

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
1 March 2007 (01.03.2007)

PCT

(10) International Publication Number  
**WO 2007/024972 A2**

(51) International Patent Classification:  
A61K 9/70 (2006.01)

(74) Agent: ELMAN, Gerry, J.; ELMAN TECHNOLOGY  
LAW, P.C., P.O.box 209, Swarthmore, PA 19081-0209  
(US).

(21) International Application Number:  
PCT/US2006/032953

(81) Designated States (unless otherwise indicated, for every  
kind of national protection available): AE, AG, AL, AM,  
AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,  
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI,  
GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP,  
KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT,  
LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ,  
NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU,  
SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR,  
TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(22) International Filing Date: 22 August 2006 (22.08.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/710,131 22 August 2005 (22.08.2005) US

(84) Designated States (unless otherwise indicated, for every  
kind of regional protection available): ARIPO (BW, GH,  
GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM,  
ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI,  
FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,  
RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA,  
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(71) Applicants (for all designated States except US):  
**QUICK-MED TECHNOLOGIES, INC.** [US/US]; 3427  
SW 42nd Way, Gainesville, FL 32608 (US). **UNIVER-  
SITY OF FLORIDA RESEARCH FOUNDATION,  
INC.** [US/US]; P.O.box 115500 WAL 320, Gainesville,  
FL 32611 (US).

(72) Inventors; and

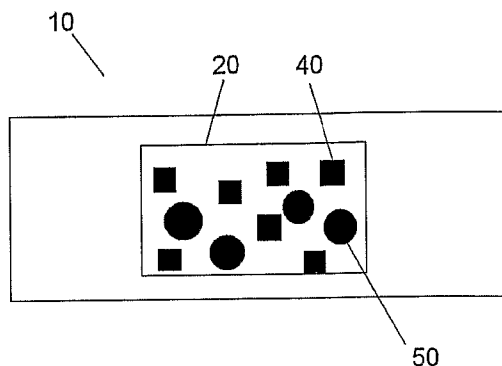
(75) Inventors/Applicants (for US only): **LIESENFELD,  
Bernd** [DE/US]; 4415 NW 70th Terrace, Gainesville, FL  
32606 (US). **TOREKI, William** [US/US]; 3427 42nd  
SW Way, Gainesville, FL 32608 (US). **MOORE, David**  
[US/US]; 3427 SW 42nd Way, Gainesville, FL 32608  
(US). **SCHULTZ, Gregory, S.** [US/US]; 832 NW 45th  
Terrace, Gainesville, FL 32605-5504 (US).

**Published:**

— without international search report and to be republished  
upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: ABSORBENT WOUND DRESSING WITH A NON-LEACHING ANTIMICROBIAL ACTIVITY AND A CON-  
TROLLED-RELEASE BIOACTIVE AGENT



(57) Abstract: An antimicrobial wound dressing (10) having a  
non-leaching antimicrobial activity (20), releasable antimicrobial and  
antiprotease agents (40), and a controlled-release bioactive agent (50),  
such as doxycycline. The wound dressing material is absorbent and  
acts as a substrate for antimicrobial and antiprotease agents, as well  
as bioactive agents. Releasable antimicrobial agents and releasable  
anti-protease agents are ionically stabilized within the device so as to be  
released from the device in a controlled manner and releasable bioactive  
agents are also attached to the substrate to assist in wound healing.

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**TITLE OF THE INVENTION**

**Absorbent wound dressing with a non-leaching antimicrobial activity and a controlled-release bioactive agent.**

**5 FIELD OF THE INVENTION**

This invention relates to wound dressings, more particularly to wound dressings having a non-leaching antimicrobial activity and providing for the controlled-release of bioactive substances.

**BACKGROUND ART**

10 Absorbents such as carboxymethyl cellulose (CMC) and alginates are commonly used in wound dressings to absorb exudate fluids and cellular debris. This absorbed wound exudate fluid is an ideal medium for microbial growth, and this microbial growth can be detrimental to wound healing in at least two ways. First, toxins produced by microorganisms growing in the wound dressing may  
15 diffuse into the wound and impair the health of eukaryotic cells. Second, microorganisms growing on the dressing may be shed into the wound and result in higher microorganism colonization, ultimately leading to infection of the wound. Even sub-clinical infections, defined as less than one million colony forming units per ml, have been shown to significantly impair or impede wound healing.

20 Some commercially available moist wound dressings endeavor to prevent the growth of microorganisms by incorporating a diffusing biocidal agent. One commonly used diffusing biocidal agent is silver. One disadvantage to this approach is that certain bacteria have been able to develop resistance to silver. (Silver S., "*Bacterial silver resistance: molecular biology and uses and misuses of silver compounds.*" FEMS Microbiology Reviews, 2003; 27:341-353). Another  
25 disadvantage to this approach is that diffusing silver may be able to enter the wound and may potentially stain the skin. An additional disadvantage of silver is the high cost of the raw material.

Several approaches to wound management rely on the release of antimicrobial agents such as silver ions. Burton et al. (US\_2005/0124724\_A1) disclose a polymer composition comprising: a hydrophilic polymer; and a bioactive agent selected from the group consisting of a metal oxide of silver, copper, zinc, and combinations thereof; wherein the bioactive agent is dispersed within the hydrophilic polymer; and wherein substantially all of the bioactive agent has a particle size less than one micron. Cooper et al. (US\_2005/0008840\_A1) disclose an antimicrobial material and a method of inhibiting infection during the treatment of a wound by applying an antibacterial material to an affected skin area on a patient, said antibacterial material including: a fibrous substrate impregnated with a carrier and a controlled release biocide, e.g. triclosan, dispersed through the carrier. The substrate is desirably a substantially cellulose fiber blend, and the biocide is desirably non-allergenic to humans, and inhibits the growth of *E. coli*, *Legionella* or *Staphylococcus*. Munro et al. (WO\_04/028255\_A1) disclose an antimicrobial composition comprising (a) an antimicrobially effective amount of a dissolved antimicrobial metal ion and (b) a dissolved halide ion. Bowler et al. (US 2004/0001880\_A1) disclose the use of an effective amount of silver in the manufacture of a wound dressing comprising an anionic, amphoteric or hydrophilic polymer, which dressing, when applied to a wound site, gives a controlled release of ionic silver into the wound fluid for the prevention of staining of the underlying tissue. Gibbins et al. (U.S. Pat. 6897349) disclose a hydrophilic antimicrobial fiber comprising a hydrophilic polymer, such as carboxymethyl cellulose, of a wound dressing for external application to a wound, wherein the hydrophilic polymer of the wound dressing contains a silver salt compound.

There are also numerous examples which provide for wound dressings with releasable bioactive agents (other than antimicrobials). Yamazaki et al. (U.S. Pat. 5098417) disclose a wound dressing for systemic administration of a physiologically- or biologically-active agent by controlled release of the agent into such wound, the wound dressing comprising a substrate in the form of a fabric or cloth, at least a

portion of which is cellulosic, which has been chemically modified to convert hydroxyl groups in said cellulosic portion to ionic-adsorbing sites; an ionic form of a physiologically- or biologically-active agent adsorbed in said substrate, namely an antibacterial agent, an antifungal agent, an analgesic agent, a tissue healant agent, a local anesthetic agent, an antibleeding agent, an enzyme or a vasoconstrictor. The patent teaches that ionic bonds hold the agent temporarily to the substrate for controlled release therefrom in proportion to the amount of exudate in contact with the substrate. The ionic bonds are formed by adsorbing the agent on the substrate at room temperature, the ionic bonds disassociating upon contact with body exudate from wounds to which the wound dressing is applied by ion exchange with ions in the body exudate, thereby to release the physiologically- or biologically-active agent in an amount in proportion to the amount of the exudate in contact with the substrate.

