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(56) Documents Cited:
GB 2422664 A **GB 2393120 A**
WO 2008/070865 A2 **WO 2006/030232 A2**
WO 2003/058237 A1
Macromolecular Bioscience, Vol. 10, No. 10, Oct 2010, AG Patrick et al., "Hydrogels for the detection and management of protease levels.", pages 1184 - 1193.
Wound Repair and Regeneration, Vol. 7, No. 5, Sept-Oct 1999, JF Tarlton, et al., "Prognostic value of markers of collagen remodeling in venous ulcers", pages 347-355
International Wound Journal, Vol. 5, No. 2, Jun 2008, R Smeets, et al., "Effect of oxidised regenerated cellulose/collagen matrix on proteases in wound exudate of patients with chronic venous ulceration", pages 195-203.
Archives of Dermatological Research, Vol. 302, No. 6, Aug 2010, C Wiegand, et al., "Protease and pro-inflammatory cytokine concentrations are elevated in chronic compared to acute wounds and can be modulated by collagen type I in vitro", pages 419-428.
Wound Repair and Regeneration, Vol. 15, No. 6, Nov-Dec 2007, B Cullen, et al., "A comparison of collagen containing wound dressings to modify the chronic wound environment", page A148

(58) Field of Search:
INT CL **C12N, C12Q, G01N**
Other: **EPODOC, WPI, BIOSIS, MEDLINE**

(54) Title of the Invention: **Wound prognosis**
Abstract Title: **Measurement of matrix metalloproteinase (MMP) and elastase in wound fluid**

(57) A diagnostic apparatus for simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase (MMP) in a wound fluid sample is disclosed, wherein the amount of protease inhibitors in the sample is not determined. Also claimed is a diagnostic apparatus for simultaneously or sequentially determining the amount of elastase, MMP-1 (collagensase) and MMP-9 (gelatinase) in a sample of wound fluid. Methods of predicting whether patients will respond to a particular treatment of a wound (e.g. an oxidized cellulose (ORC) dressing) using measurement of the levels of such proteases, along with methods of treating wounds following measurement of the above proteases, are also claimed. Kits comprising the aforementioned diagnostic apparatus and a wound dressing (e.g. an oxidized cellulose (ORC) dressing) are also claimed.

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FIGURE 1

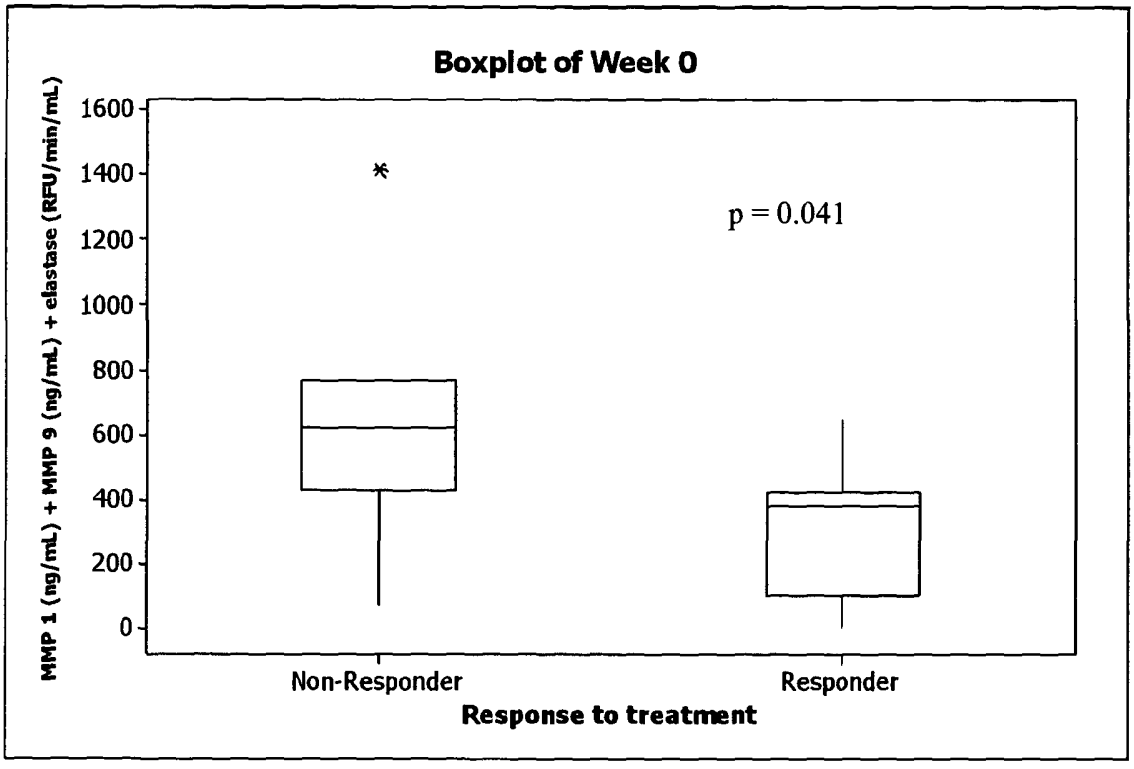

Boxplot of Week 0
MMP 1 (ng/mL) + MMP 9 (ng/mL) + elastase (RFU/min/mL)
1600
1400
1200
1000
800
600
400
200
0
*
p = 0.041
Non-Responder
Responder
Response to treatment

FIGURE 2

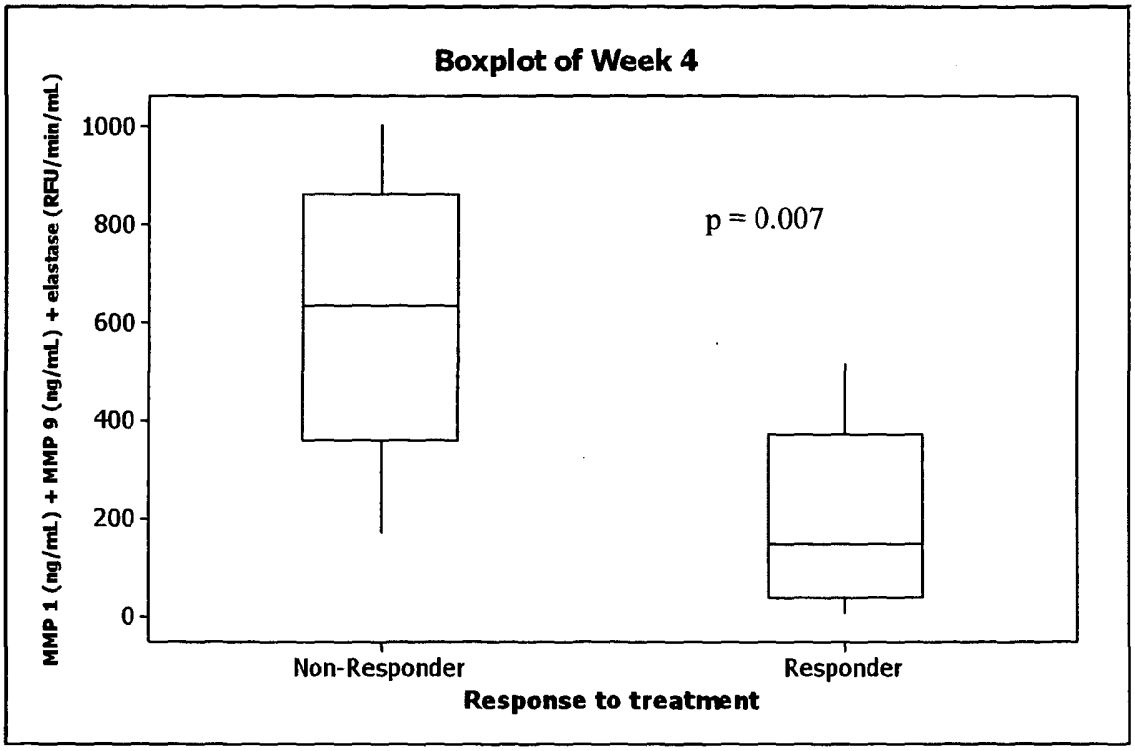

Boxplot of Week 4
MMP 1 (ng/mL) + MMP 9 (ng/mL) + elastase (RFU/min/mL)
1000
800
600
400
200
0
p = 0.007
Non-Responder
Responder
Response to treatment

FIGURE 3

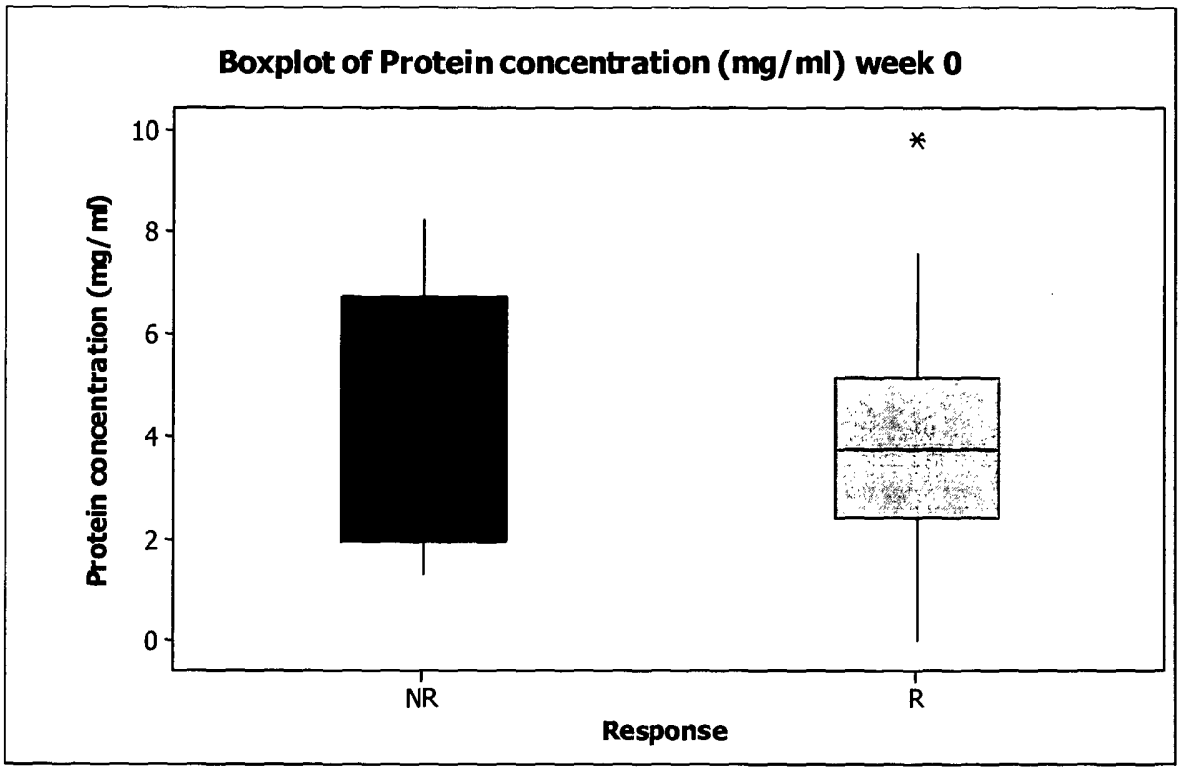

Boxplot of Protein concentration (mg/ml) week 0
Protein concentration (mg/ml)
10
8
6
4
2
0
*
NR
R
Response

