porous non-absorbent and inert is designed to allow the passage of wound exudate for absorption by a secondary dressing.	Shallow or superficial wounds with minimal to moderate exudate.	Not recommended for cleaning the wound.

Gauze packing	Cotton gauze used both as a primary and secondary wound dressing. Gauze is manufactured as bandages, sponges, tubular bandages, and stockings. Improvement in low-linting and absorbent properties. Gauze is still a standard of care for chronic wounds.	Cotton gauze may be wetted with saline solution to confer moist properties. Possess a slight negative charge that facilitates uptake of cationic proteases. Absorbent and elastic for mobile body surfaces.	For chronic wounds it fills deep wound defects and is useful over wound gel to maintain moist wound; needs to be packed lightly. May traumatize wound if allowed to try.	Avoid multiple small dry dressing wads in wound cavity.
Wound vacuum assisted closure	Polyurethane foam accompanied by vacuum negative pressure in the wound bed.	Wound filled with foam and sealed with a film. Vacuum tubing is inserted and used continuously or as	Deep wound to stimulate the growth of granulation tissue.	Infected wounds and wounds with fistulae.

synthesis and degradation, cell division, movement, and metabolism. Cytokines are regulators of inflammation and have potent stimulatory and inhibitory action on inflammatory cells. Chemokines are proteins and peptides that regulate the trafficking of leukocytes, activate inflammatory cells as neutrophils, lymphocytes, and macrophages. However, the following quote taken from a current special issue of Wound Regeneration and Repair reflects the current relationship of growth factors, cytokines, and chemokines:

Growth factors, cytokines, and chemokines are key molecular regulators of wound healing. They are all proteins, or polypeptides, and are typically synthesized and released locally, and primarily influence cells by paracrine actions. The initial concepts that growth factors were mitogens only for wound cells, that cytokines regulated inflammatory cells, and that chemokines only regulated chemoattraction of inflammatory cells were too narrow and it is now recognized that there are substantial overlaps in target cell specificity and actions between these three groups [17].

Central to understanding the future of wound dressing fibers in wound healing is an understanding of how progress in wound healing science is reshaping the design of wound dressings. Wound healing is a complex cascade of molecular and cellular events [10]. During the coagulation phase following injury, platelets initiate healing through the release of growth factors, which diffuse from the wound to recruit inflammatory cells to the wound. Thus, growth factors are responsible for the activation of immune cells, extracellular matrix deposition, collagen synthesis, and keratinocyte and fibroblast proliferation and migration. Neutrophils arrive on the scene early, and serve to clear the wound of bacteria and cellular debris. The arrival of neutrophils marks the onset of the inflammatory phase of wound healing and under acute healing conditions lasts only a few days. However, in the chronic wound the period of growing neutrophil population is extended indefinitely. Inflammation is the second phase of healing and it is mostly regulated by cytokines that are secreted by macrophages. Cytokines control cellular chemotaxis, proliferation, and differentiation. Macrophages also migrate to the wound site to destroy bacteria. However, an overabundance of cytokines and neutrophils prolong the inflammatory phase and has a negative influence on healing. Granulation tissue, which consists of fibroblasts, epithelial cells, and vascular endothelial cells, is formed about 5 days after injury. Fibroplasia is the last restorative stage of healing. Fibroplasia involves the combined effect of reepithelialization, angiogenesis, and connective tissue growth and it has been termed “a dynamic reciprocity of fibroblasts, cytokines, and extracellular matrix proteins”. In a healthy person healing occurs in 21 days from coagulation, and the remodeling phase consisting of scar transformation based on collagen synthesis continues for months following injury.

When wounds fail to heal, the molecular and cellular environment of the chronic wound requires conversion to an acute wound so the ordinary sequential phases of wound healing can proceed. In June 2002, a meeting of wound healing experts formulated an overview of the current status, role, and key elements of wound bed preparation [17]. The subsequent reports in the literature from this meeting articulate well the concept of a systematic approach to wound bed preparation, which is based on an emphasis to decrease inflammatory cytokines and protease activity while increasing growth factor activity. Thus, a challenge of current wound dressing development is to promote the clinical action of wound bed preparation through addressing issues of high protease and cytokine levels and increasing growth factor levels.

2.2 The origins of moist wound dressings and the ideal wound dressing

The concept that wounds heal best when kept dry was chiefly espoused in wound management up until the late fifties because it was thought that bacterial infection could best be prevented by absorbing and removing all wound exudate. Consequently, most wounds were treated with cotton or viscose fiber material under dry conditions. However, in the early sixties Winter [6] and Hinman and Maiback [7] showed that the rate of reepithelialization increases in a moist wound versus a wound kept dry.

