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(54) Title: ANTIMICROBIAL AND ANTIPROTEOLYTIC WOUND DRESSING

(57) Abstract: This invention comprises a novel wound dressing and a method for making and using the wound dressing comprising an immobilized matrix metalloproteinase inhibitor (MMPI) with or without immobilized microbicidal or other biologically active compounds grafted onto the wound dressing. In one embodiment, the microbicidal function may comprise quaternary amines or polymers of quaternary amines grafted onto a cellulosic substrate.

TITLE OF THE INVENTION

ANTIMICROBIAL AND ANTIPROTEOLYTIC WOUND DRESSING

FIELD OF THE INVENTION

This invention pertains to the field of wound care and in particular to wound care scenarios where it is critical to maintain an aseptic environment while at the same time reducing the amount of proteolytic degradation that occurs in and around a wound.

BACKGROUND OF THE INVENTION

Those skilled in the art have been aware for some time of methods for producing antimicrobially effective wound dressings. In particular, reference is made to patent publication WO 00/33778 for an "Intrinsically Bactericidal Absorbent Dressing and Method of Fabrication", which published on June 15, 2000. As disclosed in that publication, it is possible to produce substrates onto which have been grafted antimicrobially effective compounds. In one specific embodiment, the substrate is a cellulosic substrate and the atnimicrobially effective compound is a quaternary amine, or a polymer of quaternary amines.

Those skilled in the art have also been aware, for some time, of methods for inhibiting matrix metalloproteinases, which includes a number of enzymes which effect the breakdown of structural proteins and which are structurally related metalloproteases. These include human skin fibroblast collagenase, human skin fibroblast gelatinase, human sputum collagenase and gelatinase, and human stromelysin. These are zinc-containing metalloprotease enzymes, as are the angiotensin-converting enzymes and the enkephalinases.

Collagenase and related enzymes are important in mediating the symptomology of a number of diseases, including rheumatoid arthritis (Mullins, D. E., et al., Biochim Biophys Acta (1983) 695:117-214); the metastasis of tumor cells (ibid , Broadhurst, M.

J., et al., EP application 276436 (1987), Reich, R., et al., Cancer Res (1988) 48:3307-3312); and various ulcerated conditions. Ulcerative conditions can result in damage to the cornea as the result of alkali burns or as a result of infection by Pseudomonas aeruginosa, Acanthamoeba, Herpes simplex and vaccinia viruses. Other conditions that may be exacerbated by unwanted matrix metalloprotease activity include: periodontal disease, epidermolysis bullosa, scleritis, ulcerative conditions including but not limited to chronic wounds, leg ulcers (whether venous or arterial in origin), pressure sores, diabetes related sores and ulcers, burn injuries, and similar conditions.

In view of the involvement of collagenase and related matrix metallo-proteinases (MMPs) in a number of disease conditions, attempts have been made to prepare inhibitors to this enzyme. A number of such inhibitors are disclosed in EP applications 126,974 (published 1984) and 159,396 (published 1985) assigned to G. D. Searle. These inhibitors are secondary amines which contain oxo substituents at the 2-position in both substituents bonded to the amino nitrogen. See also U.S. Pat. Nos. 4,599,361 and 4,743,587, also assigned to G. D. Searle. These compounds are hydroxylamine dipeptide derivatives which contain, as a part of the compound, a tyrosine or derivatized tyrosine residue or certain analogs thereof. Other compounds that contain sulfhydryl moieties as well as residues of aromatic amino acids such as phenylalanine and tryptophan are disclosed in PCT application WO88/06890. Some of these compounds also contain i-butyl side chains. MMP inhibitors have also been disclosed for the related protease, thermolysin. These include hydroxamic peptide derivatives described by Nishino, N., et al., Biochemistry (1979) 8:4340-4347; Nishino, N., et al., Biochemistry (1978) 17:2846-2850. Tryptophan is also known to be therapeutic in various conditions, some of which may involve collagenase (see, for example, JP 57/058626; U.S. Pat. No. 4,698,342; 4,291,048). Also, inhibitors of bacterial collagenases have been disclosed in U.S. Pat. No. 4,558,034. Reference is also made to MMP inhibitors (MMPI's) and methods of use thereof as disclosed and claimed in US Patent Nos. 5,189,178; 5,270,326; 5,183,900; 5,239,078; 5,268,384;5,696,147; 5,892,112; 5,773,438; 6,420,408; 6,417,229; 6,403,632; 6,399,612; 6,387,901; 6,376,665; 6,376,506; 6,372,758; 6,365,587; 6,358,980; 6,352,976; 6,350,907; 6,350,885; 6,344,189; 6,342,508; 6,340,691. The disclosure of these patents