FIGURE 4

5

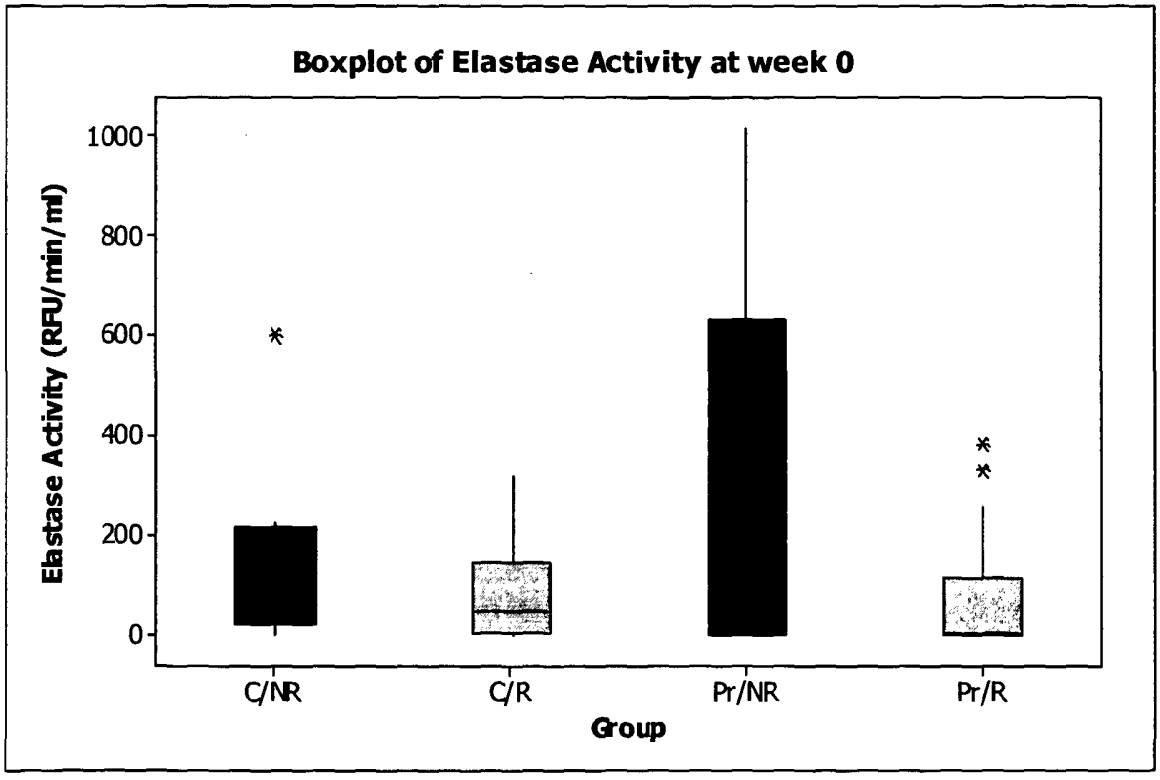

Boxplot of Elastase Activity at week 0
Elastase Activity (RFU/min/ml)
1000
800
600
400
200
0
C/NR
C/R
Pr/NR
Pr/R
Group

FIGURE 5

5

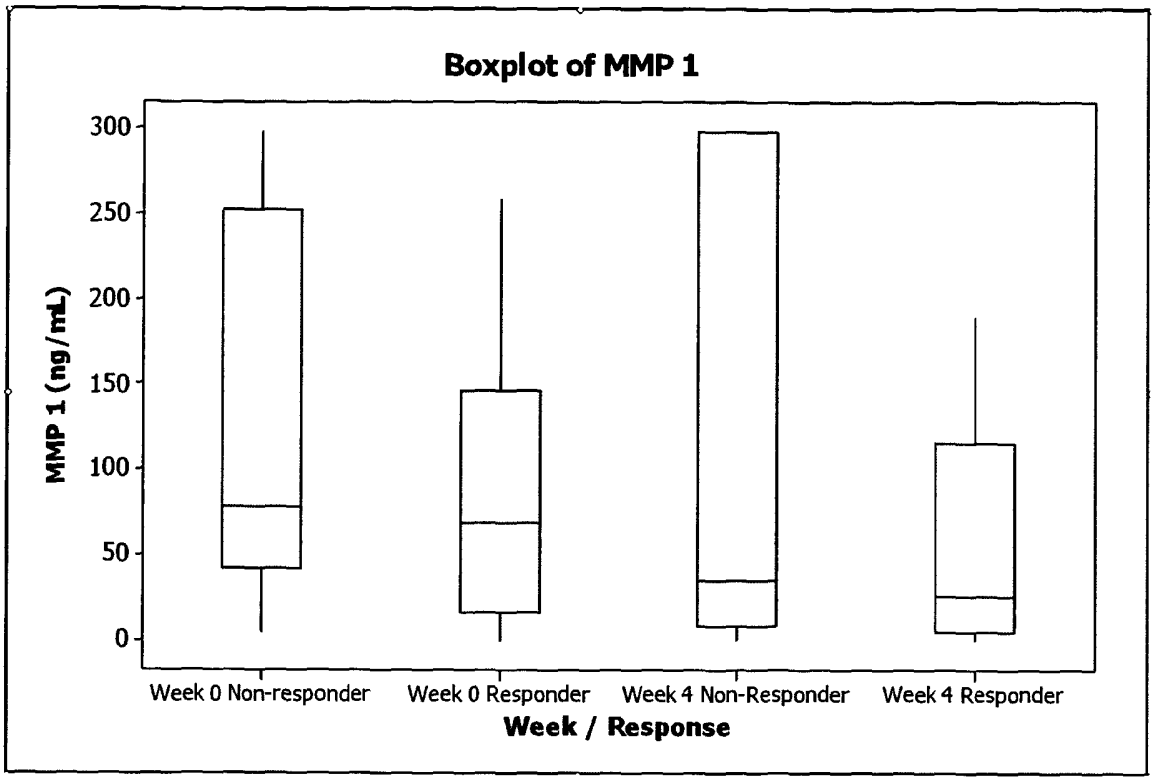

Boxplot of MMP 1
MMP 1 (ng/mL)
300
250
200
150
100
50
0
Week 0 Non-responder
Week 0 Responder
Week 4 Non-Responder
Week 4 Responder
Week / Response

FIGURE 6

5

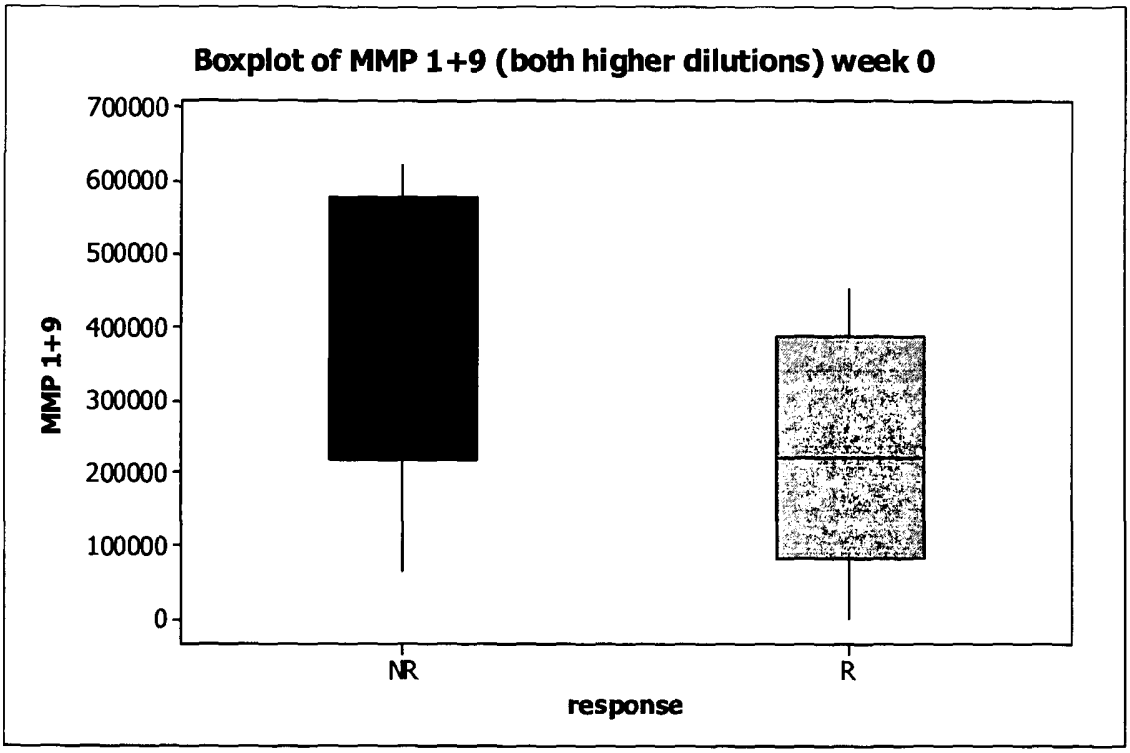


Fig 6: Combined MMP-1 and MMP-9 in ng/mL.

FIGURE 7

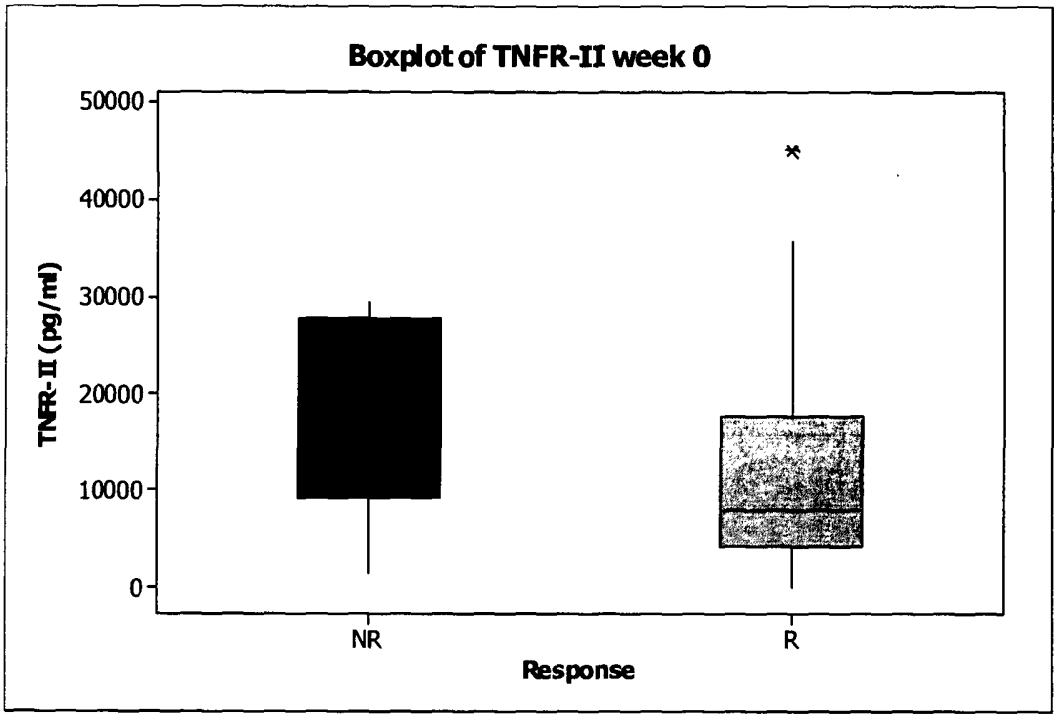
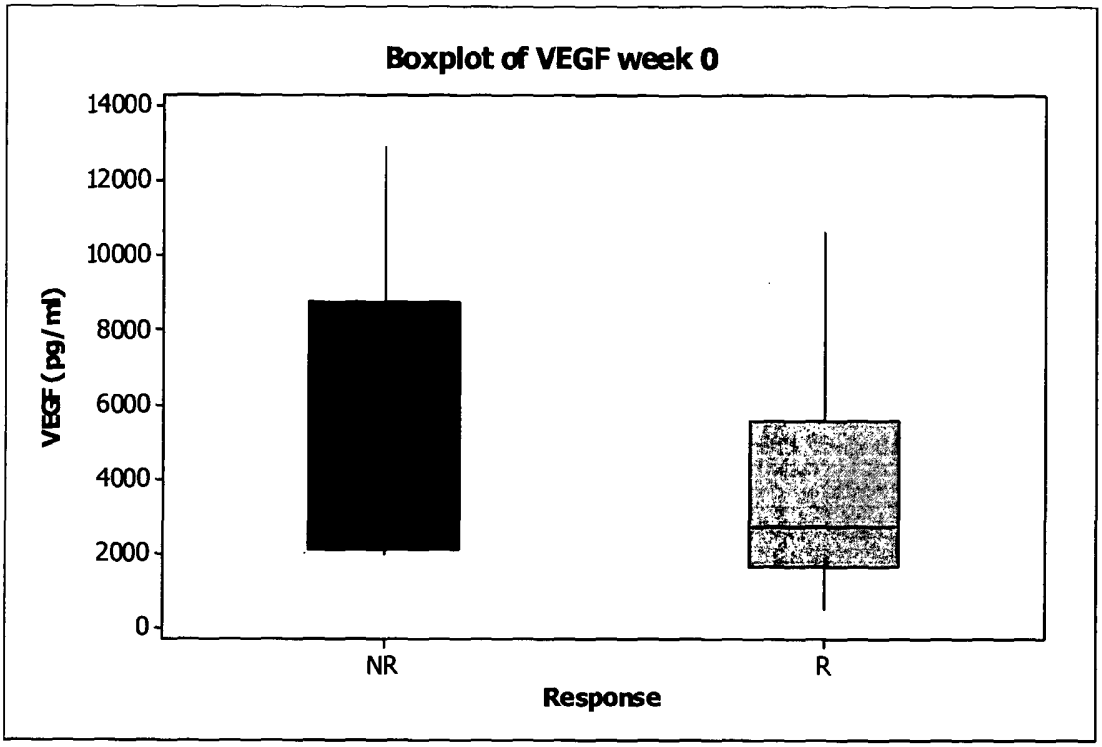