Occlusion is a concept in wound management that prompted a revolution during the 1970s in the production of new types of wound dressings that are still being developed. Occlusion is the regulation of water vapor and gases from a wound to the atmosphere promoting a moist environment, which allows epidermal barrier function to be rapidly restored. However, wound occlusion does require careful regulation of the moisture balance of the wound with vapor permeability to avoid exceeding the absorbency limits of the dressing. Thus, the occlusive dressing types have been developed depending on the nature of the wound and accompanying wound exudate as illustrated in Figure 2.2. The theory of moist wound healing led to approximately eight to nine separate types of wound dressing materials and devices (Table 2.1) useful for different wound treatment indications. Each of the material types that represent these distinct groups have molecular and mechanical characteristics that confer properties to promote healing under specifically defined clinical indications. For example, it has been recommended that wounds with minimal to mild exudate be dressed with hydrocolloid, polyurethane, and saline gauze, and wounds with moderate to heavy exudate be dressed with alginate dressings. Dressings may also be selected based on wound tissue color, infection, and pressure ulcer grade [11].

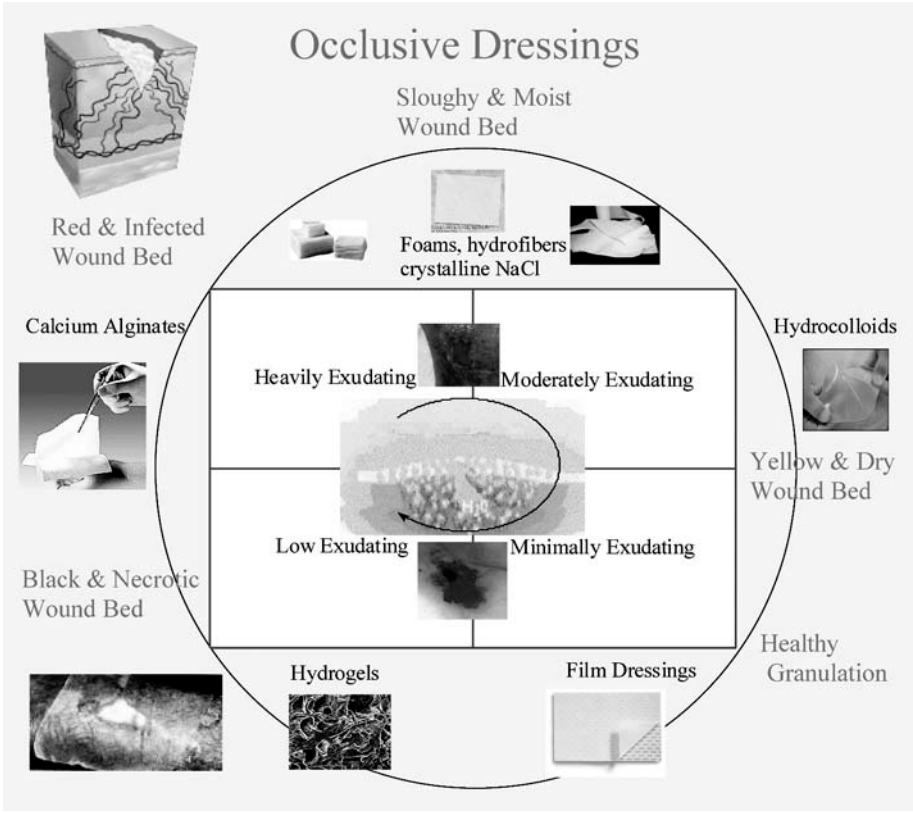

Figure 2.2. Occlusive dressings promote moist wound healing by regulating water vapor and gases in the chronic wound environment. The selection of an occlusive wound dressing depends on the degree of hydration in and around the wound tissue, its color, the presence of infection, and the pressure ulcer grade. Table 2.1 discusses the design, composition, and indications of different classes of occlusive wound dressings. For an in-depth treatment on selecting occlusive dressings see *Occlusive wound dressings. Why, when, which? By Vincent Falanga, Arch. Dermatol. 1988, June; 124(6), 872–877*

When taken as a composite of material characteristics the combined properties of the dressing materials given in Table 2.1 would approximate an ideal wound dressing. A comparison of some of the ideal properties found in both cotton and alginate wound dressings are outlined in Table 2.2. Combination of cotton and alginate in a dressing material has been reported and represents an attempt to integrate properties found in each of these two types of dressings into a single dressing [12]. Improvements in wound dressings that function at a molecular or cellular level to accelerate wound healing or monitor wound function are included among the ideal characteristics and may be termed

