(11) Application No. AU 2002359529 B2

(19) AUSTRALIAN PATENT OFFICE

(54) Title

Treatment of wounds and compositions employed

(51)[6] International Patent Classification(s)

A61K 9/08 (2006.01) 8BMEP **A61K** 33/14 20060101ALI2005122
A61K 31/075 (2006.01) 0BMJP **A61K** 33/24 20060101ALI2005100
A61K 31/19 (2006.01) 8BMEP **A61K** 33/30 20060101ALI2005100
A61K 33/00 (2006.01) 8BMEP **A61K** 45/00 20060101ALI2005122
A61K 33/06 (2006.01) 0BMJP **A61K** 47/12 20060101ALI2005122
A61K 33/14 (2006.01) 0BMJP **A61K** 47/34 20060101ALI2005122
A61K 33/24 (2006.01) 0BMJP **A61L** 15/18 20060101ALI2005100
A61K 33/30 (2006.01) 8BMEP **A61L** 15/44 20060101ALI2005100
A61K 45/00 (2006.01) 8BMEP **A61P** 17/02 20060101ALI2005122
A61K 47/12 (2006.01) 0BMJP
A61K 47/34 (2006.01) PCT/US02/38175
A61L 15/18 (2006.01)
A61L 15/44 (2006.01)
A61P 17/02 (2006.01)
A61K 9/08 20060101AFI2005122 0BMJP **A61K** 31/075 20060101ALI2005100 8BMEP **A61K** 31/19 20060101ALI2005100 8BMEP **A61K** 33/00 20060101ALI2005100 8BMEP **A61K** 33/06 20060101ALI2005100

(21) Application No: 2002359529 (22) Application Date: 2002.11.27

(87) WIPO No: WO03/045366

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/334,337	2001.11.29	US

(43) Publication Date : 2003.06.10

(43) Publication Journal Date : 2003.08.21

(71) Applicant(s)

Greystone Medical Group, Inc.

(72) Inventor(s)

Hoekstra, Hans; Monroe, Stephen H

(74) Agent/Attorney

Cullen & Co, 239 George Street, Brisbane, QLD, 4000

(56) Related Art

WO 1994011010 A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 June 2003 (05.06.2003)

PCT

(10) International Publication Number
WO 03/045366 A1

(51) International Patent Classification[7]: A61K 31/075, 31/19, 33/24, 33/30

(21) International Application Number: PCT/US02/38175

(22) International Filing Date:
27 November 2002 (27.11.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/334,337 29 November 2001 (29.11.2001) US

(71) Applicant *(for all designated States except US)*: **GREYSTONE MEDICAL GROUP, INC.** [US/US]; 3251 Poplar Avenue, Suite 200, Memphis, TN 38111 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MONROE, Stephen H.**, [US/US]; 2082 Linden, Memphis, TN 38104 (US). **HOEKSTRA, Hans** [NL/NL]; Mensinge 44, 1083 HD Amsterdam (AN).

(74) Agents: **HODGES, Paul, E.** et al.; PITTS & BRITTIAN, P.C., P.O. Box 51295, Knoxville, TN 37950-1295 (US).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
with international search report

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

WO 03/045366 A1

(54) Title: TREATMENT OF WOUNDS AND COMPOSITIONS EMPLOYED

(57) Abstract: A synthesized composition containing zinc ions, calcium ions, rubidium ions and/or potassium ions in a pharmaceutically acceptable carrier, which, when applied to an open wound, effectively modulates the activity of at least MMP-2 and/or MMP-9 in the wound. A method for treatment of wounds is disclosed.

TITLE OF THE INVENTION

TREATMENT OF WOUNDS AND COMPOSITIONS EMPLOYED

RELATED APPLICATIONS

[0001] This application is a non-provisional application claiming priority based on Provisional application Serial No. 60/334,337, filed November 29, 2001, said Provisional application, in its entirety, being incorporated herein by reference.

FIELD OF INVENTION

[0002] This invention relates to the treatment of wounds, particularly open wounds which resist healing, such as decubitus ulcers and diabetic ulcers. It further relates to the use of inorganics as an aid in the establishment and/or control over the chemical environment associated with extra cellular matrices.

[0003] More particularly, this application relates to synthetic compositions for the modulation of matrix metalloproteinases (MMPs), especially in the treatment of open chronic wounds and other skin disorders.

[0004] In the prior art it is known that there exist within the human body a plurality of metal metalloproteinases. It has been suggested that at least certain of these MMPs lie relatively dormant ("Pre-MMP") until activated, whereupon various of the MMPs affect cellular growth or lack of growth, the MMPs acting at least in part through the extracellular matrix (ECM) of the cells

[0005] MMP-2 has been particularly indicated in the healing of wounds. In its inactive state, Pro-MMP-2 includes a ribbon of protein which covers its active site. Removal (cleavage) of this protein must occur before this MMP can become activated. This has been termed a "Cysteine switch". Zinc ions at the active site have been noted to activate MMP-2. Also, calcium ions at a secondary site are believed to provide the MMP with the proper geometry in its active state. Inhibitors of metalloproteinase (TIMP) have been identified.

SUMMARY OF THE INVENTION

[0006] The present inventors have identified MMP-2 and MMP-9 in increased quantities both in the peripheral region and particularly within the deep recesses of a chronic wound. It has also been a noted increase in these MMPs in "difficult to heal" open wounds. Further the present inventos have discovered a synthesized composition containing zinc ions, calcium ions, rubidium ions and/or potassium ions in a pharmaceutically acceptable carrier, which, when applied to an open wound, after two weeks of treatment, effectively shuts down the activity of MMP-2 and/or MMP-9 in the wound as evidenced by analysis of wound cultures for the presence of MMPs 2 and 9, and resulting visually observable improvement in the healing of the wound. The visually observable improvement in the healing process of the wound is dramatic and takes place within an unexpectedly short time frame.

[0007] Moreover, continued application of the composition of the present invention to a chronic wound

site has been found effective in bringing about complete healing of chronic wounds, often within a matter of weeks. Especially, the composition containing the effective ingredients of the present invention has been determined effective to modulate the presence, hence the activity of, MMPs within the deeper inner recesses of the wound, as opposed to the outer peripheral regions of the wound. The present composition further appears to act as an oxygen scavenger and thereby eliminating or materially reducing the ill effects of oxygen radicals within the inner recesses of the wound.

[0008] Wounds such as decubitus ulcers, and deep burns have been effectively treated employing the concepts of the present invention.

[0008a] Definitions of the specific embodiments of the invention as claimed herein follow.

[0008b] According to a first embodiment of the invention, there is provided a composition when used for enhancing the healing of chronic open wounds comprising a mixture of pharmaceutically effective amounts of each of ions of zinc and rubidium in physiologically inert carrier.