Boxplot of TNFR-II week 0
50000
40000
30000
20000
10000
0
TNFR-II (pg/ml)
NR
R
Response

FIGURE 8


Boxplot of VEGF week 0
VEGF (pg/ml)
14000
12000
10000
8000
6000
4000
2000
0
NR
R
Response

WOUND PROGNOSIS

TECHNICAL FIELD

The present invention relates to an apparatus for monitoring the status of wounds. The invention also provides a method of treating a wound that exudes a wound fluid and a method of prognosing a wound that exudes a wound fluid. Diagnostic apparatus and kits comprising a diagnostic apparatus and a wound dressing are also provided for use in the methods of the invention.

BACKGROUND ART

WO98/00180 and EP-A-1153622 describe the use of freeze-dried sponges comprising oxidized regenerated cellulose (ORC), optionally admixed with collagen, for the treatment of chronic wounds. Dressings based on oxidized cellulose have been found to give outstanding results in the treatment of chronic wounds, including diabetic ulcers, venous ulcers and decubitis ulcers.

WO 2004/024197 and EP-A-1536845 describe the use of wound dressing materials comprising complexes of anionic polysaccharides with silver. In particular, wound dressing materials comprising complexes formed between anionic polysaccharides, such as ORC, and silver, and to the uses thereof for the treatment of wounds are disclosed.

C.N. Rao *et al.* in the Journal of Investigative Dermatology, vol. 105(4), pages 572-578 (1995) describe the results of analysing chronic and acute wound fluids for elastase, alpha-1-antitrypsin (AAT) and fibronectin. It was found that the elastase level was 10 to 40 times higher in the chronic wound fluid.

GB-A-2393120 describes the use of wound dressings based on ORC in combination with chitosan for the treatment of chronic wounds. The dressings are shown to reduce the levels of elastase and collagenase in the wound fluids.

US-A-2003/0119073 describes sensors for the assay of catabolic protease enzymes in wound fluid. The analyte enzymes include human neutrophil elastase (hNE). It is suggested therein that the invention can be used in a method of treating chronic wounds by detecting the presence of catabolic protease enzymes, and then treating the wound with inhibitors that are specific for the detected enzymes.

It has been found that a sub-group of chronic wound patients exhibit a particularly large improvement in wound healing when treated with collagen/ORC sponges.

WO 2006/030232 describes a diagnostic test apparatus for determining a ratio of: (a) at least one endogenous protease enzyme inhibitor, to (b) at least one endogenous protease enzyme, in a sample of a wound fluid. The apparatus is used in a method of treating a wound and for identifying prospective responders and non-responders to treatment of wounds with PROMOGRAN™ collagen/ORC wound dressing.

Thus, it is an object of the present invention to provide alternative or improved means to identify as early as possible the sub-group of patients that exhibit a particularly large improvement in wound healing when treated with oxidized cellulose so that they can receive maximum benefit from this therapy. It is a further object of the invention to avoid unnecessary oxidized cellulose therapy on other patients who may be less likely to benefit. Accordingly, it is an object of the present invention to identify which patients would be more likely to respond to treatment with oxidised cellulose before treatment with oxidised cellulose has begun.

DISCLOSURE OF THE INVENTION

It has been found that the amounts of endogenous protease enzymes are particularly good predictors of the success of treatment with oxidized cellulose therapy. However, the measurement of individual marker analytes in samples of wound fluid, such as the measurement of individual endogenous protease enzymes has so far been unsuccessful in predicting whether wounds would be responsive to treatment with oxidized cellulose therapy before the treatment has commenced (see, e.g. Figures 4-8 and Reference Examples 2-6).

The present inventors have found, surprisingly, that the combined amount of elastases and matrix metalloproteinases in a sample of a wound fluid, whether before or during treatment with a protease inhibitor dressing, such as an oxidized cellulose dressing, correlates to the likelihood of (and rate of) healing by means of this therapy. In particular, it has been found, surprisingly, that the combined amount of elastase, MMP-1 and MMP-9 as determined from a sample of wound fluid is an especially good predictor of the success of treatment with oxidized cellulose therapy.

Accordingly, in a first aspect, the present invention provides a diagnostic apparatus for simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in a sample of a wound fluid wherein the amounts of protease inhibitors in the sample are not determined.

It will be appreciated that the concentration of more than one elastase and matrix metalloproteinase may be measured. In certain embodiments of the first aspect, the concentrations of at least two, three or four of these proteases are monitored. Typically, three of these proteases are measured.

In an embodiment of the first aspect, the at least one matrix metalloproteinase is at least two matrix metalloproteinases. Suitably, the at least one matrix metalloproteinase is selected from the group consisting of MMP-1, MMP-8, MMP-9 and MMP-12. Typically, the matrix metalloproteinases are MMP-1 and MMP-9.

In a second aspect, the invention provides a diagnostic apparatus for simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in a sample of a wound fluid.

In the above aspects or embodiments, the amount of elastase may be indicated by total elastase-like activity. In some embodiments, the elastase may be selected from the group consisting of neutrophil elastase, latent elastase and / or active elastase. For example, the elastase may be neutrophil elastase. Typically, the elastase is active elastase.

For example, in one embodiment of the first or second aspects, the proteases determined are neutrophil elastase, MMP-1 and MMP-9.

In another embodiment, of the first or second aspects, the proteases determined are active elastase, MMP-1 and MMP-9.

In the aspects and embodiments defined herein above and below, the at least one elastase and at least one endogenous matrix metalloproteinase may be endogenous proteases. Typically, said endogenous proteases are human endogenous proteases.

In another embodiment of the first aspect, the invention provides a diagnostic apparatus according to the first aspect, wherein the step of determining comprises establishing whether the combined amount of said proteases falls within a predetermined range.

In another embodiment of the first aspect, the invention provides a diagnostic apparatus according to the first aspect, wherein the step of determining comprises comparing the combined amount of said proteases with a control standard.

In a further embodiment, the step of determining comprises the additional step of providing an output signal indicating the result of the comparison between the combined amount of said proteases and said control standard. Output signals may be of any suitable form as apparent to the skilled person. Suitable signals are disclosed herein. For example, output signals may be visual or auditory, and may be immediately recognisable (*e.g.* written text) or may require further interpretation by reference to a standard (*e.g.* a colour signal).

The apparatus or devices according to the present invention may contain diagnostic test devices specifically adapted for detecting the proteases. For example, the apparatus may comprise a first device specifically adapted to measure the level of one protease enzyme, and one or more further devices specifically adapted to measure the level of each remaining protease. Suitably, the apparatus comprises a single device specifically adapted to measure the level of all proteases. For example, in one embodiment, the present invention provides a diagnostic apparatus according to any preceding aspect or embodiment, wherein the apparatus comprises a single diagnostic device specifically adapted for detecting each of said proteases in said sample.

The term "**specifically adapted**" herein signifies that the device comprises at least one substance that reacts selectively with the protease analyte.

The substance may, for example, comprise a selective binding partner such as an immunological binding partner, for the protease analyte. In other embodiments, the substance may comprise a specific substrate for the analyte, for example a peptide sequence that is cleaved selectively by an analyte protease enzyme. Suitably, the selective reagent is immobilized in the device, for example by chemical or physical bonding to a solid substrate in said device, as described in more detail below.

As noted above, the diagnostic apparatus according to the present invention may contain one or more selective binding partners to bind the one or more analyte molecules present in the sample. Suitable immunological binding partners include polyclonal antibodies and monoclonal antibodies.

If polyclonal antibodies are desired, a selected mammal, such as a mouse, rabbit, goat or horse, may be immunised with the monitored marker. The monitored marker used to immunise the animal can be obtained by any suitable technique, for example, it can be purified from a wound fluid sample from an infected wound, it can be derived by recombinant DNA technology or it can be synthesized chemically. If desired, the monitored marker can be conjugated to a carrier protein. Commonly used carriers to which the monitored markers may be chemically coupled include bovine serum albumin, thyroglobulin and keyhole limpet haemocyanin. The optionally coupled monitored marker is then used to immunise the animal. Serum from the immunised animal is collected and treated according to known procedures, for example by immunoaffinity chromatography.

Monoclonal antibodies to the monitored marker can also be readily produced by one skilled in the art. The general methodology for making monoclonal antibodies using hybridoma technology is well known.

Panels of monoclonal antibodies produced against the monitored marker can be screened for various properties, *i.e.*, for isotype, epitope, affinity, etc. Alternatively, genes encoding the monoclonal antibodies of interest may be isolated from hybridomas, for instance by PCR techniques known in the art, and cloned and expressed in appropriate vectors.

Chimeric antibodies, in which non-human variable regions are joined or fused to human constant regions may also be of use. Humanised antibodies may also be used. The term "humanised antibody", as used herein, refers to antibody molecules in which the CDR amino acids and selected other amino acids in the variable domains of the heavy and/or light chains of a non-human donor antibody have been substituted in place of the equivalent amino acids in a human antibody. The humanised antibody thus closely resembles a human antibody but has the binding ability of the donor antibody.

In a further alternative, the antibody may be a "bispecific" antibody, that is, an antibody having two different antigen binding domains, each domain being directed against a different epitope.

Phage display technology may be utilised to select genes which encode antibodies with binding

activities towards the monitored marker either from repertoires of PCR amplified V-genes of lymphocytes from humans screened for possessing the relevant antibodies, or from naive libraries. The affinity of these antibodies can also be improved by chain shuffling.

Where antibodies generated by the above techniques, whether polyclonal or monoclonal, are employed as reagents in immunoassays, radioimmunoassays (RIA) or enzyme-linked immunosorbent assays (ELISA), the antibodies can be labelled with an analytically-detectable reagent such as a radioisotope, a fluorescent molecule or an enzyme.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')2 and Fv, which are capable of binding to the antigenic determinant in question. Such antibodies thus bind to the monitored marker.