Table 2.2. Some ideal properties of a wound dressing as compared between cotton and alginate materials. (G) Good, (E) Excellent, and (P) Poor

Comparative properties of alginate (A) and cotton (C) dressings	C	A		C	A
Absorbency	(G)	(E)	Ease of application and removal	(G/P)	(E)
Adherence	(G)	(E)	Elasticity	(E)	(P)
Bacterial barrier	(G)	(G)	Gaseous exchange	(G)	(G)
Comfort	(G)	(E)	Hemostatic	(G)	(G)
Conformability	(G)	(E)	Non-antigenic and non-toxic	(E)	(E)
Drug delivery	(G)	(G)	Sterilizability	(E)	(E)
Durability	(E)	(G)	Water vapor transmissibility	(G)	(E)

interactive and intelligent wound dressings, respectively. For example, a wound dressing that removes harmful proteases from the wound to enhance cell proliferation is an example of an interactive wound dressing. A dressing having a detection device in the material signaling "time-to-change" from a defined colorimetric reaction as a molecular signal that the dressing has reached capacity of deleterious protein levels, and pH or temperature imbalance may be termed "intelligent". It seems likely that the future development of intelligent wound dressings that give beneficial clinical information on the wounds healing status will be in sync with the development of interactive dressings that perform a specific molecular or cellular function in the complex cellular and biochemical wound environment.

2.3 Interactive chronic wound dressings

The design and preparation of interactive chronic wound dressings [13] have become increasingly important as part of a solution to addressing the critical worldwide health crisis of the growing number of chronic wound patients. In the United States alone, there are over five million patients a year who suffer from chronic wounds due to the formation of decubitus bedsores brought on in the elderly nursing home or spinal chord paralysis patient. In addition, diabetes accounts for at least 60,000 patients annually who also suffer with foot ulcers. Since the mid 1990s, the number of wound care products in the well-recognized groups outlined in Table 2.1 has expanded and new groups of products have also been marketed including tissue-engineered products [14]. Recent efforts to develop wound dressings that do more than simply offer a moist wound environment for better healing have prompted most major wound dressing companies to develop research and approaches on interactive chronic wound dressings. Interactive chronic wound dressings, which possess a

Table 2.3. Carbohydrate wound dressings that stimulate growth factors and cytokines

Carbohydrate source	Associated wound dressings	Growth factor/cytokine induced and cell source	Activity	Wound healing events
Alginate [16]	Guluronic:mannuronic (80:20) dressing.	TNF-α, IL-β, IL-6 macrophages and monocytes	Collagen synthesis fibroblast and keratinocyte chemotaxis	Pro-inflammatory stimulus
DEAE sephadex [21, 22]	DEAE sephadex beads in PEG (10 mg/mL)	TGF-β platelets fibroblasts macrophages	Fibroblast activation ECM deposition, collagen synthesis TIMP synthesis MMP synthesis angiogenesis	Bead pocket increases wound breaking strength
Honey [1, 2]	Manuka and jelly bush—containing products	TNF-α, IL1β, IL-6 macrophages PMNs fibroblasts	Fibroblast and keratinocyte proliferation and chemotaxis.	Antibacterial pro-inflammatory
Aloe vera [32]	Aloeride/acemannan B—containing gels	IL-1β, TNF-α IFN-γ macrophages PMNs fibroblasts	Fibroblasts macrophages	Reduces acute radiation-induced skin reactions

mechanism-based mode of action, are targeted to biochemical events associated with pathogenesis of the chronic wound and are a part of good wound bed management.

Skin substitutes, which are being increasingly used, contain both cellular and acellular components that appear to release or stimulate important cytokines and growth factors that have been associated with accelerated wound healing [15]. Some basic materials may also play a role in up-regulating growth factor and cytokine production and blocking destructive proteolysis. In this regard, the biochemical and cellular interactions that promote more optimal wound healing have only recently been elucidated for some of the occlusive dressings described in Table 2.1. Some carbohydrate-based wound dressings that stimulate growth factor and cytokine production are outlined in Table 2.3. For example, certain types of alginate dressings have been shown to activate human macrophages to secrete pro-inflammatory cytokines associated with accelerated healing [16]. Interactive wound dressing materials may also be designed with the purpose of either entrapping or sequestering molecules from the wound bed and removing the deleterious activity from the wound bed as the wound dressing is removed, or stimulating the production of beneficial growth factors and cytokines through unique material properties. They may also

be employed to improve recombinant growth factor applications. Impetus for material design of these dressings derives from advances in the understanding of the cellular and biochemical mechanisms underlying wound healing. With an improved understanding of the interaction of cytokines, growth factors, and proteases in acute and chronic wounds [17–20], the molecular modes of action have been elucidated for dressing designs as balancing the biochemical events of inflammation in the chronic wound and accelerating healing. The use of polysaccharides, collagen, and synthetic polymers in the design of new fibrous materials that optimize wound healing at the molecular level has stimulated research on dressing material interaction with wound cytokines [16], growth factors [21, 22], proteases [23, 24, 25, 29], reactive oxygen species [26], and extracellular matrix proteins [27].