[0008c] According to a second embodiment of the invention, there is provided a composition when used for enhancing the healing of chronic open wounds comprising a mixture of pharmaceutically effective of each of ions of zinc and rubidium, and in a physiologically inert carrier, said mixture have a substantially neutral pH.

[0008d] According to a third embodiment of the invention, there is provided a composition when used for demodulating matrix metalloproteinases 2 and/or 9 associated with a wound comprising a pharmaceutically effective amount of a solution containing ions of zinc and rubidium.

2002359529 17 Jan 2008

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 is a photograph of depicting a wound having applied thereto a composition embodying the present invention;

Figures 2-5 are photographs of typical non-responding wounds;

Figures 6 and 7 are photographs of the leg wound of Example I, depicting the wound of Example I before and after treatment, respectively, in accordance with the present invention;

Figure 8 is a photograph of the leg wound of Example I before treatment in accordance with the present invention;

[Text continues on page 4]

Figure 9 is a microphotograph of a biopsy of the wound depicted in Figure 8;

Figure 10 is a microphotograph depicting the levels of MMP-2 in the upper layers of Zones A and B of Figure 9;

Figure 11 is a microphotograph depicting the levels of MMP-2 in the deeper layers of Zone C of Figure 9;

Figure 12 depicts the appearance of Zones A, B and C of Figure 9 after 14 days of treatment in accordance with the present invention;

Figure 13 is a photograph depicting an external view of the wound depicted in Figure 8 after 14 days of treatment;

Figure 14 is a microphotograph of Zone B of Figure 9 after 14 days of treatment;

Figure 15 is a photograph of the wound of Example I after 6 weeks of treatment;

Figure 16 is a microphotograph of a biopsy of the wound depicted in Figure 15;

Figure 17 is a photograph of the wound of Example II prior to commencement of treatment in accordance with the present invention;

Figure 18 is a microphotograph of the wound depicted in Figure 17 after complete healing of the wound following 4½ weeks of treatment;

Figure 19 is a photograph of the wound of Example III prior to the start of treatment in accordance with the present invention;

Figure 20 is a photograph depicting the wound of Example III as being fully healed after 7 weeks of treatment in accordance with the present invention;

Figure 21 is a photograph of the wound of Example IV prior to the commencement of treatment in accordance with the present invention;

Figure 22 is a photograph of the wound of Example IV as being fully healed at week 7 following treatment in accordance with the present invention;

Figure 23 is a microphotograph of the wound of Example IV and depicted in Figure 21;

Figure 24 is a microphotograph of the wound of Example IV taken in the superficial region of the wound prior to treatment in accordance with the present invention;

Figure 25 is a microphotograph of the wound of Example IV taken in the deep regions of the wound prior to treatment in accordance with the present invention;

Figure 26 is photograph depicting the wound of Example IV after 3 weeks of treatment in accordance with the present invention;

Figure 27 is a microphotograph of the wound depicted in Figure 26;

Figures 28 and 29 are microphotographs depicting the progress of healing of the wound of Example IV;

Figure 30 is a photograph of the wound of Example IV after 5 weeks of treatment in accordance with the present invention;

Figure 31 is a microphotograph of the wound depicted in Figure 30;

Figures 32 and 33 are microphotographs of the wound of Example IV after 5 weeks of treatment in accordance with the present invention and depicting the decrease in MMP expression;

Figure 34 is a photograph of a right let wound of Example V prior to commencement of treatment in accordance with the present invention;

Figure 35 is a photograph showing full healing of the right leg wound of Example V after 8½ months of treatment in accordance with the present invention;

Figure 36 is a photograph of a left leg wound of Example V prior to commencement of treatment in accordance with the present invention;

Figure 37 is a photograph of the left leg wound of Example V after nine months of treatment in accordance with the present invention;

Figure 38 is a pictorial representation of the wound healing process;

Figure 39 is a pictorial representation of the balancing of MMPs within a wound;

Figure 40 is a pictorial representation of ECM generation and degradation in a wound; and,

Figure 41 is a pictorial representation of collagen formation in a wound.

DETAILED DESCRIPTION OF THE INVENTION

[0009] In initial experimentation conducted with rats (partial thickness excision wounds) and Yorkshire pigs (contact burn wounds), the present inventors found that compositions containing the ingredients of the present invention promoted epithelialization,, resulting in a more "normal" epidermis. The wound bed contained less activated macrophages, cells staining positive for acid phosphatase.

[0010] Infliction of deep dermal contact wounds in domestic pig models induce defects which are not fully epithelialized, depending on the treatment applied. Tissue biopsy wounds are deep full thickness skin defects measuring 9 by 2 cm. Such biopsy wounds have a slow tendency to epithelialize. When excision biopsy wounds are filled up with granulation tissue there is a clear visible healing of the wound by contraction. These wounds are ideal test models to get a clear macroscopic impression of the efficacy of test substances applied. Compositions containing the ingredients of the present invention have been found to convert such wounds, which mainly healed by epithelialization starting a couple of days after the first application. Also, such biopsy wounds showed clear epithelialization instead of contraction in comparison with wounds treated with the present compositions.

[0011] Employing the domestic pig model, compositions containing the ingredients of the present invention were compounded and tested. These tests showed clear expression of MMP-2 in untreated wounds. Only minimal expression of MMP-2 was observed in comparative wounds treated with a composition containing the ingredients of the present invention.

[0012] The foregoing tests were followed by *in vitro* human studies employing a composition containing the ingredients of the present invention. In these tests, the composition was impregnated onto an ethylene vinylacetate carrier for form an impregnated dressing for the wound site.

[0013] In the present studies, 31 patients were initially involved in the study. Five patients dropped out of the study and eight patients are receiving continuing treatment. Of these patients, the wound(s) of 18 patients were completely healed with an average healing time of 10 weeks. All of the patients in the study responded positively.

[0014] The following specific examples are provided as exemplary of the results observed in the human studies. In each instance, a composition in accordance with the present invention, on an EVOH carrier defining a bandage was applied to the wound site. The bandage was removed at various intervals and replaced with a fresh bandage. A sufficient quantity of the composition of the present invention was placed on the carrier to substantially fully fill the wound cavity.

Example I

Female 74 years of age

History:

Rheumatoid Arthritis.

Medication:

High doses of steroids.

Type wound:

Post traumatic ulcer on lateral lower leg after infected hematoma.

Duration of Wound

Wound had existed for more than one year prior to commencement of present treatment.

Earlier Treatments

DUODERM

HYDROGEL

Vacuum system

Honey and SSD,

[0015] Figure 6 depicts this wound at the time of commencement of treatment. Prior to entry into the present study. Figure 7 depicts the healed wound after 30 weeks of treatment. It is noted that after 12 weeks of treatment with the composition, this patient was treated with steroids. This action was noted to delay the healing process and was discontinued. Thus, without the intervention of the steroid treatment, the healing time for this patient would have been shorter.