is hereby incorporated by reference for purposes of disclosing various MMPI's known in the art and methods of use and manufacture thereof.

Finally, it is noted that Johnson & Johnson Medical (J&J) has released a new commercial product identified by the trade-name PROMOGRAN, and which is described by that company as being a lyophilized mixture of oxidized regenerated cellulose (ORC) and typle I collagen. J&J's online literature describes this product as follows: "PROMOGRAN™ Matrix is a unique advanced wound care device comprised of a sterile, freeze-dried matrix composite of 45 percent ORC and 55 percent collagen. ORC is a plant material that has been chemically altered to be absorbed by the body. Collagen is a natural structural protein found in all three phases of wound healing. By binding to matrix metallo-proteases (MMPs), and growth factors, ORC/Collagen creates a moist wound healing environment, which is conducive to new tissue growth.... MMPs are inflammatory enzymes that degrade proteins in various tissues. Recent scientific research has shown elevated levels of MMPs in chronic wound exudate, the fluid that bathes the wound bed. These excess MMPs cause degradation of important extracellular matrix proteins and inactivation of vital growth factors, elements that are essential in the wound healing process. This may contribute to a sub-optimal healing environment resulting in delayed wound healing (Ability of chronic wound fluids to degrade peptide growth factors is associated with increased levels of elastase activity and diminished levels of proteinase inhibitors; D. Yager, S. Chen, S. Ward, O. Olutoye, R. Diegelmann, K. Cohen. Wound repair and regeneration, 1997, vol 5, pp23-32)...ORC/Collagen binds to MMPs in chronic wound exudate, without altering the activity of essential tissue growth factors, at the same time creating an optimal milieu for moist wound healing....PROMOGRAN™ Matrix maintains a physiologically moist microenvironment at the wound surface. This environment is conducive to tissue granulation; epithelialization and rapid wound healing. In the presence of exudate, the PROMOGRAN™ Matrix transforms into a soft, conformable, biodegradable gel, thus allowing contact with all areas of the wound. PROMOGRAN™ Matrix should not be used on patients with known hypersensitivity to ORC and/or collagen."

SUMMARY OF THE INVENTION

This invention comprises a novel wound dressing and a method for making and using the wound dressing comprising an immobilized matrix metalloproteinase inhibitor (MMPI) with or without immobilized microbicidal or other biologically active compounds grafted onto the wound dressing. In one embodiment, the microbicidal function may comprise quaternary amines or polymers of quaternary amines grafted onto a cellulosic substrate.

Accordingly, it is one object of this invention to provide a novel wound dressing comprising an immobilized MMPI.

It is a further object of this invention to provide a wound dressing comprising immobilized MMPI molecules in addition to additional immobilized biologically active molecules, selected from, but not limited to: antimicrobially active compounds, hemostatic compounds, anti-hemostatic compounds, anti-neoplastic compounds, and combinations and variations thereof.

Other objects of this invention will be apparent to those skilled in the art based on this disclosure and the claims appended hereto.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE PRESENT INVENTION

In a first embodiment of the invention, the composition of the present invention comprises a matrix metalloproteinase inhibitor (MMPI) immobilized on a substrate which is used as a wound covering, particularly for a chronic wound such as a leg ulcer or the like. The substrate in one embodiment of this invention is a superabsorbent substrate as is known in the art, which is capable of absorbing many times its own weight in wound exudate. The immobilized MMPIs bound to the substrate preferably has nanomolar, micromolar or millimolar affinity for various matrix metalloproteinases present in wound

exudate. As wound exudate is absorbed onto the substrate, the matrix metalloproteases are bound and inhibited by the immobilized MMPI's.