2.3.1 Sequestration of wound proteases and approaches to treating chronic dermal ulcers

The prolonged inflammatory phase characteristic of chronic wounds results in an over exuberant response of neutrophils, which contain proteases and free radical generating enzymes that have been implicated in mediating much of the tissue damage associated with chronic inflammatory diseases. Since neutrophils mediate a variety of chemotactic, proteolytic, and oxidative events that have destructive activities in the chronic wound, therapeutic interventions have been proposed based on the proteolytic and oxidative mechanisms of neutrophil activity in the wound. Neutrophils contain both matrix metalloproteases and cationic serine proteases, which are two families of proteases that have been associated with a variety of inflammatory diseases, and have been implicated as destructive proteases that impede wound healing. The presence of elevated levels of these proteases in non-healing wounds has been associated with the degradation of important growth factors and fibronectin necessary for wound healing [28]. There is also a synergistic effect of further oxidative inactivation of endogenous protease inhibitors, which leads to unchecked protease activity.

A protease sequestrant dressing's design for activity may be couched in a number of molecular motifs based on the structural features of the protease, which interferes with the healing process. The molecular features of the material may be targeted to the protein's size, charge, active site, and conformation to enhance selective binding of the protein to the dressing material and removal of the detrimental protein from the wound bed. Active wound dressings that have been designed to redress the biochemical imbalance of the chronic wound in this manner are composed of collagen and oxidized regenerated cellulose [23], nanocrystalline silver-coated high-density polyethylene [29], deferrioxamine-linked cellulose [30], and electrophilic and ionically derivatized cotton [24].

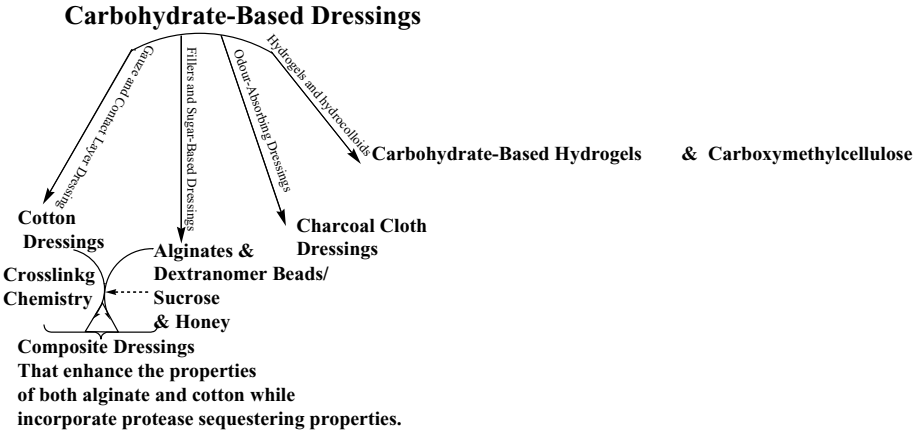

Figure 2.3. Schematic of some types of carbohydrate-based dressings and the application of cross-linking chemistry to combine two families of carbohydrate-based dressings into a more ideal composite dressing [12]

2.4 Carbohydrate-based wound dressings

Carbohydrate-based wound dressings (Figure 2.3) have received increased attention in recent years for their mechanical and molecular interactive properties with chronic and burn wounds. Traditionally, the use of carbohydrate-based wound dressings including cotton, xerogels, charcoal cloth, alginates, chitosan, and hydrogels has afforded properties such as absorbency, ease of application and removal, bacterial protection, fluid balance, occlusion, and elasticity. Focus will be given here to the design, preparation, and assessment of carbohydrate-based wound dressings as an effort to improve cotton medical textiles.

2.5 Prototype design of active cotton wound dressings

Cotton gauze has been manufactured and utilized for the last two centuries as a standard wound dressing in the care of both acute and chronic wounds. Although it is still used in much the same manner as originally conceived, there have been some fiber modifications that have improved its quality and versatility in medical applications.