[0016] Referring to Figures 8 and 9, at Day One, the wound of this patient was about 6 cm long and about 2 cm wide. The wound extended deeply into the leg. A biopsy of the wound is depicted in Figure 9 wherein a cross-section of the wound is depicted as including Zones A, B and C. Zone A consists of a broad fibrin layer with necrotic cellular debris. Zone B is a rather broad zone with breakdown of matured collagen and inflammation. Zone C is adjacent the bottom of the wound and depicts a decline of

inflammation at this location. Examination of the Day One biopsy for MMP-2 prior to the treatment showed fibroblasts in the upper layers of the wound to be expressing high levels of MMP-2 (Figure 10). This same biopsy depicted no more than a single fibroblast staining positive for MMP-2 in the deeper layers of the wound. As depicted in Figures 12 and 13, after 14 days of treatment with the composition, all zones are readily identifiable, with the fibrin cap depicting large accumulations of neutrophils. Zone B at this time of treatment is identifiable directly beneath the fibrin cap and shows less old collagen and the appearing of neo-dermis. Figure 13 shows the overall appearance of the wound after 14 days treatment and clearly indicates both a "cleaner" wound and reduction in the overall size of the original wound. Biopsies of the wound after 14 days of treatment showed no clear change in the expression of MMP-2 in Zone B (Figure 14). As shown in Figures 15 and 16, after 6 weeks of treatment, the wound was further decreased in size and healing was progressing. A biopsy of the wound at this time showed that the necrotic cap had vanished and the neo-dermis was healthy. Further, the biopsy the expression of MMP-2 within the wound had declined to near zero, coinciding with the healthy appearance of the neo-dermis.

[0017] Between the 6^{th} and 12^{th} weeks of treatment of the present patient, steroid treatment was conducted. At week 12, a biopsy of the wound clearly showed that the fibroblasts began again to express MMP-2. Treatment of the wound using steroids was ceased and the wound fully healed within a total treatment time of 30 weeks as shown in Figure 7.

10

Example II

Male 75 years of age

History

Decompensation cordis

Vascular insufficiency

Diabetes Mellitus

Wound type

Post traumatic

Lacerations

Duration of Wound

Wound had existed for weeks

Earlier Treatments

ADAPTIC

SSD (FLAMMAZINE)

[0018] Figure 17 depicts the wound of Example II at Day One, prior to the commencement of treatment with the composition. Figure 18 depicts that portion of the arm treated with the composition as being completely healed after 4 ½ weeks of treatment.

Example III

Male 81 years of age

Type of wound

2nd and 3rd degree burns by electricity

Duration of wound

Wound had existed 16 days

Earlier Treatments

SSD (FLAMMAZINE)

ELASTO-GEL

[0019] Figure 19 depicts this wound at Day One of the commencement of treatment of the wound with the composition. Figure 20 depicts the completely healed wound after 7 weeks of treatment.

Example IV

Male 57 years of age

History

Diabetes Mellitus

Pilonfracture

Osteosynthesis

Type of wound

Post traumatic ulcer lateral malleoulus

Duration of wound

Wound had existed for more than one year

Earlier Treatment

KALTOSTAT

[0020] Figure 21 depicts the present wound at Day One and prior to commencement of treatment. Figure 22 depicts the completely healed wound at week 7.

[0021] A biopsy of a cross-section of the wound depicted in Figure 21 is shown in Figure 23. Of note is the fibrous

cap consisting of fibrin and dead cells on top of the wound. Figures 24 and 25 show the expression of MMP-2 being high in the superficial and deep regions of the wound, at Day One.

[0022] At week 3 of treatment, the wound showed clear progress in epithelial outgrowth (Figure 26) and reduction in the size of the fibrinous cap (Figure 27). Examination of biopsies of the wound at week 3 further showed an increase in fibroblast and blood vessels (Figure 28) and diminished MMP-2 expression in the fibroblast (see also Figure 29).

[0023] At week 5, the wound was almost fully closed and the fibrinous cap had further diminished (Figures 30 and 31). Biopsies of the wound at week 5 showed slightly more active stellate fibroblasts, an increase in inflammation and a decrease of MMP-2 expression. (Figures 32 and 33).

[0024] As noted, complete healing of the wound occurred after only 7 weeks of treatment.

Example V

Female 76 years of age

History

Rheumatoid arthritis

Morbus Reynaud

Lumbal sympatechtomy

Amputation of first digit

Venous insufficiency

Type of Wound

Leg ulcer right medial ulcus

Leg ulcer left medial ulcus

Duration of wound

Wounds had existed for more than 4 years (open/healed)

Earlier Treatment

SSD (FLAMMAZINE)

BIATIN foam

Compression bandage

[0025] Figures 35 and 36 depict the two wounds involved in Example V. Figures 35 and 37 depict the same two wounds after fully healed. Full healing of the wound of Figure 34 was effected after 8 ½ months of treatment, and full healing of the wound of Figure 36 was effected after 9 months of treatment.

[0026] In one embodiment, the composition of the present invention includes zinc ions, rubidium ions, potassium ions, and calcium ions.

[0027] Solutions including various of the above-listed ingredients were prepared as follows:

Composition I

potassium citrate	0.895 moles/l
rubidium chloride	3.1 millimoles/l
zinc chloride	64 micromoles/l
citric acid	(sufficient to adjust the pH of the solution to 5.5)

Composition II

potassium citrate	0.895 moles/l
rubidium chloride	3.1 millimoles/l
zinc chloride	64 micromoles/l
calcium chloride	0.2 millimoles/l
citric acid	(sufficient to adjust the pH of the solution to 5.5)

Composition III

potassium hydroxide	0.895 moles/l
rubidium chloride	3.1 millimoles/l
zinc chloride	64 micromoles/l
citric acid	(sufficient to adjust the pH of the solution to 5.5)

Composition IV

potassium hydroxide	0.895 moles/l
rubidium chloride	3.1 millimoles/l
zinc chloride	64 micromoles/l
calcium chloride	0.2 millimoles/l
citric acid	(sufficient to adjust the pH of the solution to 5.5)

[0028] Preferably, pharmaceutical grade ingredients are employed in each composition of the present invention.

[0029] Compositions I and III were subjected to chemiluminescence assay (indicative of inhibition of production of reactive oxygen species, complement assay (classical pathway, indicative of complement activity). These compositions of the present invention exhibited IC-50 values as follows:

TABLE A

	Chemiluminescence Assay	Complement Assay
Example I	10μl/ml	9μl/ml
Example II	36μl/ml	28μl/ml

[0030] Composition II which included potassium hydroxide required a greater amount of citric acid to produce a pH of 5.0, indicating that the potassium citrate employed in Example I was more active, hence the lower IC-50 values exhibited by Composition I. In any event the complement assay results clearly show the effectiveness of the present composition in the modulation of MMPs found in chronic wounds such as diabetic ulcers, decubitus ulcers, and other wounds.