Suitably, the immunological or other binding partners are immobilised on a solid support material, for example by avidin-biotin linking, or dialdehyde derivatization of the support material, followed by cross-linking to a peptide binding partner. The apparatus may further comprise other immunological binding partners and/or reagents or indicator molecules may for example in a solution that is added to the wound fluid sample.

In some embodiments of any previous embodiment or aspect, the apparatus comprises a solid support material having an immunological binding partner for an analyte moiety covalently linked thereto. The solid support materials bearing immunological or other binding partners may be used in a range of immunoassays to analyse the presence of the analytes of interest. For example, the support having antibodies or antibody fragments bound thereto may be used in sandwich immunoassay-type analysis. Alternatively, the support may have analog ligands bound to the antibodies, whereby the molecules present in the wound fluid are detected by affinity displacement immunoassay. Various other immunoassays will be apparent to persons skilled in the art.

The analytes of interest are protease enzymes that can modify substrates such as proteins or polypeptides, by cleavage. Such modification of peptide substrates can be detected to determine the presence or absence of the analyte in a sample. Accordingly, in one embodiment, the present invention provides a diagnostic apparatus according to any preceding aspect or embodiment wherein the apparatus comprises an indicator moiety that is immobilized or inhibited by a chemical moiety, wherein the chemical moiety comprises an exogenous peptide substrate for the protease enzyme, and the exogenous peptide substrate is cleavable by the analyte protease enzyme to release or activate the indicator moiety.

Suitably, the indicator moiety comprises an indicator enzyme, an enzyme cofactor, a dye, a radioactive moiety, a spin label, a luminescent moiety or a fluorophore. Suitably, the indicator moiety comprises an indicator enzyme or a fluorophore. Suitable indicator enzymes may for example be selected from the group consisting of a laccase (CotA enzyme), alkaline phosphatase, p-galactosidase, acetylcholinesterase, green fluorescent proteins, luciferases and horseradish peroxidases. Suitable fluorophores include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, coumarin derivatives such as 7-amino-4-methyl coumarin, and phycoerythrin. Suitable luminescent moieties include luminol, luciferase, luciferin, and aequorin.

In the devices in which the indicator moiety comprises an enzyme, the device suitably further comprises a substrate that interacts with the indicator enzyme to give a detectable spectrophotometric, colorimetric, fluorimetric, luminescent, electrochemical or radioactive signal.

In certain embodiments, the indicator moiety comprises an indicator enzyme and the chemical moiety inhibits the indicator enzyme by sterically hindering an active site of the indicator enzyme, or by causing the indicator enzyme to fold into an inactive conformation. Alternatively or additionally, the chemical moiety may tether the enzyme to a solid substrate, whereby release from the substrate by action of the analyte protease activates the enzyme and/or allows the enzyme to migrate to a remote substrate location where it reacts with a suitable substrate (which may be immobilized at the remote location) to give a detectable signal. In yet other embodiments, the device comprises two indicator enzyme moieties linked by the chemical moiety, and cleavage of said peptide by the host-derived protease enzyme results in activation of both enzyme moieties.

In other embodiments, the device comprises an indicator enzyme, and a cofactor for the enzyme that is immobilized or inhibited by the chemical moiety, whereby cleavage of peptide releases or activates the cofactor.

In certain embodiments, the indicator moiety is tethered to a solid substrate by said chemical moiety, and is released from said substrate by cleavage of said exogenous peptide substrate by said protease.

One method for detecting the modification of a substrate by an enzyme is to label the substrate with two different dyes, where one dye serves to quench the fluorescence of the other dye by fluorescence resonance energy transfer (FRET) when the dye molecules are in close proximity. A typical acceptor and donor pair for resonance energy transfer consists of 4-[[-(dimethylamino)phenyl]azo]benzoic acid (DABCYL) and 5-[(2-aminoethylamino] naphthalene sulfonic acid (EDANS). EDANS is excited by illumination with a wavelength of 336 nanometers, and emits a photon with a wavelength of 490 nanometers. If a DABCYL moiety is located within 2 nanometers of the EDANS, this photon will be efficiently absorbed. DABCYL and EDANS can be attached to opposite ends of a peptide in the diagnostic material used in the systems of the present invention. If the peptide is intact, FRET will be very efficient. If the peptide has been cleaved by an enzyme analyte, the two dyes will no longer be in close proximity and FRET will be inefficient. The cleavage reaction can be followed by observing either a decrease in DABCYL fluorescence or an increase in EDANS fluorescence (loss of quenching).

Another suitable diagnostic material for use in the systems of the present invention comprises a chromogenic dye conjugated to a solid support by a suitable cleavable substrate moiety, such as a peptide. The chromogenic dye will change color when the linker group is cleaved by the enzyme of interest. For example, para-nitrophenyl is colorless when linked to the support, and turns yellow when cleaved. The analyte concentration can be determined by measuring absorbance at 415 nanometers. Other dyes that produce detectable color change upon cleavage are known to those skilled in the art.

In yet another embodiment, the diagnostic material may comprise a colored support having a differently-colored molecule conjugated thereto by a linker moiety that can be cleaved by an enzyme in the sample. Cleavage of the dye from the colored support can thereby result in a color change of the diagnostic material.

The solid support materials used for the above identified assays of enzyme activity and immuno-assays may comprise any suitable natural or synthetic polymer, including insoluble polysaccharides such as cellulose, and synthetic polymers such as polyacrylates. The cleavable cross-linkages, where present, generally comprise cleavable oligopeptidic sequences or cleavable oligosaccharides, each typically of twenty residues or fewer, for example from 3 to 15 residues.

The sensitivity of the diagnostic material will depend on a number of factors, including the length of the cleavable linker sequences. Steric hindrance may also be reduced by coupling the cleavable oligopeptidic sequence to the polymer by means of an appropriate spacer. Thus, the oligopeptidic sequences may couple the polymers directly (in which case the cross-linkage consists of the oligopeptidic sequence) or by means of an appropriate spacer. Suitable conjugation methods incorporating spacers are described in US-A-5770229.

Particularly preferred chemical systems for use in the devices of the present invention are described in WO03/063693 and WO2005/021780, the entire contents of which are incorporated herein by reference.

In one embodiment, the indicator enzyme is a laccase that has been inhibited by the peptide substrate. Laccase (diphenol oxidase) is a member of the multi-copper oxidase family of enzymes. Generally, these enzymes require oxygen to oxidize phenols, polyphenols aromatic amines, and other non-phenolic substrates by one electron to create a radical species. It is a suitable indicator enzyme in part due to its stability and oxidation properties. The oxidation of species results in an upaired electron which generates a color change. CotA is highly thermostable.

CotA can be used in the apparatus and devices of the present invention by modifying the sequence to generate a proenzyme form. Analysis of the structure of CotA indicates that an extension of suitable length appended onto the N-terminus of CotA can allow an appended inhibitor to be placed in the active site of the enzyme. The extension peptide is selected to be a cleavage target of the analyte protease. This will allow the blocking extension to be cleaved in the presence of the analyte protease. Analysis of the x-ray structure of CotA has shown that the length of the amino acid chain needed to reach the shortest distance around the structure is about 3nm.

The modified enzymes with the peptide extension block can be prepared and screened for suitability using standard recombinant methods as described in more detail in WO2005/021780.

As already noted, the endogenous proteases to be detected include elastase. For elastase, suitable substrate linkers may include one or more of the oligopeptidic sequences Lys-Gly-Ala-Ala-Ala-Lys-Ala-Ala-Ala-, Ala-Ala-Pro-Val, Ala-Ala-Pro-Leu, Ala-Ala-Pro-Phe, Ala-

Ala-Pro-Ala or Ala-Tyr-Leu-Val. For example, the substrate may be MeOSuc-Ala-Ala-Pro-Val-AMC, wherein MeOsuc is a succinyl methyl ester residue and AMC is a 7-amino-4-methyl coumarin residue.

The proteases to be detected also include matrix metalloproteinases, such as MMP-1 and MMP-9. Suitable cleavable linkers for matrix metalloproteinases may comprise the oligopeptidic sequence -Gly-Pro-Y-Gly-Pro-Z-, -Gly-Pro-Leu-Gly-Pro-Z-,-Gly-Pro-Ile-Gly-Pro-Z-, or -Ala-Pro-Gly-Leu-Z-, where Y and Z are amino acids.

Fragments and sequence variants of the polypeptides and nucleic acids described above may also be used in the apparatus and methods of the present invention. Functional variants can contain substitution of similar amino acids that result in no change or an insignificant change in function. Alternatively, such substitutions may positively or negatively affect function to some degree.

In certain embodiments, the apparatus or device(s) according to the present invention comprise, or consist essentially of a wound dressing, dipstick or swab. In one embodiment, the invention provides a diagnostic apparatus according to any preceding aspect or embodiment, comprising a dip-stick or swab for sampling of said wound fluid.

Immobilisation of reaction components onto a dipstick, wound mapping sheet or other solid or gel substrate offers the opportunity of performing a more quantitative measurement. For example, in the case of a reaction linked to the generation of a colour the device may be transferred to a spectrometer. Suitable methods of analysis will be apparent to those of skill in the art.

Immobilisation of the reaction components to a small biosensor device will also have the advantage that less of the components (such as enzyme and substrate) are needed. The device will thus be less expensive to manufacture than a dressing that needs to have a large surface area in order to allow the mapping of a large wound area.

Methods for the incorporation of the components of the assay reaction onto a clinical dressing, "dipstick", sheet or other biosensor are routine in the art. See for example Fägerstam and Karlsson (1994) *Immunochemistry*, 949-970.

In one embodiment, the apparatus or device of the invention comprises a reference assay element for determining the total protein content of the sample, so that the measured levels of marker can be normalised to constant total protein level in order to increase accuracy.

In certain embodiments, the apparatus or device(s) in the apparatus according to the present invention comprises a housing containing one or more reagents and having an inlet provided therein for introduction of the sample. The housing may be at least partially transparent, or may have windows provided therein, for observation of an indicator region that undergoes a color or fluorescence change. In certain embodiments, the device operates on the lateral flow principle. That is to say, said device comprises a housing having an inlet for the sample and side walls defining a fluid lateral flow path extending from the inlet.

By "**lateral flow**", it is meant liquid flow in which the dissolved or dispersed components of the sample are carried, suitably at substantially equal rates, and with relatively unimpaired flow, laterally through the carrier. Suitably, the fluid flow path contains one or more porous carrier materials. The porous carrier materials are suitably in fluid communication along substantially the whole fluid flow path so as to assist transfer of fluid along the path by capillary action. Suitably, the porous carrier materials are hydrophilic, but suitably they do not themselves absorb water. The porous carrier materials may function as solid substrates for attachment of reagents or indicator moieties. In certain embodiments of the present invention, the device further comprises a control moiety located in a control zone in said in said device, wherein the control moiety can interact with a component of the wound fluid sample to improve the accuracy of the device.

The size and shape of the carrier are not critical and may vary. The carrier defines a lateral flow path. Suitably, the porous carrier is in the form of one or more elongate strips or columns. In certain embodiments, the porous carrier is one or more elongate strips of sheet material, or a plurality of sheets making up in combination an elongate strip. One or more reaction zones and detection zones would then normally be spaced apart along the long axis of the strip. However, in some embodiments the porous carrier could, for example be in other sheet forms, such as a disk. In these cases the reaction zones and detection zones would normally be arranged concentrically around the center of the sheet, with a sample application zone in the center of the sheet. In yet other embodiments, the carrier is formed of carrier beads, for example beads made from any of the materials described above. The beads may suitably be sized from about 1 micrometer to about 1mm. The beads may be packed into the flow path inside the housing, or may be captured or supported on a suitable porous substrate such as a glass fiber pad.