The protease human neutrophil elastase found in high concentration in the chronic wound creates considerable protein destruction and prevents the wound from healing [25]. The design of wound dressings that selectively sequester proteases from the chronic wound is couched in the concept that molecular

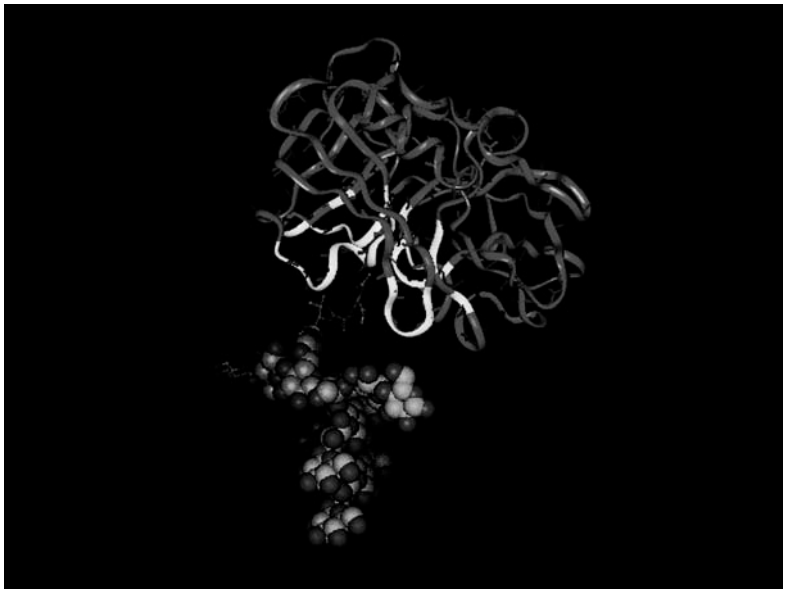

Figure 2.4. Computer graphic model of peptide-bound cellulose docked at the active site of human neutrophil elastase. Cellulose is depicted as the green and red CPK model. The peptide portion of the conjugate is a ball and stick model shown docked within the yellow highlighted ribbon depicting the active site of elastase

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, overall charge, and active site mechanism for binding substrate may be employed to create the appropriate fiber design that might best bind the enzyme selectively. The design approach of the prototype for selective sequestration is a molecular model of a cellulose conjugate containing an active site recognition sequence docked to the active site of human neutrophil elastase as shown in Figure 2.4. The subsites of enzyme active site interaction consist of the sequence conjugate H-Val-Pro-Glycine-O-ester-Cellulose.

2.6 Preparation and assay of the prototype active cotton-based wound dressing

The preparation of the prototype cotton wound dressing containing the conjugate shown in Figure 2.4 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 [25]. Assay of the peptide conjugate on cotton was

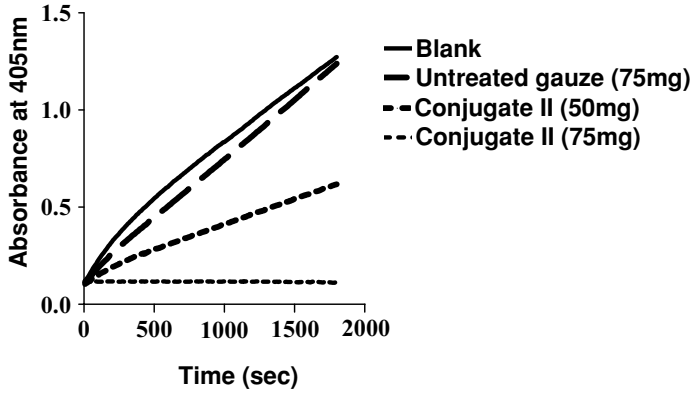

Figure 2.5. Reaction progress curves for a peptide conjugate [25] as illustrated in the molecular model in Figure 2.2 in solutions of elastase that have been treated with interactive cotton wound dressing fibers. The reaction is an enzyme hydrolysis of the elastase substrate left in solution following treatment. A slower reaction as seen with the peptido–cellulose conjugates versus the untreated cotton dressing reflects increased removal of elastase from solution

completed by incubating the cotton wound dressing in a solution of elastase for an hour and assessing the sequestration activity of the modified 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 Figure 2.5. The elastase substrate is employed in the assay as a putative protein associated with healing. Thus, a smaller amount of substrate left in solution and a slower reaction progress curve is associated with 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.