Silcock et al. (WO 04/080500 A1) disclose a wound dressing material comprising a low-moisture hydrogel matrix having oxidized cellulose distributed therein, and wherein the hydrogel matrix comprises a hydrogel selected from the group consisting of modified celluloses, modified starches, alginates, plant gums, gelatins, glycosaminoglycans, polyacrylates, polyurethanes, and mixtures thereof, and wherein the hydrogel is selected from the group consisting carboxymethyl cellulose salts, alginate salts, gelatins, hyaluronic acid and its salts, xanthan gum, guar gum, and mixtures thereof, and wherein said wound dressing material further comprises one or more therapeutic agents. Bootman et al. (EP 1120112 A2) disclose a wound dressing and drug-delivery device for controlled-release of a bioactive agent comprising a cross-linked biopolymer and a bioactive agent reversibly bound thereto, and wherein said biopolymer comprises collagen or gelatin, and wherein said bioactive agent comprises silver ions, and wherein said bioactive agent comprises a peptide and/or protein, growth factor, or immune-modulating factor. Soerens et al. (WO 04/011046 A1) disclose an absorbent binder composition comprising a water-soluble ionic polymer capable of sufficient non-radiative crosslinking within about

10 minutes at a temperature of about 120°C or less, to reach an absorbent capacity of at least one gram per gram, and wherein the ionic polymer comprises a carboxyl group-containing monomer, and wherein the ionic polymer comprises a quaternary ammonium group-containing monomer, and wherein the binder is applied as a coating to a substrate, and wherein the substrate comprises a person's skin, an article of clothing, a wound dressing, and wherein the absorbent binder coating further comprises at least one of a fragrance additive, odor-absorbing particles, an antimicrobial additive, a wound healing agent, or a fungicide. Berthold et al. (U.S. Pat. 6399091) disclose a wound dressing having a layered structure for the controlled release of active substance to wounds comprising two polymer-containing layers each comprising a hydrocolloid-containing swellable hydrogel as an absorbent; two woven layers; and at least one active substance in at least one of the layers, the polymer-containing layers and the woven layers being superposed in alternating sequence, and wherein the polymer is a cellulose, such as carboxymethyl cellulose, and wherein the active substance is a biologically active peptide, protein, or growth factor, and wherein the woven layer is based on polyester, polyurethane or cellulose.

Other approaches combining a non-leaching antimicrobial activity with a releasable bioactive agent include the following. Toreki et al. (US 2005/0033251 A1) disclose a material comprising a substrate and an enhanced surface area, the enhanced surface area comprising a multitude of non-hydrolyzable, non-leachable polymer chains covalently bonded by non-siloxane bonds to said substrate; wherein said non-hydrolyzable, non-leachable polymer chains comprise a multitude of antimicrobial groups attached to said non-hydrolyzable, non-leachable polymer chains by covalent bonds; and wherein a sufficient number of said non-hydrolyzable, non-leachable polymer chains are covalently bonded to sites of said substrate to render the material antimicrobial when exposed to aqueous fluids, menses, bodily fluids, skin, cosmetic compositions, or wound exudates, wherein said material has associated therewith a plurality of anionically charged biologically or chemically

active compounds, and wherein said antimicrobial groups comprise at least one quaternary ammonium structure. The disclosed substrate is comprised, in whole or in part, of cellulose, or other naturally-derived polymers or comprises all or part of a wound dressing.

5 Proteases are enzymes that have catalytic activity (that is typically highly specific) against certain proteins. Proteases help mediate the degradation of protein based structures, such as tissue structures the body needs to remodel, as occurs in wound healing, the blood clotting cascade, and in apoptosis pathways. In the case of chronic wounds the protease concentration at the wound site is out of balance,  
10 leading to retardation or complete arrest of wound healing because the rate of tissue degradation (protease mediated) overwhelms the rate of tissue formation. Protease inhibitors have been applied with significant success to aid the healing and closure of chronic wounds. Protease inhibitors bind proteases and therefore prevent their action. A simpler approach to protease control that is used due to simplicity is to  
15 provide a sacrificial substrate such as the mixture of collagen and oxidized regenerated cellulose of Johnson and Johnson's Promogran™.

There exists a need for an absorbent material that can resist colonization by microorganisms and can additionally provide for the controlled-release of a bioactive substance.

20

## DISCLOSURE OF THE INVENTION

The current invention provides a device for the treatment of wounds which is comprised of an absorbent wound dressing material having inherent non-leachable antimicrobial and inherent non-leachable anti-protease activity or agent  
25 incorporated therein. In addition, the device also contains releasable antimicrobial and releasable anti-protease agents that are ionically-stabilized within the wound dressing, and may be released from the device in a controlled manner. In addition, the device also contains releasable bioactive agents which aid in wound healing, such as growth factors, vitamins, and/or nutrients.

"Microbe" or "microorganism" refers to any organism or combination of organisms such as bacteria, viruses, protozoa, yeasts, fungi, molds, or spores formed by any of these.

5 "Antimicrobial" refers to the microbicidal or microbistatic properties of a compound, composition, article, or material that enables it to kill, destroy, inactivate, or neutralize a microorganism; or to prevent or reduce the growth, ability to survive, or propagation of a microorganism.

10 By "polymeric quaternary ammonium" is meant a polymer wherein multiple quaternary ammonium moieties are covalently bonded to the polymer molecule, or attached to the molecular structure by covalent chemical bonds and form part of the polymer molecular structure, and that said multiple quaternary ammonium moieties are located in the main-chain of the polymer or in side-groups of the polymer. The terms "main-chain" and "side-groups" are commonly used to describe polymer molecular structure and are familiar to one skilled in the art. Sufficient  
15 negative counter-ion such as chloride is typically present to balance the positive charge of the quaternary ammonium moieties.

"Polyquaternium" refers to a polymeric quaternary ammonium substance as the term is used in the International Nomenclature of Cosmetic Ingredients (INCI).

20 By "hydrogel" is meant a gel structure comprised of a crosslinked network structure of a polymer, such as a polymeric quaternary ammonium molecule and water, or a gel formed by a polyelectrolyte complex or network upon absorption of water.

"Incorporated" means bonded to a substrate in a non-leachable manner.

25 "Inherent non-leachable" means that the agents responsible for the particular effect (such as antimicrobial, anti-protease, or bioactive agents) are immobilized within the wound dressing, and are not eluted or leached when exposed to aqueous fluids, menses, bodily fluids, skin, cosmetic compositions, or wound exudates.

"Releasable" means that the agents responsible for the particular effect (such as antimicrobial, anti-protease, or bioactive agents) may be eluted, leached, or

migrate from the wound dressing when exposed to aqueous fluids, menses, bodily fluids, skin, cosmetic compositions, or wound exudates.

“Ionically-stabilized” means that the agents (such as antimicrobial, anti-protease or bioactive agents) show a propensity to remain within the wound dressing as a result of ionic interactions between a component of the wound dressing and the agent, resulting in the retention of the agent within the dressing to a greater extent than would otherwise be expected. Ionic stabilization manifests as decreased solubility or a decreased diffusion rate for the stabilized agent.

“Controlled manner” means that the release of the ionically-stabilized agents (such as antimicrobial, anti-protease or bioactive agents) are released from the wound dressing in a manner that may be controlled or adjusted via appropriate modifications to the composition of the dressing or use conditions, or that the release rate of the ionically-stabilized agents is reduced compared to non stabilized agents.

A “cellulose-based material” means a natural material made in whole or in part of cellulose or a synthetic material derived from cellulose or having chemical and physical properties similar to cellulose.

“Substantially carboxymethylated cellulosic substrate” means a cellulose-based material that has been derivitized to contain carboxymethyl groups at a degree of substitution greater than 0.25. “Degree of substitution” refers to the number of carboxymethyl groups per anhydroglucose unit”.

It is an advantage of the invention that the wound dressing is capable of absorbing at least 10 times, preferably 20 times, and more preferably greater than 20 times its dry weight of blood, wound exudates, bodily fluid, or 1% saline solution.

It is an aspect of this invention that the inherent non-leachable antimicrobial and/or antiprotease activity is provided by quaternary ammonium moieties which are non-leachably bonded to the wound dressing via covalent chemical bonds.

It is an aspect of this invention that the inherent non-leachable antimicrobial and/or antiprotease activity is provided by polymeric quaternary ammonium

molecules which are non-leachably bonded to the wound dressing via covalent chemical bonds.

It is an aspect of this invention that the inherent non-leachable antimicrobial and/or anti-protease activity is provided by polymeric quaternary ammonium molecules, each of which is non-leachably bonded to the wound dressing via a multiplicity of ionic bonds.

It is an aspect of this invention that said polymeric quaternary ammonium molecules form a crosslinked network structure that is capable of forming a hydrogel (a gel structure comprised of said crosslinked network structure and water).

It is an aspect of this invention that said crosslinked network structure is formed via chemical crosslinking of polymeric quaternary ammonium molecules.