[0031] In one embodiment, the composition of the present invention may be incorporated into a pharmaceutically acceptable carrier such as WHITFIELD'S ointment or other suitable crème.

[0032] A composition of the present invention, preferably in a crème-type carrier, may be applied directly to an open wound or the like or through the use of a gauze bandage to which the composition is applied.

[0033] A preferred composition for use in the treatment of various open wounds comprises 0.895 moles/l potassium citrate, 3.1 millimoles rubidium chloride, 0.2 millimoles/l calcium chloride and 64 micromoles zinc chloride in a solution employing distilled water. The solution is acidified to pH 5.0 employing citric acid.

[0034] Whereas the compositions of the present invention function to scavenge superoxide anions, addition of other pharmaceutically acceptable scavengers of superoxide anions may be employed. Naturally occurring polyether or caffeic acid may be beneficial in the treatment of wounds, particularly burn wounds. Such additives may reduce the chemiluminescence and/or DPPH assay (anti-oxidant activity by donating electrons or hydrogen) values of the composition into the microgram range.

[0035] The preferred composition of the present invention may be modified by eliminating calcium ions, but with some reduction in the efficacy of the composition in treating at least certain wounds. As noted, substitution of potassium hydroxide for potassium citrate in the present composition is permissible, but not preferred, due to the increased need for acid to adjust the pH of the solution to 5.0. Though present in a relatively small amount, the presence of zinc ions in the solution appear to be important to the desired level of effectiveness of the present composition. This same factor appears true for rubidium ions. Whereas the sources of the inorganic ions of the present composition are given herein, it is to be recognized that other sources of these ions may be acceptable for given applications of the composition. Initial tests have indicated that the quantity of the

several inorganic ions in the composition may be varied from the preferred composition without destruction of, but with possible reduction of, the wound healing efficacy of the composition. In all instances, preferably, the pH of the solution is adjusted to substantially 5.0 thereby imparting desirable buffering properties to the composition.

[0036] In any event, the active ingredients of the present composition have been found to include zinc, potassium, rubidium and/or calcium. Calcium does not appear to be critical to the desired healing process, it does not appear to be detrimental when included in the present composition, and in certain instances is considered desirable. On the other hand, zinc appears to be essential to the healing qualities of the present composition, and rubidium is also strongly indicated for those compositions employed in cancer, ulcer and others of those maladies for which the present compositions have been found useful as healing agents.

[0037] Citric acid, preferably, when included in the present composition for pH control purposes has been found effective in such role. Other acids for normalizing the pH of present solution, for example hydrochloric acid, may be employed.

[0038] Polyethylene glycol has been found particularly effective as a component of the present solution, in part due to its oxygen scavenging properties.

[0039] In one embodiment of the present invention, a channeling agent, such as monoxidil, has been found to be effective in lieu of the potassium ions.

[0040] Whereas the compositions of the present invention may include other inactive ingredients which are biologically relatively inert or inactive relative to the healing process of a wound, the present inventors have found that the absence of ions of zinc, potassium, rubidium and calcium (in certain compositions) are essential to obtaining the aforenoted dramatic results of wound healing.

[0041] During wound repair, different MMPs are produced by multiple cell types. MMP-2 is produced only by inflammatory cells. MMP-9 is produced by keratinocytes as well as inflammatory cells. MMP-2 and MMP-9 act on cleaved collagen better than other MMPs. MMPs are not actively expressed in uninjured skin either in the epidermis or dermis. The idea exists that MMPs are stored in the matrix awaiting activation by migrating cells. Inflamed tissues in chronic wounds exhibit excessively high MMP levels in comparison to normal healing wounds, the excess being in the range of 30% greater MMP levels in chronic wounds.

[0042] In accordance with one aspect of the present invention, the compositions of the present invention exhibit those properties which are known to increase tissue regeneration of chronic open wounds, providing full wound closure of demonstrated non-responding or slow-healing wounds.

[0043] At a first level, compositions of the present invention clearly modulate the expression of one or more MMPs, particularly MMP-2 and MMP-9, thereby reducing the levels of these MMPs in chronic wounds to normalize wound healing. At second and further levels, compositions of the present invention function to scavenge oxygen radicals from wound sites, normalizing the pH levels within a wound and

thereby developing an environment within the wound which is favorable to healing. Still further, the compositions also can reduce inflammation, scavenge free oxygen radicals, reduce scar tissue, and act as a powerful antimicrobal.

[0044] Dermal wound healing is recognized as a complex, but orderly process which takes place in injured tissue. Subsequently the injured tissue respond with inflammation, granulation tissue formation, extracellular matrix (ECM) deposition, contraction and remodeling of the deposited collagen. This process is depicted in Figure 37. The present inventors have found that remodeling results when there is a balance between ECM-synthesis and ECM-degradation. Many different circumstances can influence these processes thus shifting the balance toward a state of excess or shortage of ECM, thereby inhibiting the remodeling process (See Figure 38). As seen in Figure 39, fibroblast synthesis of collagen, the major constituent of the dermal tissue, is stimulated by growth factors and cytokines. Soluble pro-collagen peptides are released in the environment of the fibroblasts. Pro-collagen peptidase cleaves of the terminal peptide chains allow true collagen fibrils to form. Lysyl-oxidase promotes the cross-linking of these fibrils rendering structural stability to the matrix. In the ECM, several types of collagen can be recognized, along with other substances which contribute to the ECM.

[0045] The production of MMPs, enzymes that serve to degrade collagen, are also under the influence of growth factors. Stimulating and inhibition factors result I the release of pro-metalloproteinases. These pro-forms are activated by plasmine. Activated metalloproteinases are

quickly deactivated by Tissue Inhibitors of metalloproteinases (TIMPs) so that the spatial actin of the proteolytic enzyme is limited. The main action of the MMPs is to degrade the collagen. It has to be borne in mind that this scheme is likely to be an oversimplification of what is happening *in vivo*. For example, (a) plasmine release from plasminogeen is regulated by the action of plasminogeen activator (PA) and plasminogeen Activator Inhibitor (PAI) both of which are also produced by fibroblasts under the influence of growth factors and cytokines; (b) Metalloproteinases can also be activated by other substances as HOCL- from the oxidative burst of granulocytes (H_2O_2 + MPO + Cl^- → HOCl- which is strongly anti-bacterial); (c) metalloproteinases can also be activated by other than TIMP, for instance alpha2-Macroglobulin (anti-protease in serum); and/or (d) metalloproteinases can cleave other molecules than collagen for instance other ECM molecules by cleavage capacity can perhaps also lead to activation of the complement system (C5a and C3A).