2.7 Design of active cotton-based wound dressings

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. This is necessary so economical methods can be adopted in the textile mill to modify the cotton. An active site protease sequestrant 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, and as illustrated in Figure 2.4. On the other hand, a charge sequestrant material is based on binding of the enzyme to the cotton fiber through ion pairing: elastase is positively charged, thus a negatively

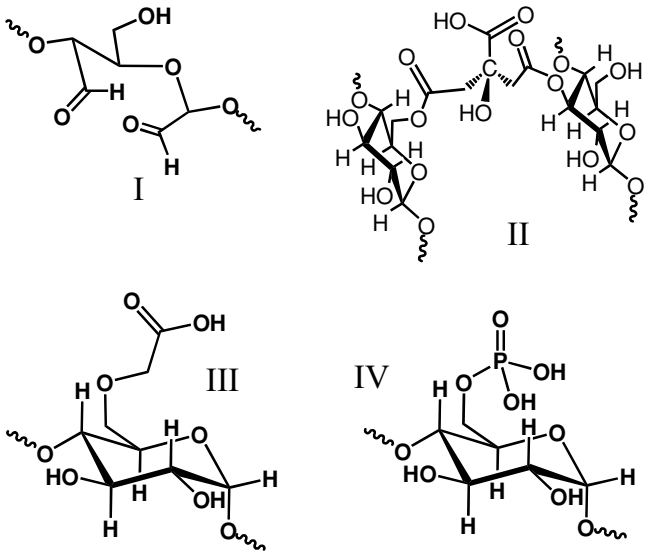

Figure 2.6. Representative structures of modified anhydroglucose monomer units in the cellulose chain upon treatment of the cotton gauze. I, structure of dialdehyde cotton cellulose; II, polycarboxylic acid cross-linked cotton; III, carboxymethylcellulose cotton; and IV, phosphorylated cotton

charged fiber would ion pair with the enzyme. The types of sequestrant motifs might be based on structures of modified cellulose as shown in Figure 2.6. These cotton cellulose modifications are dialdehyde, carboxymethylated, phosphorylated, and polycarboxylate cross-linked modifications. The active site sequestrant is the dialdehyde functional group (I), and the negatively charged modifications are the two forms of carboxylated and phosphorylated cellulose (II–IV). The preparation of these functionally finished cotton wound dressings has been previously reported [24].

The proposed mechanism of action of the dialdehyde cotton wound dressing is shown in Figure 2.7. 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.