It is an aspect of this invention that said crosslinked network structure comprises a polyelectrolyte complex formed between polymeric quaternary ammonium molecules and polymeric anionic molecules.

It is an aspect of this invention that said polymeric anionic molecules comprise carboxymethyl cellulose (CMC), alginate, or polyacrylate.

It is an aspect of this invention that said polymeric quaternary ammonium molecules are covalently bonded to a substrate.

It is an aspect of this invention that said substrate is comprised of cellulose or a cellulose derivative (such as cotton, rayon, carboxymethyl cellulose (CMC), hydroxyethyl cellulose, paper, or woodpulp); a polysaccharide (such as dextran, chitosan, alginate, or starch); a fabric or textile; gauze; fibers; a synthetic polymer; a superabsorbent material; or a protein such as collagen.

It is an aspect of this invention that said polymeric quaternary ammonium molecules comprise poly(dimethyldiallylammonium chloride)- also known as polyDADMAC; quaternary ammonium derivatives of poly(acrylic or methacrylic) acid; poly(vinylbenzyl)trimethylammonium chloride; or compounds generally known as polyquaternium.

It is an aspect of this invention that said inherent non-leachable anti-protease activity is provided by a polymer with a multitude of anionic sites such as carboxymethyl cellulose (CMC), alginate, collagen or polyacrylate.

It is an aspect of this invention that said releasable antimicrobial agent is at least one selected from the group consisting of antibiotics, tetracycline, doxycycline, minocycline and poly(DADMAC).

It is an aspect of this invention that said releasable antiprotease agent is at least one selected from the group consisting of doxycycline, minocycline, tetracyclines, collagen, CMC and poly(DADMAC).

It is an aspect of this invention that said releasable or inherent (non leachable) antimicrobial agent and said releasable or inherent non-leachable anti-protease agent is one and the same, and is at least one selected from the group consisting of CMC, polyDADMAC and doxycycline.

It is an aspect of this invention that said releasable antimicrobial or anti-protease agent exhibits an ionic interaction with said polymeric quaternary ammonium molecules, or with said polymer with a multitude of anionic sites, resulting in ionic stabilization of said agent.

It is an aspect of this invention that said releasable bioactive agents are at least one selected from the group consisting of antibiotics, tetracycline, doxycycline, minocycline, growth factors, epidermal growth factor (EGF), platelet derived growth factor (PDGF), or vascular endothelial growth factor (VEGF), vitamins, nutritive factors, matrix metalloproteinase inhibitors (MMPIs), ilomastat, and steroids.

It is an aspect of the invention that it is an absorbent antimicrobial material comprising a substantially carboxymethylated cellulosic substrate and a plurality of polymeric diallyldimethylammonium chloride molecules non-leachably attached to said substrate, wherein a sufficient amount of said polymeric molecules are attached to said substrate to form a polyelectrolyte network, and wherein said polyelectrolyte network permits the degree of swelling of said material to range from about 10 times up to about 20 times of the dry material, and wherein said

polyelectrolyte network diminishes the dissolution of the material upon exposure to aqueous fluids, and wherein said polyelectrolyte network permits the incorporation and release of a bioactive agent in a controlled manner.

It is an aspect of the invention that said polymeric quaternary ammonium molecules impart antimicrobial activity to said material before, during, and after exposure of said material to skin, aqueous biological fluids, bodily fluids, sweat, tears, mucus, urine, menses, blood, or wound exudates.

It is an advantage of the invention that said wound dressing when applied to a mammal reduces the potential for development of infection, inflammation, and malodor.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Diagrammatic representation of a wound dressing with non-leachable and leachable agents attached.

Figure 2: Graph illustrating the controlled release of doxycycline by its ability to inhibit bacterial growth following repeated daily inoculation with *E. coli* and *S. aureus*.

Figure 3: Graph of absorbance readings from azocoll assays of polymer treated CMC material, untreated base material, doxycycline loaded treated CMC material, and a commercial dressing.

Figure 4: Graph of transmittance values from azocoll assays of doxycycline containing superabsorbent polymer (NIMBUS<sup>TM</sup>-SAP doxy), unloaded superabsorbent polymer (NIMBUS<sup>TM</sup>-SAP), untreated Sof-Wick rayon dressing (Sof-Wick), and no substrate (control).

Figure 5: Graph of cumulative and time point concentration of Epidermal Growth Factor released from NIMBUS<sup>TM</sup>-SAP matrix over time.

Figure 6: Graph of cumulative and time point concentration of Vitamin C released from NIMBUS<sup>TM</sup>-SAP matrix over time.

Figure 7: Graph of cumulative and time point concentration of Trolox (water soluble Vitamin E analog) released from NIMBUS<sup>TM</sup>-SAP matrix over time.

## DETAILED DESCRIPTION

5           Figure 1 is a diagrammatic representation of various components present in an embodiment of a wound dressing in accordance with the present invention.

Wound dressing 10 is made of cellulosic material which acts as a substrate for non-leachable agents 20, releasable agents 40, and releasable bioactive agents 50. The non-leachable agents 20 comprise one or more kinds of molecule that impart  
10 antimicrobial and anti-protease activity into wound dressing 10. Releasable agents 40 and releasable bioactive agents 50 are ionically stabilized within cellulosic material 20, so as to be released from the device in a controlled manner. Releasable agents 40 are comprised of one or more kinds of molecule that impart antimicrobial or anti-protease activity. Releasable bioactive agents 50 aid in wound healing.

15           Releasable agents are ionically stabilized due to the nature of the polyionic substrates prepared according to various aspects or modifications of this invention. Thus, with respect to wound dressings, a wide variety of antibiotics, proteins, peptides, matrix metalloproteinase inhibitors, analgesics, anti-inflammatory compounds, and the like exhibit net anionic charge at physiological pH, or pH's  
20 encountered at a wound site. By contacting these anions with polyquaternary amine functionalized substrates, prepared according to the methods of the present disclosure, the association of these anionic compounds with the substrate is stabilized. Through mass action, displacement of ions and similar mechanisms, the anions associated with the polycationic substrate of this invention are released over  
25 time, to exhibit desirable biological effects over a more extended period than would be the case if the biologically active compound were merely absorbed or adsorbed in, on, or to a substantially ionically neutral substrate.

It is an aspect of one exemplary embodiment of the invention that the attachment of polymeric cationic molecules, such as polyquaternary ammonium

compounds, to a cellulosic substrate imparts to that substrate an antimicrobial activity effective against a broad range of microorganisms. As used herein, "antimicrobial" refers to the microbicidal or microbistatic properties of a compound, composition, article, or material that enables it to kill, destroy, inactivate, or neutralize a microorganism; or to prevent or reduce the growth, ability to survive, or propagation of a microorganism. As used herein, "microbe" or "microorganism" refers to any organism or combination of organisms able to cause infection, such as bacteria, viruses, protozoa, yeasts, or molds.

A polyelectrolyte network is an arrangement of ionically-charged polymer chains (polyelectrolyte molecules) linked, bonded, or bridged together to form a three dimensional structure. This bonding or bridging of different chains is referred to as "crosslinking". If the crosslinked molecules are attached to each other by covalent chemical bonds, then the network actually consists of a single molecule. This is what is meant by the term "chemical crosslinking." The crosslinking may also be achieved by ionic interactions between the chains. For instance, a polymer molecule with multiple cationic sites (a polycation, or cationic polyelectrolyte) may easily dissolve in water or aqueous solutions. The same may be true for a polymer molecule with multiple anionic sites (a polyanion, or anionic polyelectrolyte). The cationic and anionic polymer molecules may interact or associate with each other due to electrostatic attraction of the oppositely charged sites. One cationic molecule may interact with two or more anionic polymer chains, just as an individual anionic molecule may interact with multiple cationic chains. These ionic attractions are called "complexation". The result is the formation of a three dimensional polyelectrolyte complex network, which is no longer soluble in water or aqueous solutions. Although the ionic crosslinking bonds are not generally as strong or permanent as covalent crosslinking bonds, the sum of many such interactions may give substantially the same effect as covalent crosslinking. The polyelectrolyte complex network or polyelectrolyte network will generally not be soluble in water; however, it is likely to have a great propensity to absorb fluid and swell to form a

gel (or hydrogel). An example of a polyelectrolyte network is a hydrogel comprised of covalently crosslinked polyDADMAC chains. The consistency and absorbent capacity of the hydrogel is determined by the extent of covalent crosslinking. An example of a polyelectrolyte complex network is that which is formed by linear (non-crosslinked) polyDADMAC and CMC. The CMC may be linear, branched, or even already a crosslinked polyelectrolyte network. The consistency and absorbent capacity of this hydrogel will depend on the molecular weights and ratio of the two oppositely charged polyelectrolytes. In the case where the CMC is already crosslinked to some extent, addition of polyDADMAC will tend to make the CMC hydrogel less absorbent and less soluble.