[0046] Very little appears to be known about the distribution of MMPs in time. It is known that normal skin shows basic levels of MMP-2, but shows no MMP-9 expression. The present inventors have shown elevated levels of MMPs in chronic wounds.

[0047] Irrespective of the complexity of the wound healing mechanism, the present inventors have discovered a combination of metal ions which in solution, preferably substantially at a pH of 5.0, when applied over time to a chronic or other dermal wound, dramatically enhances the healing of the wound. The composition of the present

invention is further indicated in the treatment of cancers, psoriasis, and a variety of skin infections, burns, and/or lesions.

The foregoing embodiments are illustrative only of the principles of the invention, and various modifications and changes will readily occur to those skilled in the art. The invention is capable of being practiced and carried out in various ways and in other embodiments. It is also to be understood that the terminology employed herein is for the purpose of description and should not be regarded as limiting.

The term "comprise" and variants of the term such as "comprises" or "comprising" are used herein to denote the inclusion of a stated integer or stated integers but not to exclude any other integer or any other integers, unless in the context or usage an exclusive interpretation of the term is required.

Any reference to publications cited in this specification is not an admission that the disclosures constitute common general knowledge in Australia.

The claims defining the invention are as follows:

1. A composition when used for enhancing the healing of chronic open wounds comprising a mixture of pharmaceutically effective amounts of each of ions of zinc and rubidium in physiologically inert carrier.

2. The composition of Claim 1 and including a pharmaceutically effective amount of ions of calcium.

3. The composition of Claim 1 and including a pharmaceutically effective amount of ions of potassium.

4. The composition of Claim 2 and including a pharmaceutically effective amount of ions of potassium.

5. The composition of any one of Claims 1-4 and including an acid suitable for adjusting the pH of the composition.

6. The composition of Claim 5 wherein said acid is citric acid.

7. The composition of any one of Claims 1-6 wherein said composition includes polyethylene glycol.

8. The composition of any one of Claims 1-7 and including a pharmaceutically effective amount of a channelling agent.

9. A composition when used for enhancing the healing of chronic open wounds comprising a mixture of pharmaceutically effective of each of ions of zinc and rubidium, and in a physiologically inert carrier, said mixture have a substantially neutral pH.

10. The composition of Claim 9 wherein said carrier comprises polyethylene glycol.

11. The composition of Claim 9 or 10 and including a pharmaceutically effective amount of ions of calcium.

12. The composition of any one of Claims 9-11 and including a pharmaceutically effective amount of a channelling agent.

13. A composition when used for demodulating matrix metalloproteinases 2 and/or 9 associated with a wound comprising a pharmaceutically effective amount of a solution containing ions of zinc and rubidium.

14. The composition of Claim 13 and including a pharmaceutically effective amount of a channelling agent.

15. The composition of Claim 13 and including a pharmaceutically effective amount of ions of potassium.

16. The composition of Claim 13 and including a pharmaceutically effective amount of ions of calcium.

17. The composition of Claim 13 and including a pharmaceutically effective amount of each of ions of potassium and calcium.