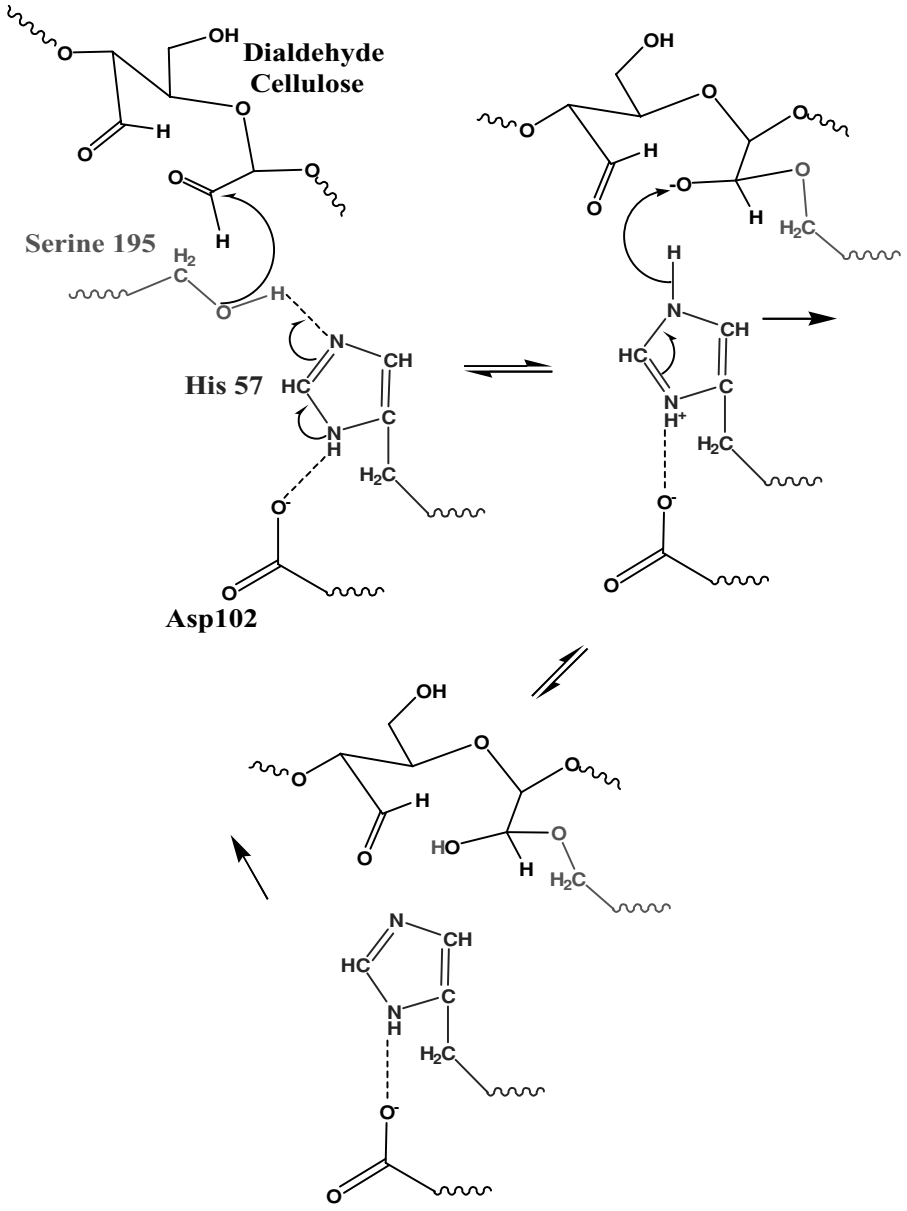

Figure 2.7. The postulated mechanism of action for protease sequestration by dialdehyde cotton [24] as an example of a modified fiber sequestrant acting at the active site of the protease to enable enhanced protease removal from the chronic wound. The broad range proteolytic activity of serine proteases released by neutrophils into the wound environment is responsible in part for the degradation of growth factors and extracellular matrix proteins. The active site of catalytic activity in serine proteases consists of a catalytic triad shown by X-ray studies to consist of a charge relay among amino acids for substrate hydrolysis. Inhibition of the active site occurs

2.8 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. The pathology of the chronic wound is even more complex than the healing wound, and difficult to mimic. One predictor for efficacy of a chronic wound dressing is testing the dressing *in vitro* with chronic wound fluid or proteins that mimic the environment and protein concentration as well as makeup of chronic wound exudate. During the course of developing a modified 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 [24]. 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 groups of modified cotton cellulose, and compare capacities for competitive protease binding. Using these types of models, we have begun to study and compare more closely the mechanisms for competitive binding through ion pairing between the enzyme and cotton as shown in Figure 2.5 with the "inhibitor-active site". Figure 2.8 shows the results of an experiment designed to evaluate the capacity of a type of charge sequestrant wound dressing currently in development that removes elastase from solution. The results of this 24-hour assay where the dressing is challenged with a constant concentration of elastase and evaluated for continued removal of the protease from solution suggest good capacity of the charge sequestrant wound-dressing motif.

2.9 Summary

Wound care products along with the clinical practice of wound care itself have rapidly matured over the past 20 years and become a molecular-biotechnology focused industry. The product market is now valued at $1.74 billion, and five million Americans suffering from chronic open wounds

←

Figure 2.7. (*Continued*) analogous to substrate peptide bond cleavage when proton transfer from Serine-195 is transferred to Histidine-57 upon attack of the Serine-195 hydroxyl oxygen at the electrophilic carbonyl of the anydrogluco-aldehye. Several classes of aldehyde and ketone-based inhibitors have been developed (*Edwards, P. D.; Bernstein, P. R. Synthetic inhibitors of elastase. Med. Res. Rev.* ***1994***, *14, 127–194*) for a variety of inflammatory diseases but few have been adapted to wound dressing

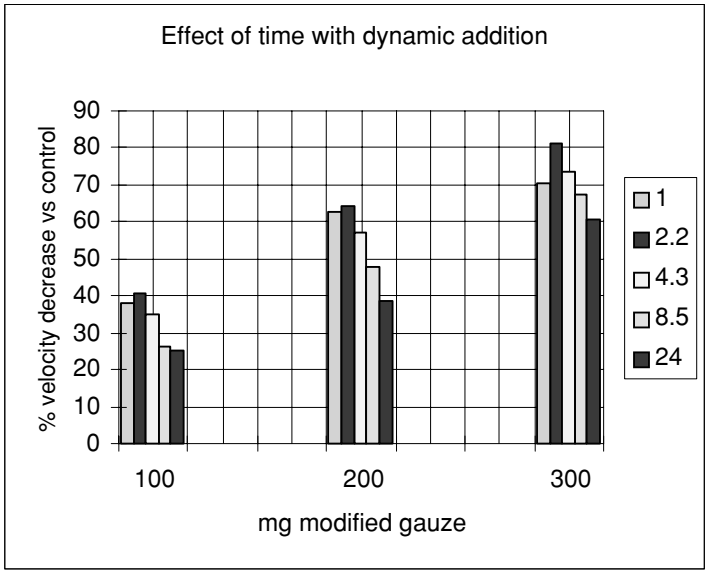

Figure 2.8. Results of a dynamic addition capacity study for a charge sequestrant dressing. The bar graph is a plot of percent decrease in elastase activity versus varying amounts of modified gauze (rep100 mg, 200 mg, and 300 mg) used over a 24-h period. Elastase levels are regenerated through out the 24-h time course to challenge the dressing material with increasing levels. A solution mimicking wound fluid was prepared consisting of 4% albumin and 2 milliunits of elastase per milligram of protein. The results show that the dressing continues to remove elastase after 24 h in the presence of protein levels found in the chronic wound