In a second embodiment of the invention, the composition of the present invention comprises an MMPI immobilized on a substrate useable as a bandage for a wound, wherein, in addition to the immobilized MMPI, there is immobilized additional biologically active molecules, selected from the group including but not limited to: antimicrobial compounds, serine protease inhibitory compounds, hemostatic agents, anti-hemostatic agents (such as heparin), analgesic compounds, and antineoplastic compounds.

In a third embodiment of the invention, the composition of the present invention comprises an MMPI bound to a polymer which is grafted onto a substrate which is used as a wound dressing.

In a fourth embodiment of this invention, the composition of this invention comprises an MMPI bound to a polymer comprising additional biologically active molecules, including but not limited to: antimicrobial compounds, serine protease inhibitory compounds, hemostatic agents, anti-hemostatic agents (such as heparin), analgesic compounds, and antineoplastic compounds.

It will be understood by those skilled in the art, based on this disclosure, that in addition to the composition of this invention, to the wound being bandaged may be added soluble factors such as MMPI's, collagen, antibiotics, analgesics, serine protease inhibitors, hemostatic agents, anti-hemostatic agents, antineoplastic agents, or the like.

The composition of this invention may be prepared, in one embodiment, by grafting quaternary amines to a cellulosic substrate utilizing cerium ion as a free-radical polymerization catalyst, and utilizing monomeric quaternary amines such as those known in the art, selected from, but not limited to: diallyldimethylammonium chloride (DADMAC), vinylbenzyltriethyl ammonium chloride (VBC), N,N,N-trimethyl-N-

(meth)acryloyloxyethylammonium chloride (TMMC), also known as the methyl chloride quaternary salt of dimethylaminoethyl methacrylate, and the like. As disclosed in WO 00/33778, substrates comprising bonded quaternary amines, whether as monomers or as polymers, exhibit anitmicrobial efficacy. To such substrates, MMPI's may be immobilized, or co-polymerized.

This is achieved, for example, with reference to a specific MMPI, known as Ilomastat, ($C_{20}H_{28}N_4O_4$, MW= 388.46, *S*-(*R**,*S**)]-N^4-Hydroxy-N^1-[1*H*-indol-3-ylmethyl)-2-(methyl-amino)-2-oxoethyl]-2-(2-methylopropyl)butanediamide; or (*R*)-N^1-Hydroxy-*N*-[(*S*)-2-indol-3-yl-1-(methylcarbamoyl)ethyl]-2-isobutylsuccinamide; *CAS-142880-36-2)*, see, for example, US Patent 5,189,178, herein incorporated by reference for this purpose, by obtaining the methyl amide group on illomastat and modifying that functionality to form a carboxylate group, which is then reacted with hexamethylene diamine to form a free primary amine active group. Both of these groups (carboxylate and amine, CA and AM forms respectively) can easily be used to couple the MMPI to either a cellulosic backbone or to pendant acrylate chains which are grafted onto the surface. Standard coupling chemistry is used, such as the reaction of an ester with an amine. For instance, the AM form is reacted with the substrate directly to form an amide bond from reaction with the acrylate ester group. Furthermore, in one embodiment, the substrate is esterified, and then reacted with the AM-form. A more active substrate is formed by reacting the cellulose with cyanogen bromide, a diisocyanate or a bis-epoxide, and that reacted with the AM-form. The bis-epoxide modified cellulose is also susceptible to reaction with the CA-form. Those skilled in the art will appreciate that a wide variety of modifications on this basic chemistry may be developed, without departing from the heart of the invention disclosed and claimed here. In a further embodiment, Ilomastat is attached to an acrylate monomer, and simply polymerized in a grafting reaction. As an example, the AM-form is reacted with acrylochloride, and the resulting monomer used for free radical induced grafting. Spacers are added as needed, such as polyethylene glycol units.