18. The composition of any one of Claims 13-17 and including polyethylene glycol.

19. The composition of any one of Claims 13-18 wherein said composition exhibits a pH of substantially 5.0.

Dated: 17 January 2008

FIG 1

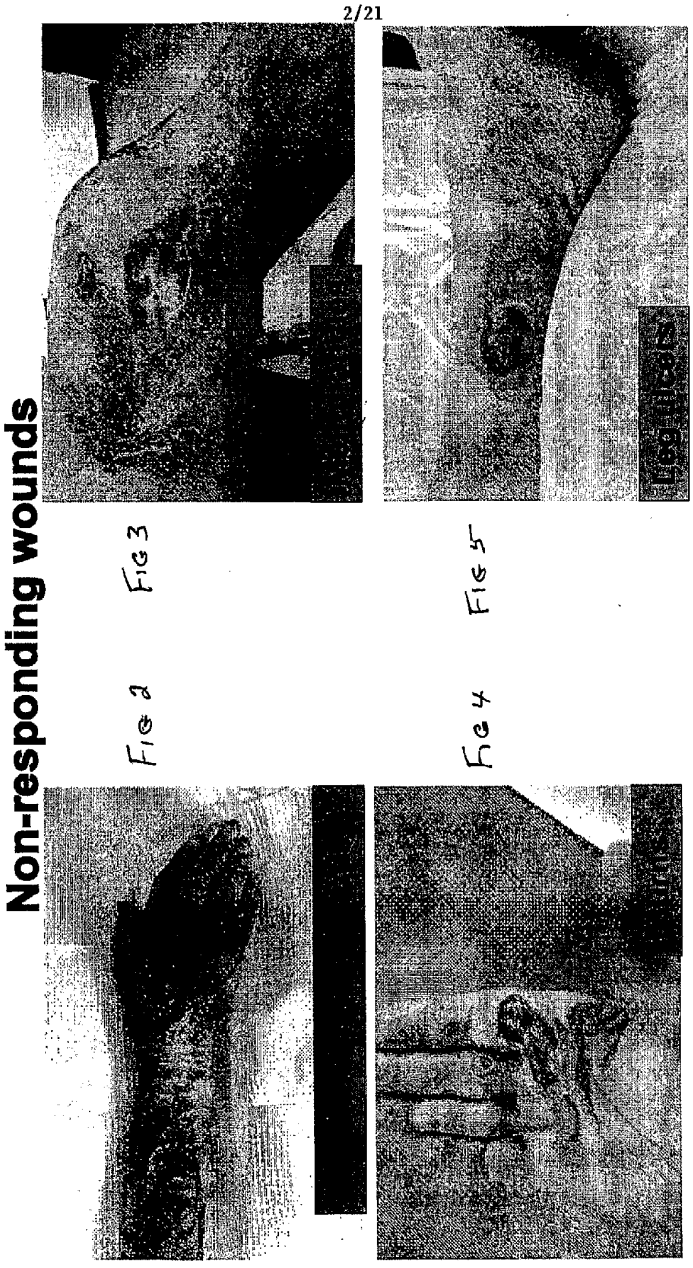


Fig 3

Fig 5

Fig 4

Fig 7

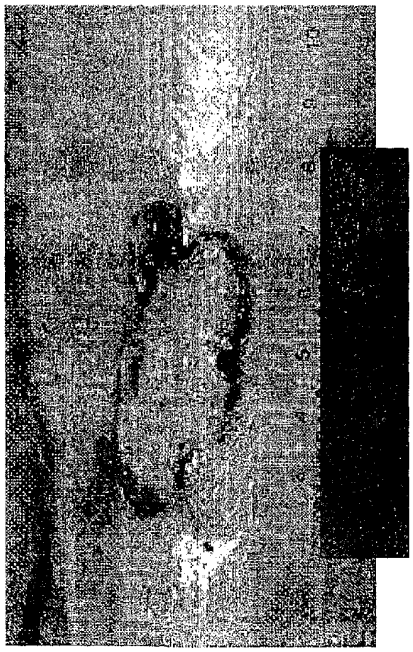


Fig 6

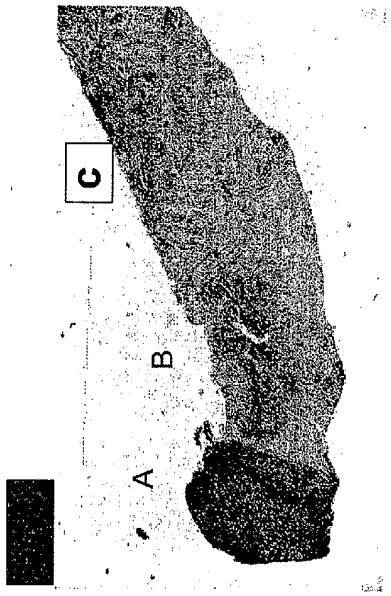


Fig 9

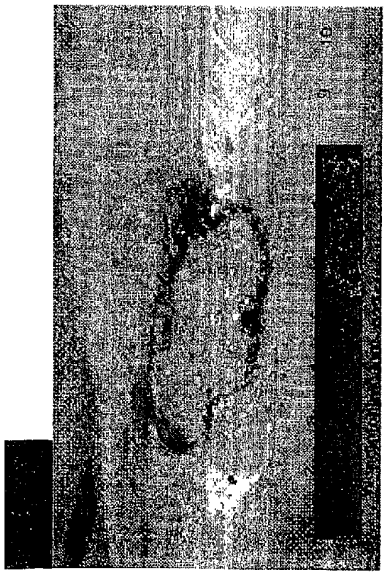


Fig 8

Top end consists of a broad fibrin layer with necrotic cellulair debris (A)

Adjacent there is a rather broad zone with breakdown of matured collagen and inflammation (B)

Toward the bottom the inflammation declines (C)