It is an aspect of this invention that a covalently crosslinked polycationic network, either alone, or attached to a substrate is used as a component of a wound dressing. This component provides inherent non-leachable antimicrobial and inherent non-leachable anti-protease activity, which is inherent to the polycationic material used. Furthermore, the multitude of cationic sites provided by the polycationic network provides reactive sites for the ionic association and stabilization of releasable antimicrobial, anti-protease, and bioactive agents, which can be released from the wound dressing in a controlled manner. The covalently crosslinked polycationic network is capable of absorbing water or aqueous fluids, resulting in the formation of a hydrogel. In a preferred embodiment, the polycationic network is comprised of poly(DADMAC).

It is an aspect of this invention that a polyelectrolyte complex network formed between a polymeric cationic molecule and a polymeric anionic molecule is used as a component of a wound dressing. This component provides inherent non-leachable antimicrobial and inherent non-leachable anti-protease activity, which is an inherent property of one or both anionic or cationic) polymeric molecules. Furthermore, the multitude of cationic and/or anionic sites provided by the polyelectrolyte complex network provides reactive sites for the ionic association and stabilization of releasable antimicrobial, anti-protease, and bioactive agents, which

can be released from the wound dressing in a controlled manner. The polyelectrolyte complex network is capable of absorbing water or aqueous fluids, resulting in the formation of a hydrogel. In a preferred embodiment, the polyelectrolyte complex network is comprised of poly(DADMAC) and CMC.

5 It is an aspect of one exemplary embodiment of the invention that after attachment of the polymeric cationic or anionic molecules to the substrate, there is a fraction of unbound cationic and/or anionic polymeric molecules remaining within, but not attached to, the treated material. It is an aspect of one exemplary embodiment of the invention that the treated material can be repeatedly rinsed  
10 with fluid until substantially all unbound cationic/and or anionic polymer is removed. Alternatively, it is an aspect of one exemplary embodiment of the invention that the unbound fraction can be retained in the treated material to provide additional, leachable, antimicrobial or anti-protease activity. Whether the unbound fraction is rinsed away or retained in the material, the attached fraction of  
15 polymeric cationic molecules will serve to form the polyelectrolyte network.

It is an aspect of the invention that the attachment of polymeric cationic molecules to cellulosic substrates forms a polyelectrolyte network with the substrate, and thereby reduces the propensity of the substrate to dissolve when it contacts aqueous fluids. For purposes of illustration of this aspect of the invention, the  
20 dissolution of an untreated carboxymethylcellulose (CMC)-based material (e.g. Aquacel®) is relatively fast, with visible dissolution in water occurring within 10-20 minutes under constant agitation and with a significant or complete loss of structure occurring within 4 hours. In contrast, the CMC-based polyelectrolyte complex network material of the current invention does not dissolve in water, or in  
25 salt solutions, but maintains its fluid-swollen form for an extended period of time, thus making it a more useful material for wound dressing applications. As used herein, cellulosic means a natural or synthetic material made up, in whole or in part, of cellulose or a cellulose derivative. As used herein, polyelectrolyte network means a network of individual polymeric molecules that interact at nodes of contact

formed by charges that coordinate to each other and thereby act as cross-linking points, with the effect of forming smaller and smaller pore size equivalents within the network as the density of the nodes of contact is increased.

It is an aspect of the invention that the attachment of polymeric cationic molecules to anionic cellulosic substrates alters the degree of swelling of the substrate material. The degree of swelling correlates with the amount of polymeric cationic molecules attached and, therefore, the degree of swelling of a treated material can be controlled by varying the amount of polymeric cationic molecules attached to a substrate. The degree of swelling is particularly important for a wound dressing, which must readily absorb wound exudates. Although the degree of swelling of an untreated CMC substrate is difficult to define, because dissolution of untreated CMC substrate begins to occur as soon as it comes into contact with aqueous fluids, in one exemplary embodiment of the current invention, the degree of swelling of a treated substrate can be tailored to be about 10 times to about 20 times its pre-swollen weight.

It is an aspect of the invention that the amount of polymeric cationic molecules attached to the substrate to establish the polyelectrolyte network preferably ranges from about 1% to about 50% of the pre-treated substrate's dry weight; however, the invention is not necessarily limited to this range, as a polyelectrolyte network could be feasibly established with an amount of polymeric cationic molecules that is less than 1% or more than 50% of the pre-treated substrate's dry weight.

It is an aspect of the invention that the controlled-release aspect of the invention is due, at least in part, to at least two factors, each of which can be varied to affect the controlled-release aspect. The first factor is that the density of the polyelectrolyte network structure impedes the transit of agents from the material. The density of the polyelectrolyte network structure can be varied, for example, by varying the amount of cationic polymeric molecules attached to the substrate. The second factor is that a non-uniform charge distribution exists in the polyelectrolyte

network due to the attached charge-dense cationic polymeric molecules and this charge-based binding impedes the transit of agents from the material.

It is an aspect of one exemplary embodiment of the invention that when the treated substrate is to be used as a wound dressing material, the material can be formulated to possess protease inhibiting activity in at least two ways. First, CMC material treated with an immobilized stoichiometric excess of polyDADMAC itself has protease binding activity owing to the high density of cationic charge. This means that the material will act in the manner of an anion exchange column. The positively charged surface would act to bind proteases that are predominantly negatively charged at physiological pH of approximately 7. Second, the protease binding activity of the treated CMC material can be augmented by the inclusion of certain controlled-release bioactive agents, such as, but not limited to, the antibiotic doxycycline, which is itself a matrix metalloprotease inhibitor (MMPI). Each of these two properties acting alone, or in concert, can reduce the overall protease activity occurring at the wound site and thereby assist in wound healing.

In one exemplary embodiment of the invention, the substrate is a hydrofiber-based CMC. A hydrofiber-based CMC is a hydrophilic fibrous material that is prepared from cellulosic fibers that are treated with chloroacetic acid in the presence of base in an alcohol solvent, which serves to prevent its gelation. An example of such a hydrofiber-based CMC substrate is ConvaTec® Aquacel®. In other exemplary embodiments of the invention, the substrate is CMC of other forms, such as pre-made gels or powdered CMC. In other exemplary embodiments of the invention, the substrate is a fibrous cellulosic substrate that has first been treated so as to carboxylate the surface, using common methods that are described among other places in U.S. Pat. 6,627,785 Edwards et al.

In one exemplary embodiment, a solution of polymerized diallyldimethylammonium chloride is applied to a dry hydrofiber substrate. The substrate material may then be dried, preferably in an oven at, for example, 50°-80°C for about two hours, but more particularly, is dried until the substrate

material has been thoroughly dried to a constant weight. Drying at elevated temperatures for a sufficient amount of time for the treated material to reach a constant weight is the preferred way to attach the cationic polymeric molecules to the surface of the substrate, but any method which allows for drying to a constant weight is also appropriate and sufficient, for example, in a vacuum desiccator or a lyophilizer. Drying is a convenient way to fabricate a useful product; however, drying is not essential to the formation of a polyelectrolyte complex network, which may spontaneously form after admixture of the two oppositely charged polyelectrolytes.

It is an aspect of the invention that the cationic polymeric molecules attached to the substrate to form the polyelectrolyte network can be any cationic polymer having a sufficient charge density. It is an aspect of one exemplary embodiment of the invention, that the attached cationic polymer is a polymeric quaternary ammonium compound, which provides additional advantages to the material. First, it provides antimicrobial activity on and within the treated material, a useful property for a material used as a wound dressing. Second, it provides at least some protease binding activity on and within the treated material, a useful property for a material used as a wound dressing.