As discussed above, the apparatus or device(s) in the apparatus according to the present invention may be adapted to detect more than one protease or other analyte. In one embodiment, a single device may be adapted to detect each of the proteases to be determined. For example, a single device may be adapted to detect each of the elastases and matrix metalloproteinases to be determined, such as active elastase, MMP-1 and MMP-9, *e.g.* neutrophil elastase, MMP-1 and MMP-9. Alternatively, a single device may be adapted to detect the protease enzymes and their respective protease enzyme inhibitors. Suitably, a single device may be adapted to detect the protease enzymes but not their respective protease enzyme inhibitors. In any of these cases, the detection of more than one protease or other analyte can be done by the use of several different reagents in a single reaction zone, or suitably by the provision in a single device of a plurality of lateral flow paths each adapted for detecting a different analyte. In certain embodiments, the plurality of lateral flow paths are defined as separate fluid flow paths in the housing, for example the plurality of lateral flow paths may be radially distributed around a sample receiving port. In some embodiments, the plurality of fluid flow paths are physically separated by the housing. In other embodiments multiple lateral flow paths (lanes) can be defined in a single lateral flow membrane by depositing lines of wax or similar hydrophobic material between the lanes.

The apparatus or device(s) of the apparatus according to the present invention may for example be incorporated into a bacterial sensing device of the kind described in UK patent application GB-A-2422664 filed on 28th January 2005, the entire content of which is incorporated herein by reference.

An absorbent element may suitably be included in the devices of the present invention. The absorbent element is a means for drawing the whole sample through the device by capillary attraction. Generally, the absorbent element will consist of a hydrophilic absorbent material such as a woven or nonwoven textile material, a filter paper or a glass fiber filter.

The apparatus or device(s) of the apparatus according to the present invention may further comprise at least one filtration element to remove impurities from the sample before the sample undergoes analysis. The filtration device may for example comprise a microporous filtration sheet for removal of cells and other particulate debris from the sample. The filtration device is typically provided upstream of the sample application zone of the fluid flow path, for example in the inlet of the housing or in the housing upstream of the inlet.

In certain embodiments, the apparatus or devices in the apparatus according to the present invention include a control moiety in a control zone of the device, wherein the control moiety

can interact with a component of the wound fluid sample to improve the accuracy of the device. Suitably, the control zone is adapted to reduce false positive or false negative results. A false negative result could arise for various reasons, including (1) the sample is too dilute, or (2) the sample was too small to start with.

In order to address false negative mechanism, the control zone suitably further comprises a reference assay element for determining the total protease content or the total protein content of the sample, that is to say for establishing that the total protease content or the total protein content of the sample is higher than a predetermined minimum. It is possible to indicate the presence of protein by the use of tetrabromophenol blue, which changes from colorless to blue depending on the concentration of protein present. It is also possible to detect glucose (using glucose oxidase), blood (using diisopropyl-benzene dihydroperoxide and tetramethylbenzidine), leukocytes (using ester and diazonium salt). These may all be useful analytes for detection in the control zone for the reduction of false negatives.

In certain embodiments, the apparatus according to the present invention may further comprise one or more components selected from: a color chart for interpreting the output of the diagnostic device, a sampling device for collecting a sample of a biological fluid such as a wound fluid, a wash liquid for carrying a sample of fluid through the device, and a pretreatment solution containing a reagent for pretreatment of the fluid sample.

Where present, the sampling device may comprise a swab or a biopsy punch, for example a shaft having a swab or biopsy punch attached thereto. Suitably, in these embodiments the diagnostic device includes a sample receiving port, and suitably the sample receiving port and the swab or biopsy punch comprise complementary fitting elements whereby the swab or biopsy punch can be secured to the device with the swab or biopsy punch received in the sample receiving port.

In certain embodiments the fitting element on the shaft may be located from 1mm to about 30mm from the base of the swab or the biopsy punch. This is consistent with the use of relatively small sample receiving port on the housing of the diagnostic device. The sample receiving port is typically located on an upper surface of the diagnostic device, and it is typically generally in the form of an upwardly projecting tube, open at the top and having the inlet to the fluid flow path located at the bottom of the tube. Suitable swabs, biopsy punches and sample receiving caps are described in detail in UK patent applications GB-A-2411230 and GB-A-2411231 both filed on 23rd February 2004, the entire contents of which are incorporated herein by reference.

The fitting element on the shaft may a tapered region of the shaft for forming an interference fit with the housing, for example it may appear as a truncated cone that is coaxial with the shaft and tapers towards the first end of the shaft. Or the whole shaft may have a diameter larger than that of the swab or biopsy punch, with a tapered region adjacent to the first end. In any case, the diameter of the tapered region where it engages with the housing is normally greater than the diameter of the swab or biopsy punch, so that the inlet port can enclose the swab or biopsy punch.

In other embodiments, the engagement element may comprise a snap-fitting projection for forming a snap-fit with one or more complementary projections on an inner surface of the housing, or a threaded projection for forming a screw fit with one or more complementary threads on an inner surface of the cap, or a Luer-lock type fitting.

The swab may be any absorbent swab, for example a nonwoven fibrous swab. Typically the diameter of the swab is about 2 to about 5mm, for example about 3mm. In certain embodiments, the swab may be formed from a medically acceptable open-celled foam, for example a polyurethane foam, since such foams have high absorbency and can readily be squeezed to expel absorbed fluids. The biopsy punch will typically be a stainless steel cylindrical punch of diameter about 1mm to about 10mm, for example about 3mm to about 8mm, suitably about 6mm.

In certain embodiments the shaft is hollow, whereby a fluid can be passed down the shaft from the second end to expel the biological sample from the swab or the biopsy punch into the diagnostic device. This helps to ensure that the entire sample passes through the device, thereby avoiding false negatives. The shaft may comprise a fitting at the second end for attachment of a syringe or other source of the fluid. In certain embodiments, the apparatus may comprise a reservoir of liquid attached to the second end of the shaft, for example a compressible bulb containing the liquid, which can be activated after use of the swab or biopsy punch. Suitable devices of this kind are described, for example in US-A-5266266, the entire content of which is incorporated herein by reference. In other embodiments, the apparatus may comprise a plunger that can be pushed down the hollow bore of the shaft to expel fluid or other specimens from the swab or biopsy punch.

Another advantage of the hollow shaft is that, where the apparatus is a biopsy punch, the biopsy sample can more readily be pushed or blown out of the punch. The biopsy punch apparatus can further comprise a homogenizing tool that can be passed down the hollow shaft to homogenize a tissue sample in the biopsy punch. This step of homogenizing can be

followed, if necessary, by passing liquid down the shaft from the second end to expel the homogenized tissue from the biopsy punch into the device for diagnostic analysis.

The swab or biopsy punch may be sterilized, and may be packaged in a microorganism-impermeable container.

In a third aspect, the present invention provides a kit comprising a diagnostic apparatus according to any preceding aspect or embodiment as described above and a wound dressing.

In a fourth aspect, the present invention provides a method for predicting whether a subject would be responsive to treatment of a wound that exudes a wound fluid, the method comprising simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid wherein the amounts of protease inhibitors in the wound sample are not determined.

In a fifth aspect, the present invention provides a method for predicting whether a subject would be responsive to treatment of a wound that exudes a wound fluid, the method comprising simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid.

In one embodiment of the fourth or fifth aspect, the treatment comprises applying a wound dressing to the wound.

In a sixth aspect, the invention provides a method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid wherein the amounts of protease inhibitors in the wound sample are not determined; and

(b) applying a wound dressing to the wound.

In a seventh aspect, the invention provides a method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid; and

(b) applying a wound dressing to the wound.

In an eighth aspect, the invention provides a method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid at a point in time, wherein the amounts of protease inhibitors in the wound sample are not determined;

(b) applying a wound dressing to the wound;

(c) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid at a subsequent point in time, wherein the amounts of protease inhibitors in the wound sample are not determined; and

(d) applying a wound dressing to the wound if the combined amount of the at least one elastase and at least one matrix metalloproteinase in the wound fluid in step (c) is indicative of a wound that would respond well to wound treatment.

In a ninth aspect, the invention provides a method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid at a point in time;

(b) applying a wound dressing to the wound;

(c) simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid at a subsequent point in time; and

(d) applying a wound dressing to the wound if the combined amount of the elastase, MMP-1 and MMP-9 in the wound fluid in step (c) is indicative of a wound that would respond well to wound treatment.

Typically, in the fifth, seventh or ninth aspects, the amounts of protease inhibitors in the wound sample are not determined.

In some embodiments of the above aspects or embodiments, the step of determining comprises establishing whether the combined amount of the proteases falls within a predetermined range; and / or comparing the combined amount of said proteases with a control standard.

In some embodiments of the above aspects, the step of applying a wound dressing to the wound is performed only when the amount of the protease is determined to fall within a

predetermined range that is indicative of a wound that would respond well to wound treatment as described above; or if a comparison of the combined amount of proteases with a control indicates that the wound would be responsive to treatment with said wound dressing. This provides the benefit of minimising the treatment of patients that would be likely to be unresponsive to said treatment.

Suitably, the wound dressings defined above in relation to any of the third to ninth aspects of the invention are as hereinbefore defined in relation to the first or second aspects of the invention. Suitably, the step of determining the amount of proteases in relation to any of the third to ninth aspects of the invention is performed by means of an apparatus or device according to the first or second aspects or of any embodiment of the first or second aspects disclosed above by one of the methods hereinbefore described in relation to the first or second aspects of the invention.

In suitable aspects or embodiments of the above aspects, the wound being assayed, monitored, sampled or treated has not previously been dressed by a wound dressing. In typical embodiments of the above aspects, the wound being assayed, monitored, sampled or treated has not previously been dressed by a wound dressing comprising oxidised cellulose, such as a wound dressing comprising collagen/ORC.

The wound dressing according to the present invention includes a wound contacting material comprising the oxidized cellulose. The term "**wound contacting material**" encompasses materials that do not contact the wound surface directly, but that contact the wound fluid e.g. through a porous top sheet. The wound contacting material is normally the wound contacting layer of the dressing in use, and may for example be selected from the group consisting of woven, nonwoven and knitted fabrics, freeze-dried sponges and solvent-dried sponges comprising the oxidized cellulose. The wound contacting material may comprise at least 10% of oxidized cellulose, for example at least 20% or at least 30% by weight of oxidized cellulose.

The methods according to the above aspects of the present invention may comprise an aqueous assay step. Wound fluid may be extracted directly from the environment of the wound, or can be washed off the wound using a saline buffer. The resulting solution can then be assayed for the concentration of the marker in, for example, a test tube or in a microassay plate.

Such a method will be preferable for use in cases in which the wound is too small or too inaccessible to allow access of a diagnostic device such as a dipstick. This method has the additional advantage that the wound exudate sample may be diluted. The values obtained for a diluted sample of wound fluid may be normalised relative to the total protein concentration in the sample. Total protein concentration can be calculated as described in Example 4.

It will be clear that an aqueous assay system is more applicable to use in a laboratory environment, whereas a diagnostic device containing the necessary reaction components will be more suitable for use in a hospital or domestic environment.

TREATMENT OF CONDITIONS

The apparatus, devices, kits and methods of the present invention are useful in prognosing or treating wounds, particularly wounds that exude a wound fluid. Any type of wound may be diagnosed for treatment using the apparatus and methods of the present invention, particularly if said wound exudes a wound fluid. For example, the wound may be a chronic or acute wound. In one embodiment the wound is an acute wound. An example of an acute wound is an acute traumatic laceration, perhaps resulting from an intentional operative incision. In another embodiment the wound is a chronic wound. Suitably, the chronic wound is selected from the group consisting of venous ulcers, pressure sores, decubitis ulcers, diabetic ulcers and chronic ulcers of unknown aetiology.

To allow measurement of concentration of a protease in the wound fluid, a sample of wound fluid must be added to the measurement apparatus. Measurement may either be made *in situ*, or fluid may be removed from the wound for analysis in the apparatus or device of the invention. Suitably, the fluid is removed from the wound for analysis.