Those skilled in the art will appreciate that, depending on the particular application desired, different ratios of MMPI to other active compounds may be desirable. Where

low incidence of microbial infection is likely, the ration of MMPI to quaternary amine monomers, for example, may be quite high. Conversely, where microbial infection is expected to be a significant problem, the ratio of MMPI to antimicrobial compounds is reduced.

In one specific embodiment, provided to further enable those skilled in the art to practice this invention and to extend the written disclosure of this invention, without intending to limit this invention to the specifics of this example, the cellulosic component of PROMOGRAN is utilized as a substrate, onto which is immobilized Ilomastat. In another embodiment, both Ilomastat and TMMC are grafted onto the cellulosic component. In a further embodiment, as discussed above, Ilomastat is co-polymerized onto the cellulosic substrate with quaternary amines. In this fashion, a wound dressing is produced which is not only beneficial because of its matrix metalloproteinase inhibitory activity, but the dressing also exhibits anti-microbial efficacy.

Having disclosed this invention in detail, including the best mode thereof, those skilled in the art will appreciate that a wide range of variations in the MMPI's used, the antimicrobial compounds used, and the combinations of various biologically active compounds may be achieved as equivalents to that which is disclosed and claimed herein without departing from the heart of this invention. For example, Ilomastat may be used as the MMPI, or a derivative or analog thereof may be utilized. Other MMPI's known in the art may have desirable properties and may be used in addition to Ilomastat or in place of Ilomastat. In addition, combinations of different MMPI's, different antimicrobial compounds and the like may be utilized.

WHAT IS CLAIMED IS:

1. A composition comprising a substrate onto which is immobilized a matrix metalloproteinase inhibitor (MMPI).

2. The composition according to claim 1 wherein said MMPI is covalently immobilized onto said substrate.

3. The composition according to claim 1 wherein, in addition to said MMPI, an antimicrobially active compound is immobilized on said substrate.

4. The composition according to claim 3 wherein said antimicrobial compound is covalently immobilized on said substrate.

5. The composition according to claim 4 wherein said antimicrobial compound is a quaternary amine.

6. The composition according to claim 5 wherein said quaternary amine is polymeric.

7. The composition according to claim 2 wherein said substrate is a cellulosic substrate.

8. The composition according to claim 7 wherein said substrate is a wound dressing.

9. The composition according to claim 7 wherein said substrate comprises cellulose, a cellulose derivative, and collagen.

10. The composition according to claim 1 wherein said MMPI is Ilomastat.

11. The composition according to claim 10 wherein, in addition to Ilomastat, there is immobilized on said substrate biologically active molecules selected from the group consisting of: antimicrobial compounds, serine protease inhibitory compounds, hemostatic agents, anti-hemostatic agents, analgesic compounds, and antineoplastic compounds.

12. The composition according to claim 11 wherein said antimicrobial compound is a quaternary amine.

13. The composition according to claim 12 wherein said quaternary amine is polymeric.

14. The composition according to claim 13 wherein Ilomastat is co-polymerized with said quaternary amines.

15. The composition according to claim 1 wherein said substrate exhibits superabsorbency.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 03/23997

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L15/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 189 178 A (GROBELNY DAMIAN ET AL) 23 February 1993 (1993-02-23) cited in the application column 2, line 59 -column 4, line 22 column 5, line 45 -column 7, line 52 ---	1-15
X	WO 00 56283 A (GOODRICH CO B F) 28 September 2000 (2000-09-28) page 4, line 14 -page 6, line 20 page 8, line 21 - line 25 page 11, line 1 - line 14 page 18, line 19 - line 23 page 24, line 4 -page 27, line 27 page 21, line 25 -page 22, line 4 --- -/--	1-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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Date of the actual completion of the international search	Date of mailing of the international search report
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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 712 635 A (KURARAY CO) 22 May 1996 (1996-05-22) page 7, line 56 -page 9, line 15 page 5, line 57 -page 6, line 26 claims 1-11	1-15
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Information on patent family members

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