It is an aspect of the invention, that one, or more than one, controlled-release bioactive agent can be incorporated into the material at the same time that the cationic polymeric molecule is applied to the substrate, or may, alternatively, be applied at any earlier or later time. It is an aspect of the invention that a diverse group of controlled-release bioactive agents can be incorporated into the treated material, including, but not limited to antibiotics (e.g. tetracyclines, including doxycycline), growth factors (e.g. epidermal growth factor (EGF) and platelet derived growth factor (PDGF)), vitamins, nutritive factors, steroids and any charged substance of interest, or MMPs (e.g. Ilomastat).

In light of the general disclosure provided herein above, with respect to the manner of practicing this invention, those skilled in the art will appreciate that this

disclosure enables the practice of the invention as defined in the attached claims. The following experimental details are provided to ensure a complete written description of this invention, including the best mode thereof. However, it will be appreciated that the scope of this invention should not be construed in terms of the specific examples provided. Rather, the scope of this invention is to be apprehended with reference to the claims appended hereto, in light of the complete description of this inventive method constituted by this entire disclosure.

**Example 1: Preparation of a treated CMC substrate material.**

A 4 inch square, hydrofiber CMC wound dressing (i.e. Aquacel®) weighing 1.1 grams and being approximately 24% carboxylated was thoroughly wetted with 20 mL of an aqueous solution of 0.15 wt % polymerized diallyldimethylammonium chloride (total polymer content ~3%). The wetted dressing was placed in a 60°C oven on a stainless steel screen mesh and allowed to thoroughly dry until successive weighings indicated no additional considerable loss of weight. To rinse out any unattached polymer, the dressing was submerged in distilled water in a beaker and stirred. The rinsate was poured off and replaced with distilled water repeatedly until the rinsate of a 5 minute soak had the same conductivity as input rinse water, indicating that the rinsate was free of unattached polymer.

**Example 2: Preparation of a treated CMC substrate material loaded with doxycycline.**

A 4 inch square, hydrofiber CMC wound dressing (i.e. Aquacel®) weighing 1.1 grams and being approximately 24% carboxylated was thoroughly wetted with 20 mL of an aqueous solution of 0.15 wt % polymerized diallyldimethylammonium chloride and 1 wt % doxycycline. The wetted dressing was placed in a 60°C oven on a stainless steel screen mesh and allowed to thoroughly dry until successive weighings indicated no additional considerable loss of weight. To rinse out any unattached polymer, the dressing was submerged in distilled water in a beaker and

stirred. The rinsate was poured off and replaced with distilled water repeatedly until the rinsate of a 5 minute soak had the same conductivity as input rinse water, indicating that the rinsate was free of unattached polymer.

5    **Example 3: Demonstration of the cationic charge character of a treated CMC substrate material using BTB dye.**

10    The anionic pH indicator dye bromothymol blue (BTB) was used to demonstrate the cationic charge character of the CMC substrate material prepared as in Example 1. The treated CMC substrate material was placed into a beaker, saturated with 0.5 wt % BTB dye solution, and allowed to fully absorb the dye for about 5 minutes. The treated CMC substrate material was then rinsed repeatedly with water, until the rinsate no longer visibly contained any BTB dye. After the final rinse, the treated substrate appeared an even, medium to dark blue color. Note that BTB dye can be rinsed from an untreated CMC sample very easily, but  
15    that this control is complicated by the relatively rapid dissolution of untreated CMC material in aqueous fluids.

**Example 4: Microbiological assay to verify the antimicrobial aspect of treated CMC substrate material.**

20    To assay antimicrobial activity, untreated CMC substrate material (control) and CMC substrate materials prepared as in Example 1 (samples) were tested for antimicrobial efficacy by inoculation with 1 mL of a  $10^6$  CFU/mL culture of the test bacteria followed by incubation at 37°C overnight. Samples and controls were then homogenized in phosphate buffered saline and dilutions of the homogenate were  
25    used to inoculate bacterial culture plates. The plates were incubated at 37°C overnight and the colonies were enumerated. Treated sample demonstrated a 3-4 log reduction in the number of bacterial colonies as compared with untreated control.

**Example 5: Microbiological assay by log-reduction method to verify the antimicrobial aspect of treated CMC substrate material loaded with doxycycline.**

5           To assay antimicrobial activity, untreated CMC substrate material (control) and CMC substrate materials prepared as in Example 2 (samples) were tested for antimicrobial efficacy per AATCC method 100-1999: "Antibacterial Finishes on Textile Materials, Assessment of". The materials were inoculated with 500 µl of a  $10^6$  CFU/mL culture of the test bacteria listed in **Table E1** below and incubated at  
10   37°C overnight. The swatches were recovered into transfer solution (with added neutralizing agent), then serially diluted and plated for log reduction quantification as per method instructions.

**Table E1: Testing of treated CMC materials for microbicidal efficacy**

| <u>Sample</u>                                  | <u>Comment</u>                 | <u>Organism</u>                                          |                  |                    |
|------------------------------------------------|--------------------------------|----------------------------------------------------------|------------------|--------------------|
| Overnight Culture                              | ATCC # 6538                    | SA: <i>Staphylococcus aureus</i>                         |                  |                    |
| Overnight Culture                              | ATCC # 15597                   | EC: <i>Escherichia coli</i>                              |                  |                    |
| Overnight Culture                              | ATCC # 13880                   | SM: <i>Serratia marescens</i>                            |                  |                    |
| Overnight Culture                              | ATCC # 15442                   | PA: <i>Pseudomonas aeruginosa</i>                        |                  |                    |
| Overnight Culture                              | ATCC # BAA-44                  | MRSA: <i>methycillin resistant Staphylococcus aureus</i> |                  |                    |
|                                                |                                |                                                          | <u>Log red'n</u> | <u>Full Kill *</u> |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SA                                                       | 6.70             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SA                                                       | 6.70             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SA                                                       | 6.70             | *                  |
| Negative control                               | Untreated Aquacel CMC dressing | SA                                                       |                  |                    |
| Negative control                               | Untreated Aquacel CMC dressing | SA                                                       | 5.00E+06         |                    |
| Negative control                               | Untreated Aquacel CMC dressing | SA                                                       |                  |                    |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | EC                                                       | 6.70             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | EC                                                       | 6.70             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | EC                                                       | 6.70             | *                  |
| Negative control                               | Untreated Aquacel CMC dressing | EC                                                       |                  |                    |
| Negative control                               | Untreated Aquacel CMC dressing | EC                                                       | 5.00E+06         |                    |
| Negative control                               | Untreated Aquacel CMC dressing | EC                                                       |                  |                    |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SM                                                       | 7.59             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SM                                                       | 7.59             | *                  |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | SM                                                       | 7.59             | *                  |
| Negative control                               | Untreated Aquacel CMC dressing | SM                                                       |                  |                    |
| Negative control                               | Untreated Aquacel CMC dressing | SM                                                       | 3.86E+07         |                    |

|                                                |                                |      |          |   |
|------------------------------------------------|--------------------------------|------|----------|---|
| Negative control                               | Untreated Aquacel CMC dressing | SM   |          |   |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | PA   | 7.63     | * |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | PA   | 7.63     | * |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | PA   | 7.63     | * |
| Negative control                               | Untreated Aquacel CMC dressing | PA   |          |   |
| Negative control                               | Untreated Aquacel CMC dressing | PA   | 4.26E+07 |   |
| Negative control                               | Untreated Aquacel CMC dressing | PA   |          |   |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | MRSA | 6.18     | * |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | MRSA | 6.18     | * |
| 3.0%wt polymer, 20%wt Doxycycline CMC dressing |                                | MRSA | 6.18     | * |
| Negative control: prewet CMC                   |                                | MRSA |          |   |
| Negative control: prewet CMC                   |                                | MRSA | 1.50E+06 |   |
| Negative control: prewet CMC                   |                                | MRSA |          |   |

**Example 6: Microbiological assay by direct inoculation to verify the antimicrobial aspect of untreated and treated CMC substrate material with incorporated controlled-release doxycycline.**

- 5 A treated sample was prepared with doxycycline as described in Example 2. The treated CMC material (sample) that had been concurrently loaded with doxycycline was compared to an Aquacel® dressing (control) that had been loaded with an equal amount (20 ml of 1 w/v % solution) of doxycycline. Sample and control were rinsed with distilled water, and then cut into 15 x 15 mm squares.
- 10 These squares were placed onto agar plates and each inoculated with 500 µl of 10<sup>6</sup> CFU/ml *E. coli* or *S. aureus*. The inoculum was placed in the center of the samples, and the samples were incubated at 37°C for 24 hours. After 24 hours the plates were examined for growth and graded for efficacy level in suppressing bacteria growth on a scale of 1 (high) through 4 (none). This procedure was repeated daily
- 15 for the periods indicated. The results are shown in **Figure 2**, which is a graph of

the efficacy level grading over several days. **Figure 2** illustrates the controlled release of doxycycline by its ability to inhibit bacterial growth following repeated daily inoculation with *E. coli* (indicated by diamond and square points) and *S. aureus* (indicated by triangle and cross points). Note that in each case the polyDADMAC (PD) treated material (indicated by square and cross points) provided a longer sustained release of doxycycline at microbicidal levels.