GENERAL

The term "**determining**" includes measuring a numerical value of said proteases; establishing if the amount or combined amount falls above or below a predetermined range; and / or comparing the numerical value with a control standard. For example, determining includes measuring the activity and or concentration of one or more analytes in a wound sample.

The term "**amount**" is used herein to signify the numerical value of a particular analyte (*e.g.* a protease) in a wound fluid. Typically, the amount of an individual analyte is expressed in terms of its free concentration or its activity. Most typically, the term amount is used to indicate the activity of a particular analyte.

When used herein, the term "**combined amount**" refers to a single numerical value that results from the application of a mathematical function to a plurality of values, for example those amounts obtained for a number of individual analytes. For example, the term "combined amount" may refer to the sum or product of a group of individual values. Typically, the term "combined amount" relates to the sum of a group of individual values. For example, in suitable embodiments, the amount of elastase refers to elastase-like activity (*e.g.* in RFU/min/mL) and the amount of metalloproteinase (MMP) refers to total concentration of the respective analyte (*e.g.* in ng/mL).

When used herein, the term "**quantifying**" refers to measuring an absolute numerical quantity of a particular analyte(s) or substrate(s) in a sample, within the margins of experimental error.

The term "**marker**" or "**analyte**" refers to any chemical entity that is identified or determined using the apparatus, devices, kits or methods defined herein. Typically, the markers or analytes determined or identified by the apparatus, devices, kits or methods of the present invention are protease enzymes.

The term "**oxidized cellulose**" refers to any material produced by the oxidation of cellulose. In suitable embodiments of the invention, the wound dressing provided in the above aspects of the invention comprises oxidized cellulose. In one embodiment, the oxidized cellulose dressing comprises oxidized regenerated cellulose. Typically, the wound dressing further comprises collagen or chitosan.

For example, oxidation may be performed with dinitrogen tetroxide. Such oxidation converts primary alcohol groups on the saccharide residues to carboxylic acid groups, forming uronic acid residues within the cellulose chain. The oxidation generally does not proceed with complete selectivity, and as a result hydroxyl groups on carbons 2 and 3 are occasionally converted to the keto form. These keto units introduce an alkali labile link, which at pH 7 or higher initiates the decomposition of the polymer via formation of a lactone and sugar ring

cleavage. As a result, oxidized cellulose is biodegradable and bioabsorbable under physiological conditions.

A particularly suitable oxidized cellulose for practical applications is oxidized regenerated cellulose (ORC) prepared by oxidation of a regenerated cellulose, such as rayon. It has been known for some time that ORC has haemostatic properties. ORC has been available as a haemostatic product called SURGICEL (Registered Trade Mark of Johnson & Johnson Medical, Inc.) since 1950. This product is produced by the oxidation of a knitted rayon material. A modification of porosity, density and knit pattern led to the launch of a second ORC fabric product, INTERCEED (Registered Trade Mark of Johnson & Johnson Medical, Inc.), which was shown to reduce the extent of post-surgical adhesions in abdominal surgery.

Thus, in particular embodiments of the present invention, the oxidized cellulose in the wound dressing material is complexed with collagen and/or chitosan to form structures of the kind described in WO 98/00180, EP-A-1153622, WO 2004/026200, EP-A-1539258, WO 2004/024197 and/or EP-A-1536845, the entire contents of which are expressly incorporated herein by reference. For example, the oxidized cellulose may be in the form of milled ORC fibres that are dispersed in a freeze-dried collagen or chitosan sponge. This provides for sustained release of the oxidized cellulose to the wound, together with certain therapeutic and synergistic effects arising from the complexation with collagen. Suitably, the weight ratio of oxidized cellulose to collagen and/or chitosan in the wound contacting material is from about 10:1 to about 1:10, for example from about 70:30 to about 30:70. Suitably, the wound contacting material comprises at least 75% on a dry weight basis of oxidized cellulose, collagen and chitosan, more suitably at least 90% and most suitably it consists essentially of oxidized cellulose, collagen and/or chitosan. Such oxidised cellulose wound dressings may also comprise silver. Suitable commercially available wound dressings comprising oxidized cellulose are PROMOGRAN™ and PROMOGRAN PRISMA™ (Systagenix Wound Management).

The term "**a wound fluid**" refers to any wound exudate or other fluid (suitably substantially not including blood) that is present at the surface of the wound, or that is removed from the wound surface by aspiration, absorption or washing. The determining, measuring or quantifying is suitably carried out on wound fluid that has been removed from the body of the patient, but can also be performed on wound fluid *in situ*. The term "wound fluid" does not normally refer to blood or tissue plasma remote from the wound site.

When used herein, the term "**predetermined range**" refers to a data range or profile that the skilled person would understand is indicative of a particular sub-class of patient. For instance, the predetermined range may be a data range or profile that is typical of a wound that would respond well to a particular wound treatment, such as oxidised cellulose therapy. Alternatively, the predetermined range may suitably refer to a data range that is typical of a wound that would not respond well to a particular wound treatment, such as oxidised cellulose therapy.

When used herein, the term "**control standard**" or "**control**" refers to a data set or profile that can be used as a reference or comparison in order to define or normalise another data point or set of data. For instance, the term "control" or "control standard" may be data set or profile that is indicative of a particular sub-class of patient. For instance, the "control" or "control standard" can be a data set or profile that can be used as a comparative tool to allow a skilled person to determine whether a wound is likely to be responsive or non-responsive to a wound treatment, such as oxidised cellulose. In one embodiment, the control standard is a data set or profile indicative of a patient that does not respond well to wound treatment. Typically, the control standard is a data set or profile indicative of a patient that responds well to wound treatment. Patients that tend to respond well to wound treatment as disclosed herein exhibit lower combined amounts of elastase and MMP than patients that tend not to respond well to the treatment. For example, patients that tend to respond well to wound treatment as disclosed herein exhibit lower combined amounts of elastase, matrix metalloproteinase MMP-1 and matrix metalloproteinase MMP-9 in a sample of wound fluid than patients that tend not to respond well to the treatment.

The skilled person would be able to easily identify whether wounds are "**responsive to treatment**" or not. In particular, the skilled person will readily be able to determine the levels of the proteases identified in the present claims that are predictive or indicative of a good response or poor response to wound treatment, particularly to treatment with wound dressings comprising oxidized cellulose. The terms "**responsive**" and "**responder(s)**" as used herein refer to wounds that are considered to respond well to wound treatment, particularly to treatment with oxidized cellulose. Similarly, "**non-responsive**" and "**non-responder(s)**" refers to wounds that are <u>not</u> considered to respond well to wound treatment, particularly to treatment with oxidized cellulose. For instance, patients who exhibit better than 50% wound closure after 4 weeks of wound treatment are considered to be responsive to said treatment.

When used herein, the term "**simultaneously**" when referring to determining or measuring a plurality markers and / or analytes refers to determining or measuring them substantially at the same time using a single apparatus or device. Alternatively, said determining or measuring simultaneously may be performed using a plurality of apparatus or devices.

When used herein, the term "**sequentially**" when referring to determining or measuring plurality markers and / or analytes refers to determining or measuring them substantially in succession using a single apparatus or device. Alternatively, said determining or measuring sequentially may be performed using a plurality of apparatus or devices.

When used herein, the term "**PROMOGRAN™**" refers to the wound dressing commercially available from Systagenix Wound Management, which can be prepared substantially as described in EP-A-1153622.

When used herein, the term "**PROMOGRAN PRISMA™**" refers to the wound dressing commercially available from Systagenix Wound Management, which can be prepared substantially as described in EP-A-1536845.

The term "**comprising**" encompasses "**including**" as well as "**consisting of**" *e.g.* an apparatus "comprising" X may consist exclusively of X or may include something additional *e.g.* X + Y.

The word "**substantially**" does not exclude "completely" *e.g.* a composition which is "substantially free" from Y may be completely free from Y. Where necessary, the word "substantially" may be omitted from the definition of the invention.

The term "**about**" in relation to a numerical value *x* is optional and means, for example, $x \pm 10$ %.

DESCRIPTION OF THE FIGURES

Figure 1 provides a box plot analysis of the sum of elastase-like activity (RFU/min/mL), MMP-1 concentration and MMP-9 concentration (ng/mL) in wound exudate taken from

patients before the wound treatment program as described in Example 4 commenced, i.e. at week 0. The data is grouped to identify patients who responded well to wound treatment (*i.e.* responders (R)) and those patients who did not respond well to treatment (*i.e.* non-responders (NR)) as defined in Example 4. The data show a significant difference ($p<0.05$) between the sum of these proteases in responders compared to non-responders before treatment.

Figure 2 provides a box plot analysis of the sum of elastase-like activity (RFU/min/mL), MMP-1 concentration and MMP-9 concentration (ng/mL) in wound exudate taken from patients after week 4 of the wound treatment program as described in Example 4. The data show a significant difference ($p<0.05$) between the sum of these proteases in responders compared to non-responders after treatment.

Figure 3 provides a box plot analysis of the total protein concentration in samples of wound fluids taken from patients before the wound treatment program as described in Example 4. The data show no significant difference between the total protein concentrations in responders compared to non-responders before treatment.

Figure 4 provides a box plot analysis of the elastase activity in samples of wound fluids taken from patients before the wound treatment program as described in Example 4. The data show no significant difference between the elastase activities in responders compared to non-responders before treatment.

Figure 5 provides a box plot analysis of the concentration of MMP-1 in samples of wound fluids taken from patients before and after the wound treatment program as described in Example 4. The data show no significant difference between the concentrations of MMP-1 in responders compared to non-responders before (week zero, $p = 0.438$) or after (week four, $p = 0.688$) treatment.

Figure 6 provides a box plot analysis of the sum of the concentrations of MMP-1 and MMP-9 in samples of wound fluids taken from patients before the wound treatment program as described in Example 4. The data show no significant difference between the total protein concentration in responders compared to non-responders before treatment.

Figure 7 provides a box plot analysis of the tumor necrosis factor receptor II (TNFR-II) concentration in samples of wound fluids taken from patients before the wound treatment

program as described in Example 4. The data show no significant difference between the total protein concentrations in responders compared to non-responders before treatment.

Figure 8 provides a box plot analysis of the vascular endothelial growth factor (VEGF) concentration in samples of wound fluids taken from patients before the wound treatment program as described in Example 4. The data show no significant difference between the total protein concentrations in responders compared to non-responders before treatment.

GENERAL METHODS

Specific wound dressing materials and methods according to the present invention will now be described further with reference to the accompanying drawings, which are described immediately above.

Preparation of the wound dressing component

The collagen/ORC sponge dressing used in these studies was commercial PROMOGRAN PRISMA™ (Systagenix Wound Management) dressing prepared substantially as described in EP-A-1536845.

Promogran Prisma is a 2% solids, freeze-dried matrix consisting of 55% Bovine Collagen, 44% Oxidised regenerated cellulose fibres (ORC) and 1% silver- ORC salt (silver acetate). To prepare the dressing ORC and silver ORC fibres are added to 0.05M acetic acid solution and mixed until evenly dispersed. To this suspension, Bovine collagen, approximately 1% solids, are added and the preparation is mixed and passed through a homogeniser to obtain a uniform slurry. The preparation is then poured into trays and freeze dried to remove the excess acetic acid and water so that the bioresorbable matrix is left behind. This is then slit to a thickness of 3mm and cut into dressing of 28 or 125 cm^2, prior to gamma sterilisation.

Clinical study and patient selection

All patients enrolled in this study had diabetic foot ulcers of at least 30 days duration and a surface area of at least 1 cm^2. Specific inclusion criteria are disclosed below:

Inclusion criteria

The patient must:

Have diabetes

Be aged between 35 to 80 years

Show no local or systemic signs of infection with normal CRP and leukocyte levels and defined as being DFU type Wagner 2-3 (>4 weeks old).

Have an ulcer of at least 30 days duration.

Be willing to return to the investigation centre for all dressing changes and wound evaluation.