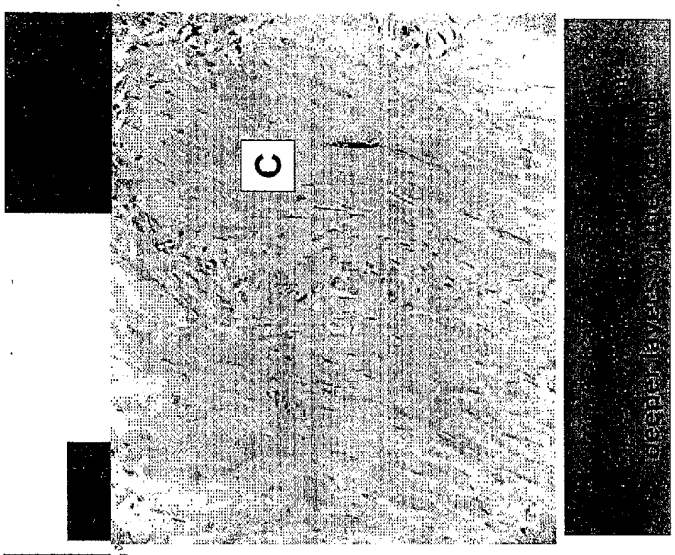


Fig 11

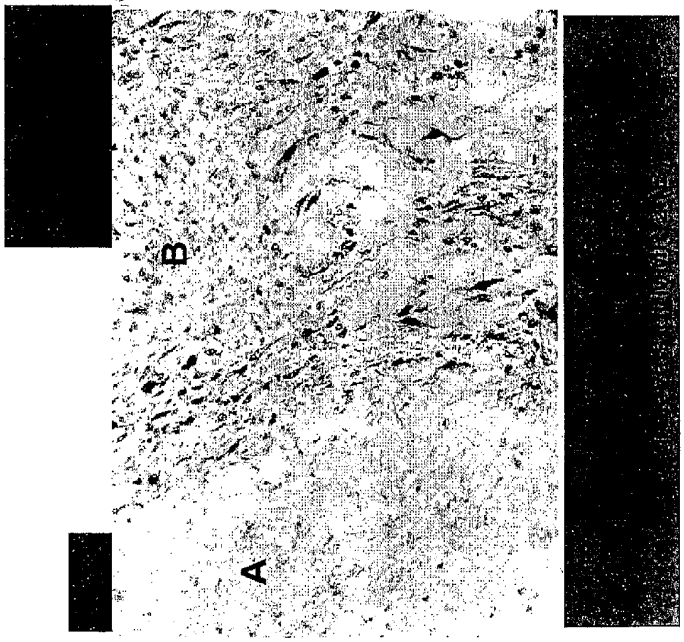


Fig 10

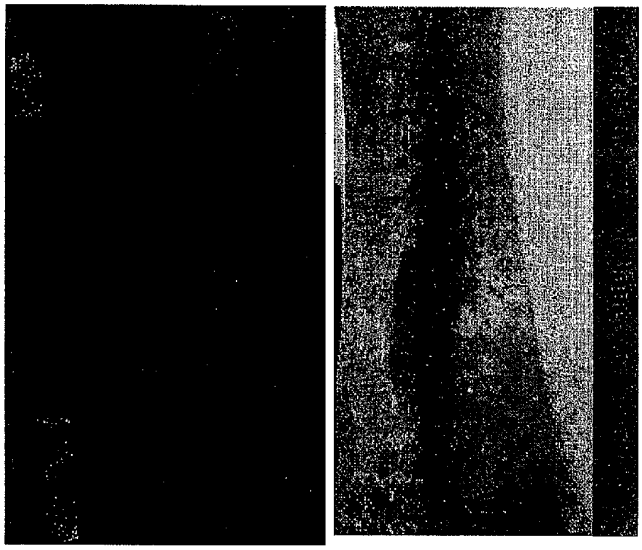


FIG 13

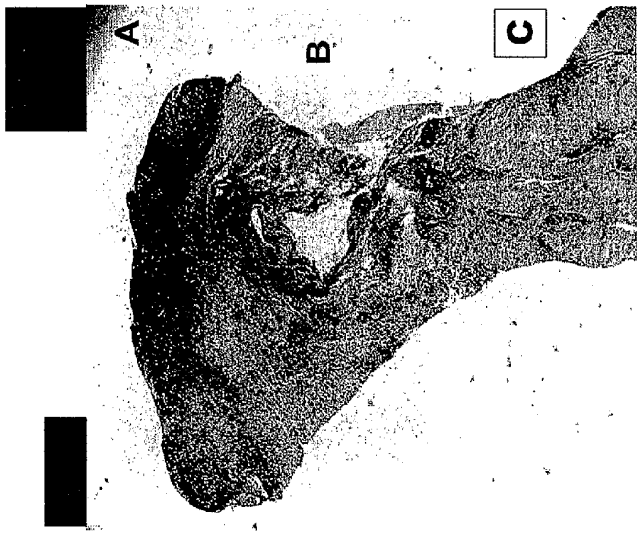


FIG 12

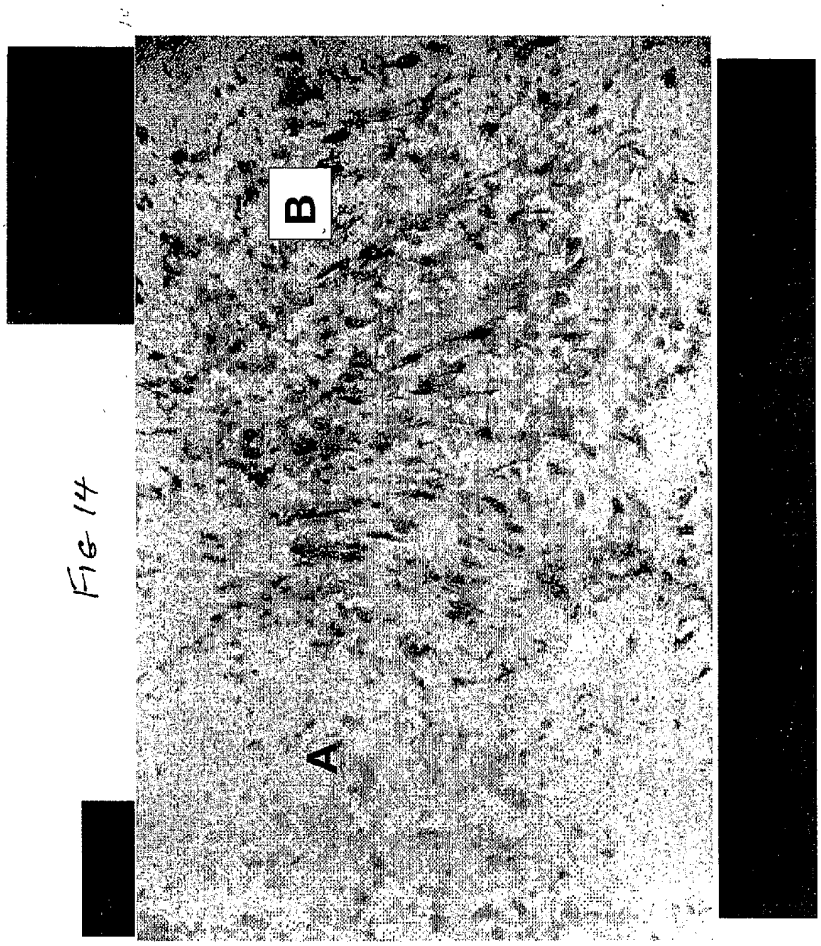


Fig 14

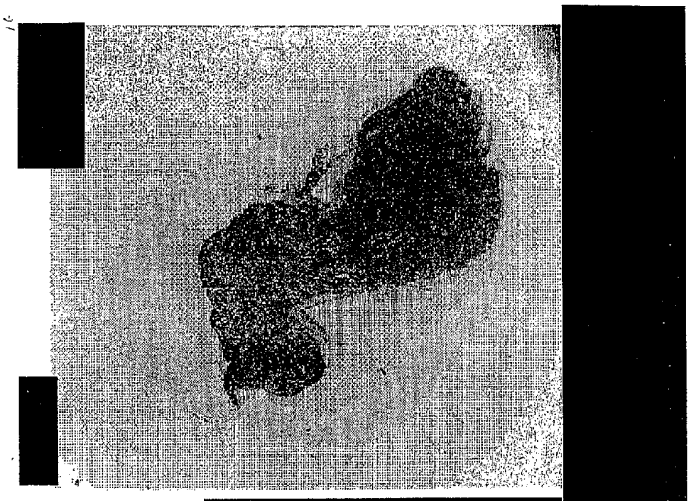


Fig 16

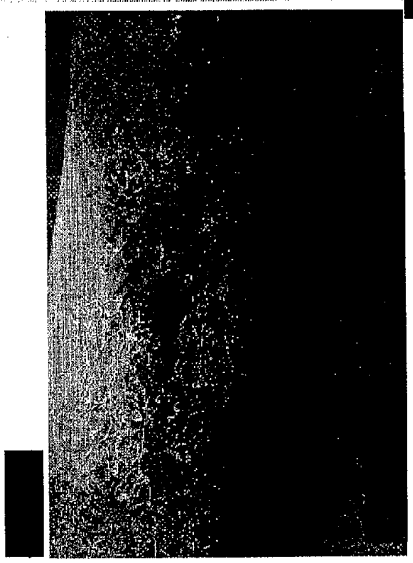
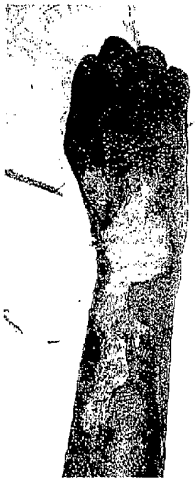


Fig 15

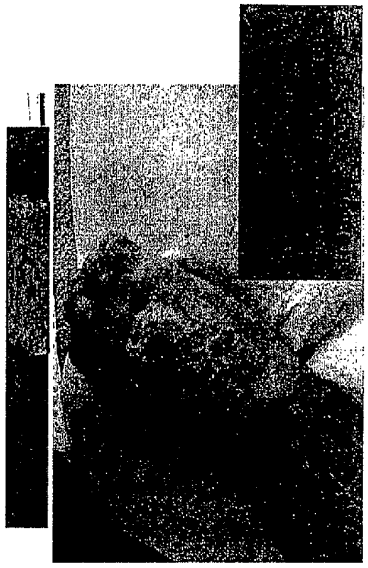


Male, 75 year

History:
- **Vascular insufficiency**
- **Decompensation cordis**
- **Diabetes mellitus**