require care that is estimated at $5–7 billion per year and increasing at an annual rate of 10% [31]. Research and development is currently underway to achieve more ideal wound dressings. As shown in the cartoon in Figure 2.9, the chronic wound dressing of the future will probably have structural features built into a single dressing. This prototype dressing would confer properties of moisture balance, protease sequestration, growth factor stimulation, "time-to-change indicator", antimicrobial activity, and oxygen permeability. Many of these properties are already present in current wound dressings; however, no single wound dressing product offers all of them. The future success of wound care products from modified traditional materials or new materials depends on continued mechanism-based research at all levels from basic through clinical assessment. As new products like those included in the interactive wound dressing category continue to become available evidence regarding their relative efficacy will be needed to provide the wound care practitioner with data in making the best product selection for the patients needs.

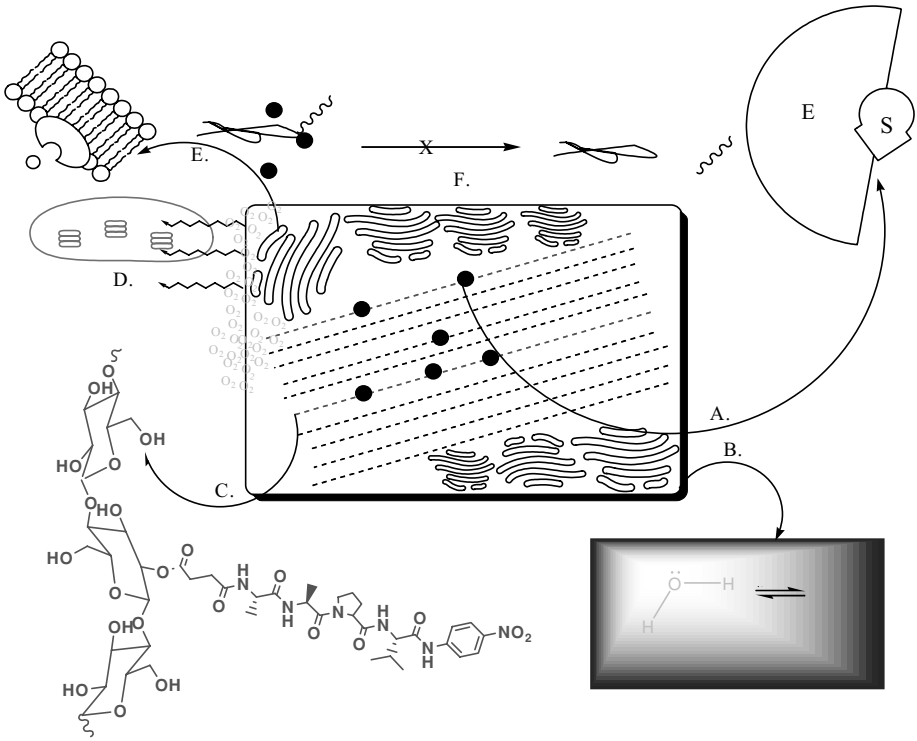

Figure 2.9. Cartoon of some of the structural properties of a wound dressing of the future. (**A**) It reduces proteases and their activity toward the degradation of growth factors (F.) by selectively binding proteases similar to the tailored fit of an enzyme–substrate complex [ES]; (**B**) It possesses absorbency that responds to exudate of the wound environment by adjusting the moist wound environment in equilibrium with optimal wound moisture for healing; (**C**) A colorimetric indicator signals the wound dressing has reached its capacity to redress biochemical imbalance in the chronic wound. A peptide-containing chromophore built into the dressing fiber might release a colorimetric signal in response to reaching its capacity to perform as a protease sequestrant. (**D**) The dressing possesses antimicrobial activity and serves as a barrier to wound contamination while remaining permeable to oxygen. (**E**) and (**F**) The dressing optimally stimulates the production of growth factors and cytokines in the wound environment while preventing their degradation. Consequently, growth factors trigger their membrane bound receptors, and proteases are blocked from degrading growth factors and their cellular receptors

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