Be willing to give written informed consent.

Patients were excluded if the target wound showed any signs of infection or if exposed bone with positive osteomyelitis was observed. Additional exclusion criteria included concomitant conditions or treatments that may have interfered with wound healing and a history of non-compliance that would make it unlikely that a patient would complete the study. Specific exclusion criteria are disclosed below:

Exclusion criteria

The patient must not:

Exhibit allergic reactions to any content of Promogran Prisma

Have clinical signs of infection as defined by clinicians

Be pregnant or lactating

Have a history of misuse of drugs or excessive alcohol consumption

Be undergoing chemotherapy

Have peripheral arterial disease and/or toe pressure $\leq$45 mm. Hg

Have haemolytic anemia and / or iron-deficiency anemia and/or malnutrition.

Be able to walk.

Have severe cardiac and/or hepatic and/or renal and/or pulmonary insufficiency; or chronic administration of cortisones for chronic inflammatory disease and /or auto-immune disease.

The wound must not be considered malignant.

Patients meeting the patient selection criteria disclosed above were enrolled (exact numbers for each assay method are disclosed below), and wound fluid collected. Informed consent was obtained from all patients or their authorised representatives prior to study enrolment and the protocol was approved by the Ethics Committee at the participating study centre prior to the commencement of the study. The studies herein were conducted in accordance with both the Declaration of Helsinki and Good Clinical Practice.

Wound fluid collection and extraction from dressings

Wound fluid was collected by absorption onto a piece of RELEASE* dressing, which was placed directly onto the wound and covered with BIOCLUSIVE, an occlusive film. These dressings are commercially available from Systagenix Wound Management Manufacturing Limited.

RELEASE* Non-Adherent Absorbent Dressing consists of a labeled, sterile pouch containing a rectangular web of viscose rayon fibres sandwiched between layers of non-woven fabric in a sleeve of perforated EMA film, which is sealed by a line of hot melt adhesive.

BIOCLUSIVE® is a hypoallergenic, transparent, adhesive film dressing with a three-part release coated facing paper which is impermeable to water and bacteria and permeable to moisture and oxygen. The dressings are designed for easy aseptic application through the use of three removable facing tabs. The facing tabs are bleached paper, coated on one side with polyethylene that is then covered by a silicone coating.

After 24 hours the dressing was removed from the wound and frozen at −70 °C until elution of wound fluid.

Wound fluid was eluted from the RELEASE* dressing by incubating the sample in 5-10 mL of wash buffer (0.1 M Tris/HCl, pH 7.4 containing 0.1% Triton X-100) per gram of dressing depending on surface area of the dressing to minimise the dilution of the wound fluid. To allow maximum recovery of fluid, the sample was incubated for 2 hours at room temperature with shaking. The elutent was then carefully removed, aliquoted and frozen at −70 °C until required for use.

EXAMPLE 1

Determining the amount of elastase by fluorogenic activity assay

A fluorogenic substrate, MeOSuc-Ala-Ala-Pro-Val-AMC (BaChem) which is cleaved by elastase to release the fluorogenic group 7-amino-4-methylcoumarin was used to measure elastase activity in the wound fluid samples.

The wound fluid samples were added to a black, flat bottomed microtitre plate. The final reaction mixture consisted of 5 µL of wound fluid, 175 µL of elastase assay buffer (0.1 M

hepes, 0.5 M sodium chloride, 10% dimethylsulphoxide, pH 7.5) and 20 µL substrate, which was added to a final concentration of 0.2 mM per well.

Using a fluorometer (excitation 380 nm, emission 450 nm) readings were taken immediately after addition of the substrate and then after 5, 10, 15, 20, 30, 45 and 60 minutes. The plate was incubated at 37 °C between readings. The rate of production of the fluorescent compound was measured, (fluorescence directly relates to elastase activity in the sample). The results were expressed as relative fluorescence units per minute per mL (RFU/min/mL).

EXAMPLE 2

Determining the amount of matrix metalloproteinase by protein microarray

MMP-1 and MMP-9 in wound samples were quantified using FAST Quant protein microarray (commercially available from Whatman).

Different sets of arrays were printed on 16-pad FAST slides in triplicates using a piezo-electric Perkin-Elmer BioChip Arrayer. The arrays consisted of monoclonal antibodies against a variety of cytokines (up to 11 different cytokines were quantified per array). Arrayed slides were then inspected and stored in a desiccated room until required.

Prior to analysis, the slides were removed from storage and a 16-pad hybridization chamber was attached to the slides, and the slides were placed into a FAST Frame (4 slides per frame) for processing.

Arrays were blocked for 15 minutes at room temperature using 70 µL S&S Protein Array Blocking buffer. The blocking buffer was removed and 70 µL of each wound fluid sample (at a 1:20 or 1:50 dilution), standard or control was added.

Arrays were incubated overnight at room temperature, with gentle agitation and then washed 5 times with TBS-T. Arrays were treated with 70 µL of an antibody cocktail, containing one biotinylated antibody corresponding to each of the arrayed capture antibodies. Following 1 hour incubation at room temperature with gentle agitation, the arrays were washed 5 times with TBS-T. Arrays were incubated with 70 µL of a solution containing streptavidin-Cy5 conjugate for 1 hour at room temperature, with gentle agitation. Arrays were washed 5 times with TBS-T, quickly rinsed in de-ionized water, and dried.

Slides were imaged in an Axon GenePix 4000B fluorescent imaging system. Array images were saved as 16-bit TIF files, with 10 micron pixel resolution. Images were analyzed using Imaging Research ArrayVision software. Briefly, spot intensities were determined by subtracting background signal. Spot replicates from each sample condition were averaged and then compared to the appropriate standard curves. Microsoft Excel was used for additional analysis and data presentation. The quantified amounts of MMP-1 and MMP-9 were provided in units of ng/mL.

EXAMPLE 3

Protein Assay

Total protein present in each extracted wound fluid sample was determined using the Bradford protein assay. The protein binding solution comprises 1 ml Coomassie Brillant Blue stock solution 200mg-Coomassie Brillant Blue G250, Sigma Chemical Co., dissolved in 50 ml ethanol-90%); 2ml orthophosphoric acid (85% w/v); in a final volume of 20 ml with distilled water. This solution was filtered (Whatman #1 filter paper) and used immediately. The protein level in a sample wound fluid was measured by mixing 10-µl sample or standard with 190-µl of the protein binding solution in a microtitre well and incubating for 30mins at ambient temperature prior to reading absorbance at 595nm. The concentration of protein was estimated from a standard calibration of BSA (bovine serum albumin prepared in distilled water; Sigma Chemical Co.) ranging from 1.0 to 001 mg/ml.

EXAMPLE 4

Wound Sampling

Twenty six patients meeting the patient selection criteria disclosed above were enrolled, and wound fluid collected. Wound fluid was collected again after four weeks.

The amount of elastase-like activity (as indicated by the method of Example 1), MMP-1 and MMP-9 (as indicated by the method of Example 2) in the wound fluid of each patient was analysed immediately before wound treatment commenced.

Eighteen of the twenty six patients were then treated by application of PROMOGRAN PRISMA^{TM} dressing to the whole surface of the ulcer, together with suitable secondary dressings to hold the PROMOGRAN PRISMA^{TM} in place. The remaining eight control

patients received "good standard care". Patients were treated with a range of different dressings, including:

Dressing	Manufacturer	Brief description
Physiotulle	Coloplast	Physiotulle is a non-adherent, non-occlusive polyester net impregnated with hydrocolloid particles suspended in Vaseline.
Physiotulle Ag	Coloplast	non-adherent, moist wound healing contact layer with silver sulphadiazine
Biatain-Ibu	Coloplast	foam dressing which releases ibuprofen.
Mesorb	Mölnlycke Health Care Ltd	Mesorb is made of two layers of a permeable, smooth nonwoven which ensures good skin compatibility and exudate permeability. The soft, thick fluff pulp core combines excellent absorption capacity with good protective cushioning and the air-permeable and fluid-repellent nonwoven backing provides an excellent exudate barrier.
Silver dressing	Information not available	
Silver Gauze		
Foam dressing		
Silver alginate		

The wound fluid from each patient was sampled again after four weeks in the same manner as disclosed above. Patients who developed symptoms of infection, or whose treatment was discontinued for other reasons, were excluded from the study.

All patients completed the trial to week 4. However, wound analysis could not be completed for three of these patients due to low abundance of wound fluid.

The groups of patients receiving treatment in this trial could be divided into two sub-groups of patients:

a) Responders, i.e. patients whom exhibited better than 50 % wound closure after 4 weeks of treatment; and

b) Non-responders, i.e. patients whom exhibited less than 50 % wound closure after 4 weeks of treatment.

Experimental data analysis

The sum amounts of elastase-like activity, MMP-1 concentration and MMP-9 concentration in responders (R) before wound treatment (i.e. week 0) with PROMOGRAN PRISMA™ are compared with the corresponding data obtained from non-responders (NR) in the box plots in **Figure 1**. Elastase-like activity was calculated as RFU/min/mL according to Example 1. Both MMP-1 and MMP-9 were calculated in ng/mL according to Example 2, above.

When testing was carried out, certain values were over or under the maximum range that the assay could detect. The samples that were "under" were allocated a value of 0. For the samples that were "over", the highest point in the data set was taken and rounded up to the nearest thousand. This value was then given to all values found to be "over" the maximum range that the assay could detect.

The sum amounts of elastase-like activity, MMP-1 concentration and MMP-9 concentration in responders (R) after week 4 of wound treatment are compared with the corresponding data obtained from non-responders (NR) in the box plots in **Figure 2**. Elastase, MMP-1 and MMP-9 were calculated as described above.

Minitab statistical analysis programme was used for all statistical analysis of these data. A 2 sample t test was used for statistical analysis and differences were considered significant if $p<0.05$. The sum amounts of elastase, MMP-1 and MMP-9 as determined above were normally distributed. However, when data was not normally distributed, a Johnson transformation using the Minitab statistical analysis programme was used before the t test. Where the data could not be transformed, a Kruskal-Wallis test was used using the Minitab statistical analysis programme.

The box plots illustrated in **Figure 1** clearly show that there is, surprisingly, a statistically significant difference ($p = 0.041$) between the sum amount of elastase-like activity, MMP-1 and MMP-9 in responders **before** commencement of wound treatment (i.e. at week 0). The data in Figure 2 also confirms that this difference is also present **after** treatment (i.e. at week 4).

In particular, the combined amount of elastase, MMP-1 and MMP-9 in the wound fluid of the responders before treatment with oxidised cellulose dressing was significantly lower than the combined amounts of these proteases in the wound fluid of non-responders.

Accordingly, from these data it can be concluded that the amount of elastase, MMP-1 and MMP-9 in wound fluid is a particularly clear and reliable prognostic tool for identifying wounds that will benefit most from therapy with oxidized cellulose and to identify those that are benefitting from therapy with oxidized cellulose.