**Example 7: Microbiological assay to verify the antimicrobial aspect of treated CMC substrate material loaded with doxycycline: time-kill assay per ASTM E-2315 method using *pseudomonas aeruginosa*.**

Materials prepared as per Example 2 were tested for time to kill as per ASTM E-2315, using *pseudomonas aeruginosa* (ATCC # 15442). The neutralizing agent utilized was a Lethen broth. Results are provided in **Table E2** below, showing that within 2 hours, >99.99 % kill was achieved.

**Table E2.** Time kill of *pseudomonas aeruginosa* (ATCC # 15442) by doxycycline loaded treated CMC materials, as per ASTM E-2315

| <u>Time</u> | <u>Average log reduction</u> | <u>% kill</u> |
|-------------|------------------------------|---------------|
| 1 min       | 0.24                         | 42.7 %        |
| 10 min      | 0.72                         | 80.1 %        |
| 60 min      | 2.82                         | 99.85 %       |
| 120 min     | 4.49                         | 99.997%       |

**Example 8: Protease inhibition by treated CMC material with and without loaded doxycycline.**

Protease inhibition testing per azocoll assay was performed on several samples and controls. A treated CMC material was prepared as in Example 1, (but treated with a 1 % polymer solution to make the total polymer content 20% rather

than 3%). Treated CMC with doxycycline was prepared as in example 2 (but loaded with a total of 10 % polymer and 20 % doxycycline). These materials were compared to untreated CMC material (Aquacel<sup>®</sup>), and to commercially available oxidized regenerated cellulose matrix wound dressing (Johnson and Johnson's Promogran<sup>™</sup>).

5 The materials were evaluated using an azocoll assay and bacterially derived clostridial collagenase. The azocoll assay is familiar to those skilled in the field, and is used to measure collagenase activity. Briefly, insoluble bovine hide (collagen) has dye molecules covalently attached. As the collagen is dissolved by collagenase, dye molecules are released into solution, permitting assessment of collagenase activity  
10 by the amount of dye released into solution – with higher absorption measurements indicating more collagenase activity. Lower absorption values indicate that collagenase activity is suppressed: this provides a good measure of protease inhibition. A 20 mg strip of each material was placed into a 1.5 ml microcentrifuge tube with filter insert (0.2 µm filter), and treated with 200 µl of collagenase solution  
15 (concentration of  $10^{-5}$ ), then incubated for 30 min. Following incubation, the samples were centrifuged to extract fluid, and the 50 µl of extract were added to 450 µl of azocoll solution (prepared at a concentration of  $10^{-5}$ ). The azocoll solution was then incubated at 37° C for 2 h, and transmission measured at 570 nm using a 96-well plate reader for triplicates of each sample. This produced the data shown in  
20 **Figure 3**. The treated CMC material produced a significant reduction in observed collagenase activity, while the treated CMC material loaded with doxycycline reduced collagenase activity on the same order as the commercial Promogran<sup>™</sup> dressing.

25 **Example 9: Protease inhibition by treated superabsorbent polymer material (NIMBUS<sup>™</sup>-SAP) with and without doxycycline loading.**

Samples referred to henceforth as “NIMBUS<sup>™</sup>-SAP” (SAP = superabsorbent polymer) were prepared as per Batich et. al. (U.S. [2002/0177828 A1](#)). This material is a graft copolymer of rayon with DADMAC, wherein the polyDADMAC is

covalently crosslinked to form a superabsorbent hydrogel when loaded with water. NIMBUS<sup>TM</sup>-SAP samples were then loaded with doxycycline by aqueous solution loading followed by drying. NIMBUS<sup>TM</sup>-SAP samples were tested for protease inhibition capacity, using azocoll assay to determine the inhibition of collagenase activity. In the case of the NIMBUS<sup>TM</sup>-SAP material the high water absorption capacity requires the use of a higher ratio of inoculum to material, with (500 µl of 10<sup>-5</sup> clostridial collagenase solution per 20 mg sample of NIMBUS<sup>TM</sup>-SAP material). Data was collected at 1 h and at 18 h. The results are shown in **Figure 4** as % transmittance at 570 nm with higher transmittance indicating more protease inhibition by the sample tested. Note the difference between the 1 h and 18 h results for the NIMBUS<sup>TM</sup>-SAP doxycycline sample is minimal, indicating very strong suppression of collagenase activity. Note also that there is a significant difference between the NIMBUS<sup>TM</sup>-SAP material and the control materials (Sof-Wick is rayon dressing material, the labeled control contained no substrate, and represents uncontrolled collagenase activity. The measured data correlated well to the observed color of the samples, as suppression of collagenase activity is inversely proportional to the color of the sample. Samples that are colorless exhibit higher suppression activity. The samples in order of most suppression to least suppression were doxycycline loaded NIMBUS<sup>TM</sup> superabsorbent polymer, unloaded NIMBUS<sup>TM</sup> superabsorbent polymer, Sof-Wick rayon substrate, and the no substrate control.

**Example 10. Incorporation of growth factor into NIMBUS<sup>TM</sup>-SAP material, with characterized release.**

Epidermal Growth Factor (EGF) was labeled with a fluorescein compound, and loaded into a NIMBUS<sup>TM</sup>-SAP superabsorbent polymer matrix, as described for doxycycline loading in Example 9. Release was characterized by performing repeated extraction cycles into PBS, and by UV-visible absorption spectroscopy of the extraction fluid. It was found that EGF concentrations within therapeutic

range could be maintained for the duration of the experiment, as illustrated by the graph in **Figure 5**.

**Example 11. Incorporation of vitamins into NIMBUS™-SAP material, with characterized release.**

Water soluble vitamins C and vitamin E analog Trolox were loaded into a NIMBUS™-SAP superabsorbent polymer matrix, as described for the material of Example 9. Release was characterized by performing repeated extraction cycles into PBS, and by UV-visible absorption spectroscopy of the extraction fluid. It was found that vitamin concentrations within therapeutic range could be maintained for the duration of the experiment, as is shown in **Figure 6 (Vitamin C)** and **Figure 7 (Trolox)**.

It is easily noticeable that the release of vitamins is faster than that of growth factor. This is reasonable in view of the relative molecular sizes. Thus, a wound dressing can be loaded with excess vitamins to compensate for easier release without adverse effects (physical or economical), allowing the dressing to be designed around the release rate of EGF.

**Example 12. Incorporation of multiple bioactive components to form an advanced wound dressings with tailored characteristics.**

The material of Example 9 was used to prepare an advanced wound care dressing with characteristics tailored to suit the wound biochemistry; for this example, a dressing for partial thickness wounds created through chemical vesicant injury. The wound dressing is created by loading the base material, which in one incarnation may be treated CMC as in Examples 1 and 2, and in another incarnation may be a superabsorbent or other material similar to the materials of Examples 9 through 11. Concurrent loading of bioactive agents is easily possible, with concentrations being designed with the release rates of the specific components, as demonstrated in the examples presented. A loading solution composed of growth

factor(s), anti-proteases, vitamins and other bioactive agents is added to the dressing, after which the dressing can be dehydrated to the state desired. Full dehydration is the optimal condition to maximize storage life. An embodiment of this dressing is prepared by making an aqueous loading solution containing 0.5 % (1  
5 part in 200) of doxycycline, 0.05 % (1 part in 2000) each of vitamins C and Trolox: a water soluble vitamin E analog, and 0.01 % (100 parts per million) epidermal growth factor (EGF). This solution is loaded into the dressing at 20 times the dry weight of the dressing. The concentrations given provide a therapeutic dose of each ingredient. Extraction testing with 20 mg samples of material, with 600 µl of PBS  
10 fluid added and extracted via centrifugation showed that the 5<sup>th</sup> extract was well above therapeutic levels (as an example, therapeutic levels for growth factors are generally in the range of 1 nM, which equates to 6 ng/ml, whereas the 5<sup>th</sup> extraction cycle shows concentrations above 12 ng/ml for the conditions given).