REFERENCE EXAMPLE 1

Total protein concentration

The total amount of protein in wound samples of the patients was determined according to the method of Example 3, above. The data are presented as a box plot in Figure 3 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between total protein concentration in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 4). Accordingly, the total protein concentration of a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

REFERENCE EXAMPLE 2

Elastase activity

The activity of elastase alone in wound samples of the patients was determined according to the method of Example 1, above. The data are presented as a box plot in Figure 4 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between elastase activity in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 4). Accordingly, the activity of elastase alone in a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

REFERENCE EXAMPLE 3

MMP-1 concentration

The concentration of MMP-1 alone in wound samples of the patients was determined according to the method of Example 2, above. The data are presented as a box plot in Figure 5 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between MMP-1 concentration in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 4). Accordingly, the amount of MMP-1 alone in a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

REFERENCE EXAMPLE 4

Concentration of MMP-1 + MMP-9

The combined amount of MMP-1 and MMP-9 in wound samples of the patients was determined according to the method of Example 2, above. The data are presented as a box plot in Figure 6 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between the combined sum of MMP-1 and MMP-9 concentration in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 4). Accordingly, the combined amount of MMP-1 and MMP-9 in a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

REFERENCE EXAMPLE 5

Concentration of the inflammatory cytokine tumor necrosis factor receptor II (TNFR-II)

The amount of TNFR-II in wound samples of the patients was determined by protein microarray substantially according to the method of Example 2, above. The data are presented as a box plot in Figure 7 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between the TNFR-II concentration in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 5). Accordingly, the amount of TNFR-II in a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

REFERENCE EXAMPLE 6

Concentration of the growth factor vascular endothelial growth factor (VEGF)

The amount of VEGF in wound samples of the patients was determined according to a method analogous to that described above in Example 2. The data are presented as a box plot in Figure 8 and clearly show that before treatment with oxidised cellulose commenced (i.e. at week 0), there was no significant difference between the VEGF concentration in patients identified as responders and those identified as non-responders (responders and non-responders are as defined in Example 5). Accordingly, the amount of VEGF in a sample of wound fluid before treatment with oxidised cellulose is not a useful indicator of the likelihood that the wound would respond well to said treatment.

CONCLUSION

Accordingly, from these data the inventors have found that the measurement of total protein in a sample of wound fluid, or the measurement of individual marker analytes in samples of wound fluid, such as the measurement of individual endogenous protease enzymes or inflammatory cytokines has so far been unsuccessful in predicting whether wounds would be responsive to treatment with oxidized cellulose therapy before said treatment has commenced, i.e. at week 0. On the other hand, the present inventors have found, surprisingly, that the combined amount of elastase, MMP-1 and MMP-9 in a sample of a wound fluid, whether before (i.e. week 0) or during (i.e. week 4) treatment with a protease inhibitor dressing, such as an oxidized cellulose dressing, correlates to the likelihood of (and rate of) healing by means of this therapy.

CLAIMS

1. A diagnostic apparatus for simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase (MMP) in a sample of wound fluid, wherein the amount of protease inhibitors in the sample is not determined.

2. A diagnostic apparatus for simultaneously or sequentially determining the amount of elastase, matrix metalloproteinase MMP-1 and matrix metalloproteinase MMP-9 in a sample of wound fluid.

3. A diagnostic apparatus of claim 1 or 2, wherein the elastase is neutrophil elastase.

4. A diagnostic apparatus of claims 2 or 3, wherein the amount of protease inhibitors in the sample is not determined.

5. A diagnostic apparatus according to any preceding claim, wherein the step of determining comprises establishing whether the combined amount of said proteases falls within a predetermined range.

6. A diagnostic apparatus according to any one of claims 1 to 5, wherein the step of determining comprises comparing the combined amount of said proteases with a control standard.

7. A diagnostic apparatus according to claim 5 or claim 6, wherein the step of determining comprises the additional step of providing an output signal indicating whether or not the combined amount of said proteases falls within said predetermined range; or indicating the result of the comparison between the combined amount of said proteases and said control standard.

8. A diagnostic apparatus according to any preceding claim, wherein the apparatus comprises a single diagnostic device specifically adapted for detecting the amount of each of said proteases in said sample.

9. A diagnostic apparatus according to any preceding claim, wherein the apparatus comprises an indicator moiety that is immobilized or inhibited by a chemical moiety, wherein said chemical moiety comprises an exogenous peptide substrate for said proteases, and said exogenous peptide substrate is cleavable by said proteases to release or activate said indicator moiety.

10. A diagnostic apparatus according to any preceding claim, wherein the apparatus comprises a solid support material having an immunological binding partner for an analyte moiety covalently linked thereto.

11. A diagnostic apparatus according to any preceding claim, comprising a dip-stick or swab for sampling of said wound fluid.

12. A diagnostic apparatus according to any preceding claim, wherein the sample of wound fluid is diluted before determining the amount of said proteases in said sample.

13. A kit comprising a diagnostic apparatus according to any preceding claim and a wound dressing.

14. A method for predicting whether a subject would be responsive to treatment of a wound that exudes a wound fluid, the method comprising simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid wherein the amount of protease inhibitors in the sample of wound fluid is not determined.

15. A method for predicting whether a subject would be responsive to treatment of a wound that exudes a wound fluid, the method comprising simultaneously or sequentially determining the amount of elastase, matrix metalloproteinase MMP-1 and matrix metalloproteinase MMP-9 in a sample of the wound fluid.

16. A method of claim 14 or 15, wherein the treatment comprises applying a wound dressing to the wound.

17. A method for treating a wound that exudes a wound fluid comprising the steps of:

(a) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid wherein the amount of protease inhibitors in the sample of wound fluid is not determined; and

(b) applying a wound dressing to the wound.

18. A method for treating a wound that exudes a wound fluid comprising the steps of:

(a) simultaneously or sequentially determining the amount of elastase, matrix metalloproteinase MMP-1 and matrix metalloproteinase MMP-9 in a sample of the wound fluid; and

(b) applying a wound dressing to the wound.

19. A method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid at a point in time, wherein the amounts of protease inhibitors in the wound sample are not determined;

(b) applying a wound dressing to the wound;

(c) simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in the wound fluid at a subsequent point in time, wherein the amounts of protease inhibitors in the wound sample are not determined; and

(d) applying a wound dressing to the wound if the combined amount of the at least one elastase and at least one matrix metalloproteinase in the wound fluid in step (c) is indicative of a wound that would respond well to wound treatment.

20. A method for treating a wound that exudes a wound fluid, comprising the steps of:

(a) simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid at a point in time;

(b) applying a wound dressing to the wound;

(c) simultaneously or sequentially determining the amount of elastase, MMP-1 and MMP-9 in the wound fluid at a subsequent point in time; and

(d) applying a wound dressing to the wound if the combined amount of the elastase, MMP-1 and MMP-9 in the wound fluid in step (c) is indicative of a wound that would respond well to wound treatment.

21. A method of any one of claims 14 to 20, wherein the elastase is neutrophil elastase.

22. A method of any one of claims 14, 16, 17 or 19, wherein the matrix metalloproteinases determined are MMP-1 and MMP-9.

23. A method of any one of claims 14 to 22, wherein the step of determining comprises establishing whether the combined amount of the proteases falls within a predetermined range.

24. A method of any one of claims 14 to 23, wherein the step of determining comprises comparing the combined amount of said proteases with a control standard.

25. A method of claim 23, wherein the step of applying a wound dressing to the wound is performed only when the combined amount of the proteases falls within the predetermined range.

26. A method of claim 24, wherein the step of applying a wound dressing to the wound is performed only when the comparison of the combined amount with the control indicates that the wound would be responsive to treatment with said wound dressing.

27. A kit of claim 13 or a method of any one of claims 14 to 26, wherein the wound dressing comprises oxidized cellulose.

28. A kit or method of claim 27, wherein the wound dressing comprises a combination of oxidized regenerated cellulose with collagen and/or chitosan in the dry weight ratio of from about 10:1 to about 1:10.

29. A method of any one of claims 14 to 28, wherein a wound dressing has not previously been applied to the wound.

30. A method of any one of claims 14 to 28, wherein a wound dressing comprising oxidised cellulose has not previously been applied to the wound.

31. An apparatus for use, kit for use, or method according to any preceding claim wherein the wound is a chronic or acute wound.

32. An apparatus, kit or method according to claim 31, wherein the chronic wound is selected from the group consisting of pressure sores, diabetic ulcers, venous ulcers and decubitis ulcers.

33. An apparatus, kit or method according to claim 31 or 32, wherein the acute wound is a traumatic laceration.

34. A method of any one of claims 14 to 33, wherein the step of simultaneously or sequentially determining the amount of the proteases is undertaken using a diagnostic apparatus as defined in any of claims 1 to 12 and 22 to 24.

35. A diagnostic apparatus according to any one of claims 1 to 12, or a kit according to any one of claims 13, 27 and 28, for use in a method of any one of claims 14 to 33.

36. A diagnostic apparatus or kit for use in a method as defined in claim 35, wherein said diagnostic apparatus or kit is used for simultaneously or sequentially determining the amount of at least one elastase and at least one matrix metalloproteinase in said sample of wound fluid.

37. An apparatus, kit or method of any preceding claim wherein the proteases determined are endogenous proteases.

38. An apparatus, kit or method of claim 37, wherein the endogenous proteases determined are human endogenous proteases.

Application No:	GB1101663.1	**Examiner:**	Dr Philip Mountjoy
Claims searched:	1-38	**Date of search:**	31 May 2011

Patents Act 1977: Search Report under Section 17

Documents considered to be relevant:

Category	Relevant to claims	Identity of document and passage or figure of particular relevance
X,Y	X: 1-26, and 29-38; Y: 27 and 28	WO03/058237 A1 (KIMBERLY CLARK CO) - See entire document, particularly the abstract, page 5, lines 2-16, page 9, lines 14-17, page 10, lines 1-5, page 11, lines 7-11, and the claims.
X	1-26, and 29-38	Macromolecular Bioscience, Vol. 10, No. 10, Oct 2010, AG Patrick et al., "Hydrogels for the detection and management of protease levels.", pages 1184 - 1193. See entire document, particularly the abstract.
X,Y	X: 1-26, and 29-38; Y: 27 and 28	Wound Repair and Regeneration, Vol. 7, No. 5, Sept-Oct 1999, JF Tarlton, et al., "Prognostic value of markers of collagen remodeling in venous ulcers", pages 347-355 See entire document, particularly the abstract.
X,Y	X: 1-26, and 29-38; Y: 27 and 28	GB2422664 A (ETHICON INC) - See entire document, particularly page 1, lines 3-5, and the claims.
X,Y	X: 1-26, and 29-38; Y: 27 and 28	WO2008/070865 A2 (UNIVERSITY OF FLORIDA) - See entire document, particularly page 5, lines 2-14, page 9, lines 1-5, and claims 16-19.
X,Y	X: 1-26, and 29-38; Y: 27 and 28	Archives of Dermatological Research, Vol. 302, No. 6, Aug 2010, C Wiegand, et al., "Protease and pro-inflammatory cytokine concentrations are elevated in chronic compared to acute wounds and can be modulated by collagen type I in vitro", pages 419-428. See entire document, particularly the abstract, page 22 (re. assays for elastase and MMPs) and page 427 (re. effects of collagen on elastase and MMPs in wounds).
X	2, 15, 18, and 20 at least	WO2006/030232 A2 (ETHICON INC) - See entire document, particularly the abstract and the claims.
Y	27 and 28	GB2393120 A (JOHNSON & JOHNSON MEDICAL LTD) - See entire document, particularly the abstract, page 19, lines 8-10, and page 20, lines 5-7.

Y	27 and 28	International Wound Journal, Vol. 5, No. 2, Jun 2008, R Smeets, et al., "Effect of oxidised regenerated cellulose/collagen matrix on proteases in wound exudate of patients with chronic venous ulceration", pages 195-203. See entire document, particularly the abstract, and page 201 (Key Points box).
Y	27 and 28	Wound Repair and Regeneration, Vol. 15, No. 6, Nov-Dec 2007, B Cullen, et al., "A comparison of collagen containing wound dressings to modify the chronic wound environment", page A148 See entire document.

Categories:

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.

Field of Search:

Search of GB, EP, WO & US patent documents classified in the following areas of the UKC^{X} :

Worldwide search of patent documents classified in the following areas of the IPC

C12N; C12Q; G01N

The following online and other databases have been used in the preparation of this search report

EPODOC, WPI, BIOSIS, MEDLINE

International Classification:

Subclass	Subgroup	Valid From
C12Q	0001/37	01/01/2006
C12N	0009/64	01/01/2006
C12N	0009/66	01/01/2006
G01N	0033/50	01/01/2006
G01N	0033/573	01/01/2006