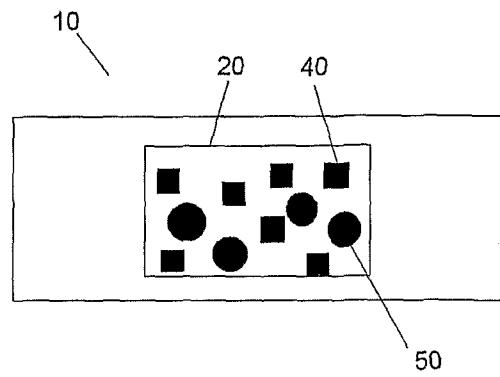
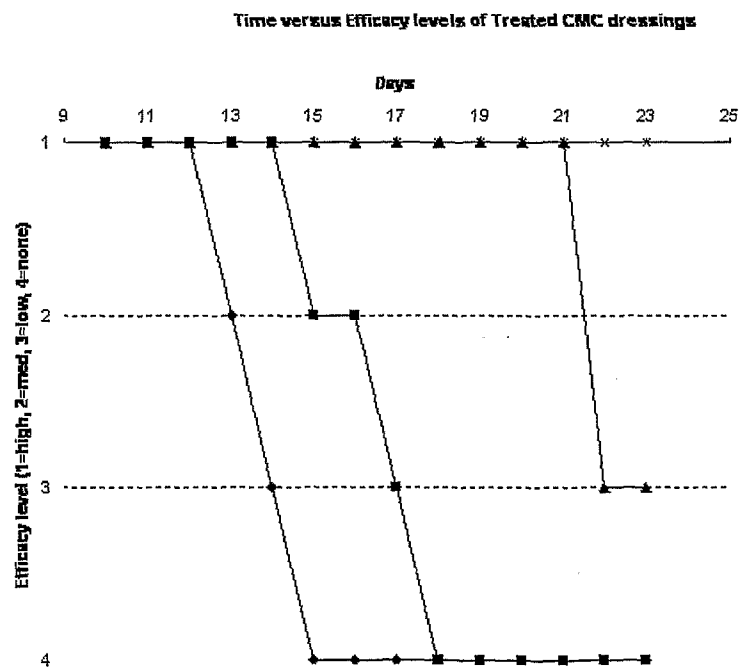
**CLAIMS**

1. A device for the treatment of wounds comprising:  
an absorbent wound dressing material having incorporated therein  
inherent non-leachable antimicrobial activity and inherent non-leachable  
anti-protease activity; and  
a releasable antimicrobial agent and a releasable anti-protease agent that  
are ionically stabilized within the device so as to be released from the  
device in a controlled manner.
2. The device of claim 1, further comprising one or more releasable bioactive  
agents which aid in wound healing, selected from the group consisting of  
growth factors, vitamins, antioxidants, anti-inflammatories,  
antimicrobials, anti-proteases, steroids and nutrients.
3. The device of claim 1, wherein the releasable antimicrobial agent and the  
releasable anti-protease agent are one and the same substance.
4. The device of claim 3, wherein the releasable antimicrobial agent and the  
releasable anti-protease agent are doxycycline.
5. The device of claim 1, wherein one or both of the inherent non-leachable  
antimicrobial activity and the inherent non-leachable antiprotease  
activity is provided by polymeric quaternary ammonium molecules.
6. The device of claim 5, wherein said polymeric quaternary ammonium  
molecules comprise poly(dimethyldiallylammonium chloride) also known  
as polyDADMAC; quaternary ammonium derivatives of poly(acrylic) acid  
or of poly(methacrylic) acid; poly(vinylbenzyl)trimethylammonium  
chloride; or a polyquaternium.

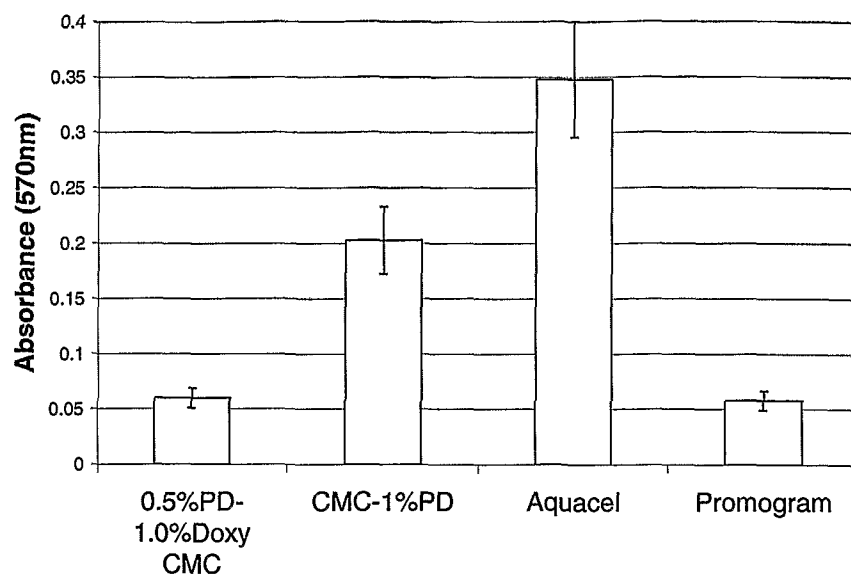
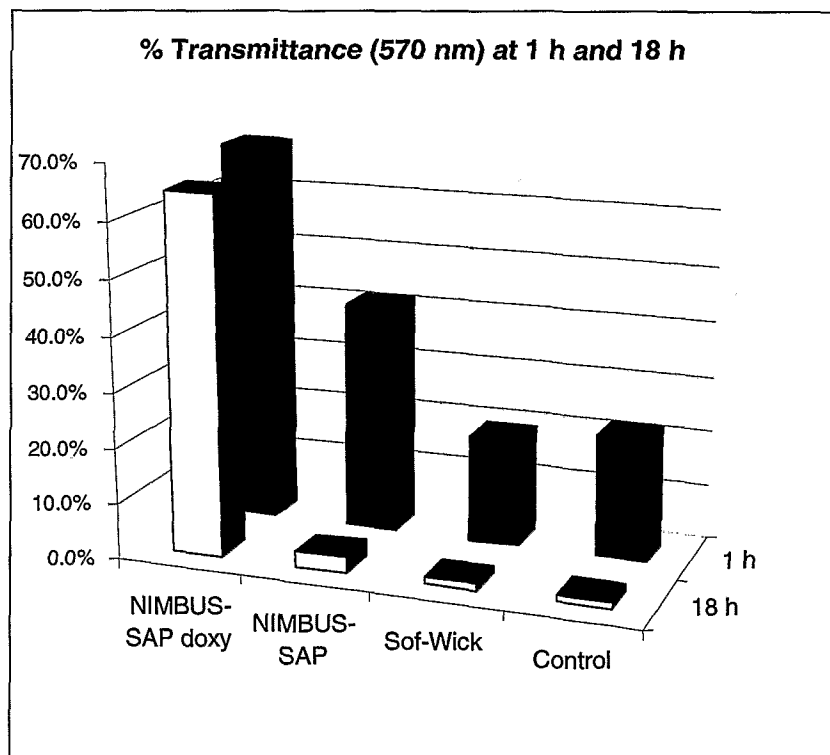
7. The device of claim 5, wherein the polymeric quaternary ammonium molecules are non-leachably bonded to the wound dressing material via covalent chemical bonds.
8. The device of claim 5, wherein the polymeric quaternary ammonium molecules are non-leachably bonded to the wound dressing material via ionic bonds.
9. The device of claim 5, wherein said polymeric quaternary ammonium molecules have a crosslinked network structure that forms a hydrogel in water.
10. The device of claim 5, wherein said polymeric quaternary ammonium molecules have crosslinked network structure is formed via chemical crosslinking of said polymeric quaternary ammonium molecules.
11. The device of claim 9, wherein said polymeric quaternary ammonium molecules have crosslinked network structure which comprises a polyelectrolyte complex formed between said polymeric quaternary ammonium molecules and polymeric anionic molecules.
12. The device of claim 11, wherein said polymeric anionic molecules comprise one or more of carboxymethyl cellulose, also known as CMC, alginate, or polyacrylate.
13. The device of claim 1, wherein said wound device comprises one or more of cellulose, a cellulose-based material, a polysaccharide, a textile fabric, gauze, fibers, a synthetic polymer, a superabsorbent material, or a protein.

14. The device of claim 13, wherein the cellulose-based material is one or more of cotton, rayon, CMC, hydroxyethyl cellulose, paper, or woodpulp, and wherein the polysaccharide is one or more of dextran, chitosan, alginate, or starch, and wherein the protein is collagen.
15. The device of claim 1, wherein said inherent non-leachable anti-protease activity is provided by a polymer with a multiplicity of anionic sites.
16. The device of claim 15, where said polymer with a multiplicity of anionic sites is one or more of CMC, alginate, collagen or polyacrylate.
17. The device of claim 1, wherein said releasable antimicrobial agent comprises one or more antibiotic or polymeric quaternary ammonium molecules.
18. The device of claim 17, wherein said antibiotic is one or more of tetracycline or doxycycline and said polymeric quaternary ammonium molecules are poly(DADMAC).
19. The device of claim 1, wherein said releasable antiprotease agent is one or more of ilomastat, doxycycline, minocycline, collagen, CMC or poly(DADMAC).
20. The device of claim 1, wherein said inherent non-leachable antimicrobial agent and said inherent non-leachable anti-protease agent is one and the same.
21. The device of claim 1, wherein said releasable bioactive agent is one or more growth factors, vitamins, nutritive factors, and anti-inflammatories.

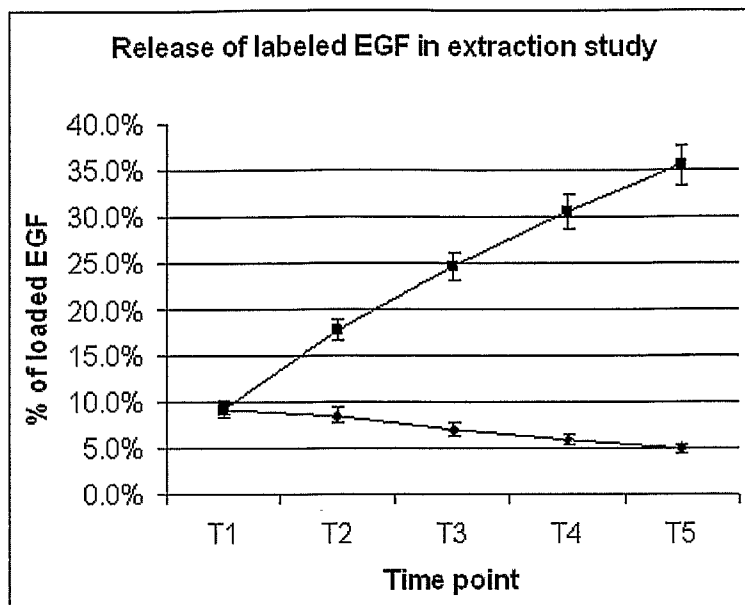
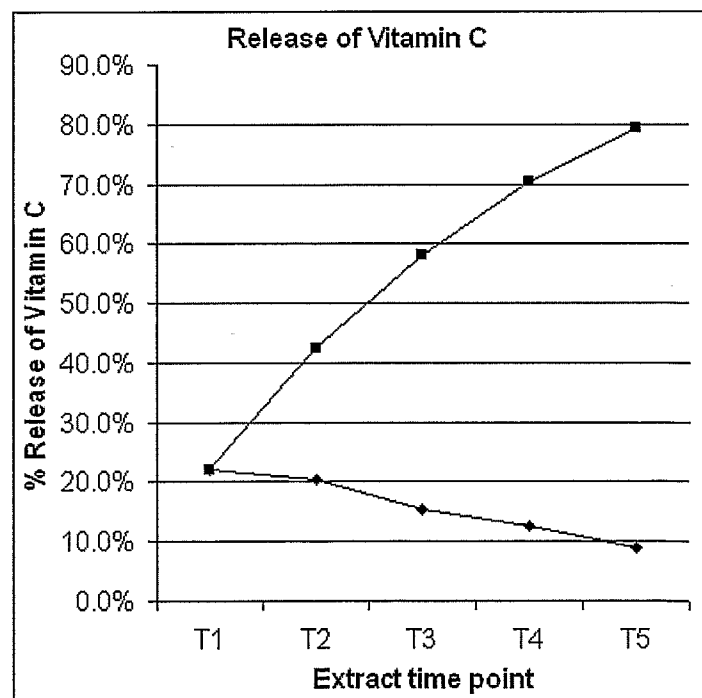
22. The device of claim 21, wherein the growth factor is epidermal growth factor (EGF), platelet derived growth factor (PDGF), or vascular endothelial growth factor (VEGF).
23. An absorbent antimicrobial material comprising a substantially carboxymethylated cellulosic substrate and poly(DADMAC) non-leachably attached to said substrate, wherein a sufficient amount of said poly(DADMAC) is attached to said substrate to form a polyelectrolyte network, and wherein said polyelectrolyte network permits the degree of swelling of said material to range from about 10 times up to about 20 times of the dry material, and wherein said polyelectrolyte network diminishes the dissolution of the substantially carboxymethylated cellulosic substrate upon exposure to aqueous fluids, and wherein said polyelectrolyte network permits the incorporation and release of a bioactive agent in a controlled manner.
24. A method of facilitating healing of a wound of a mammal comprising applying to the wound a device for the treatment of wounds, comprising an absorbent wound dressing material having incorporated therein inherent non-leachable antimicrobial activity and inherent non-leachable anti-protease activity, and a releasable antimicrobial agent and a releasable anti-protease agent that are ionically stabilized within the wound dressing material so as to be released from the device in a controlled manner, and one or more releasable bioactive agents which aid in wound healing, selected from the group consisting of growth factors, vitamins, and nutrients, whereby application of said device, reduces the potential for development of infection, inflammation, or malodor compared to an untreated wound.

**1/4****Figure 1****Figure 2**

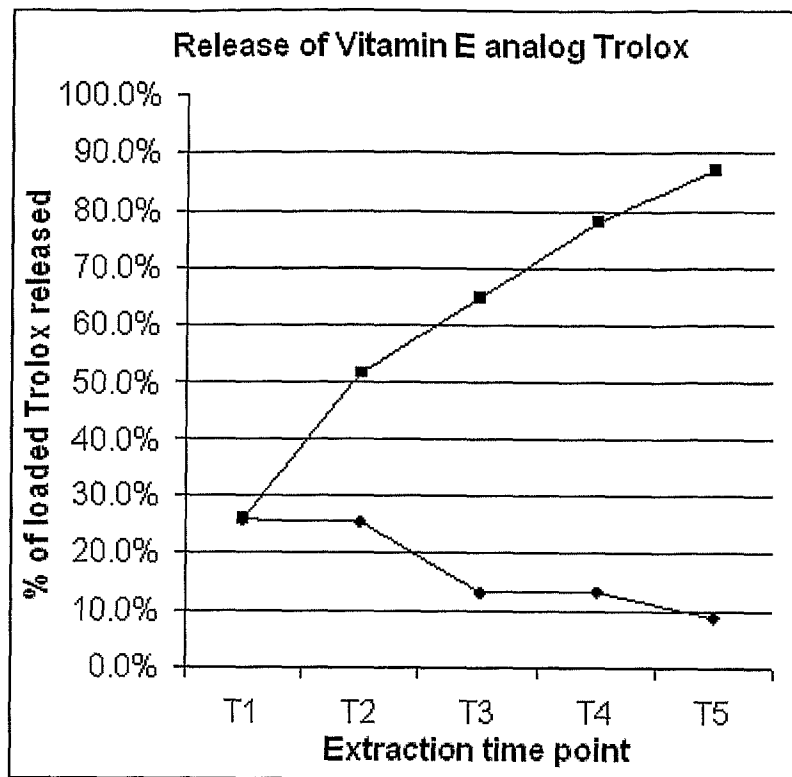
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**Figure 3****Figure 4**

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**Figure 5****Figure 6**

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**Figure 7**