Medication:
- **Cortisone usage**

Fig 17

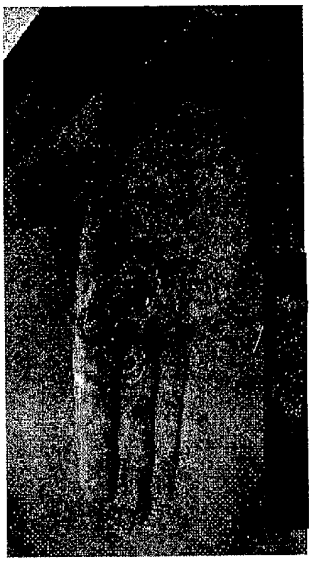


Type wound:
- **Post traumatic**
- **Lacerations**

Duration ulcer:
- **2 weeks**

Earlier treatments:
- **SSD (Flammazine)**
- **Adaptic**

Fig 18



Male, 81 year

Fig 19

Type wound:
- **2nd / 3rd Degree burns by electricity**

Duration burns:
- **16 days**

Earlier treatments:
- **SSD (Flammazine)**
- **Elasto-Gel**

Fig 20

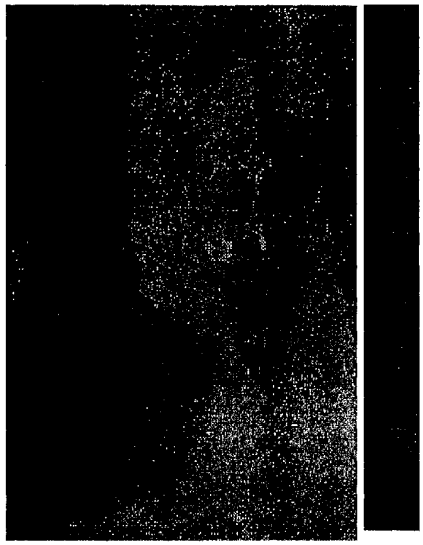


Fig 22

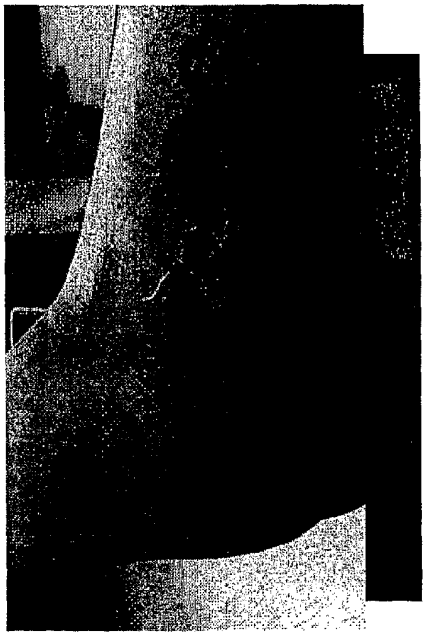


Fig 21

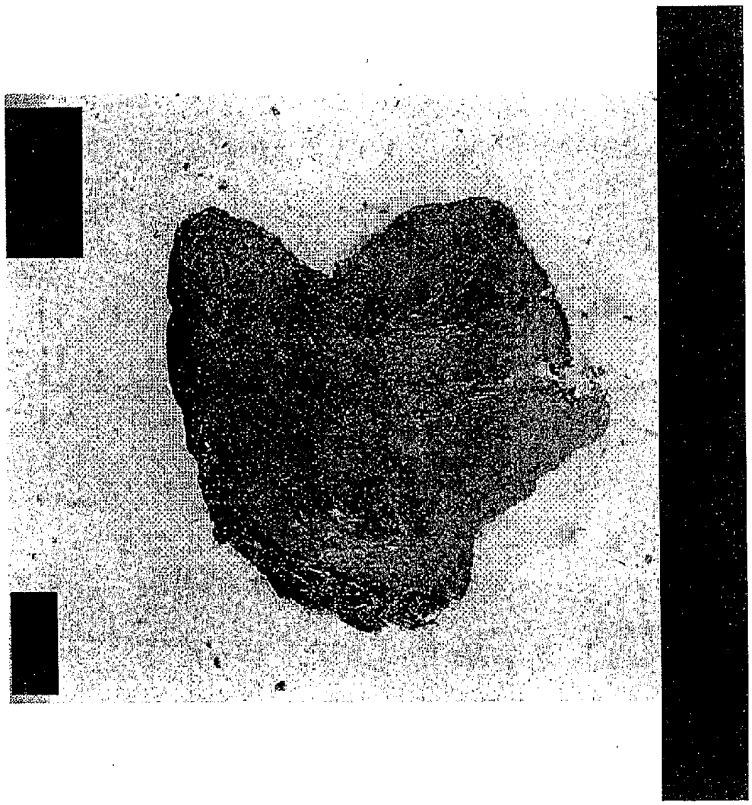


Fig 23

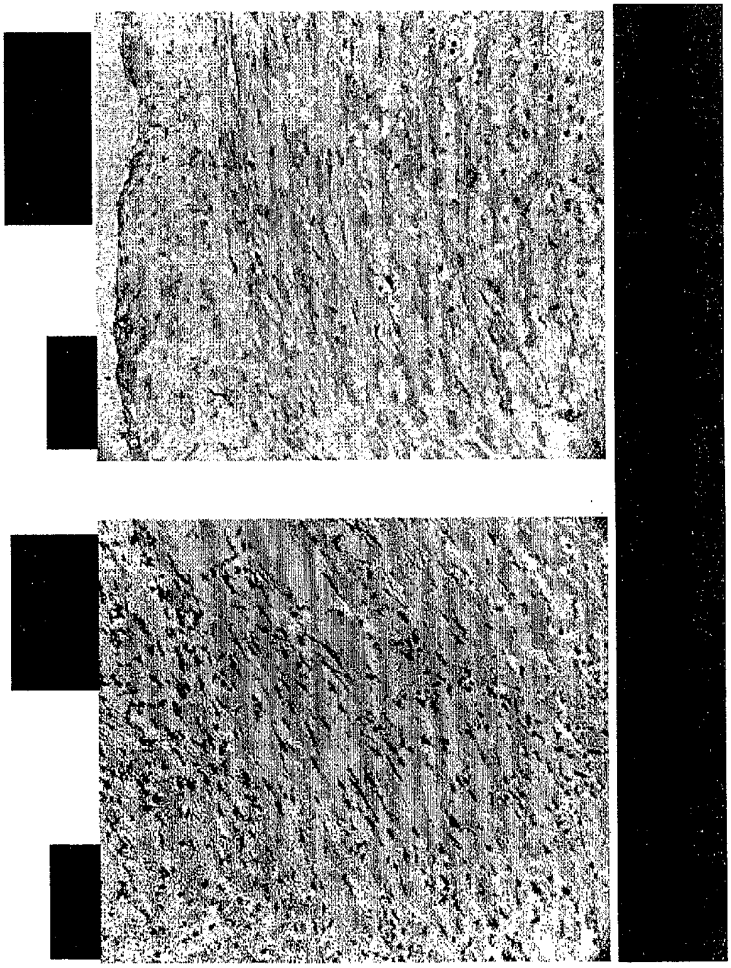


Fig 23

Fig 24

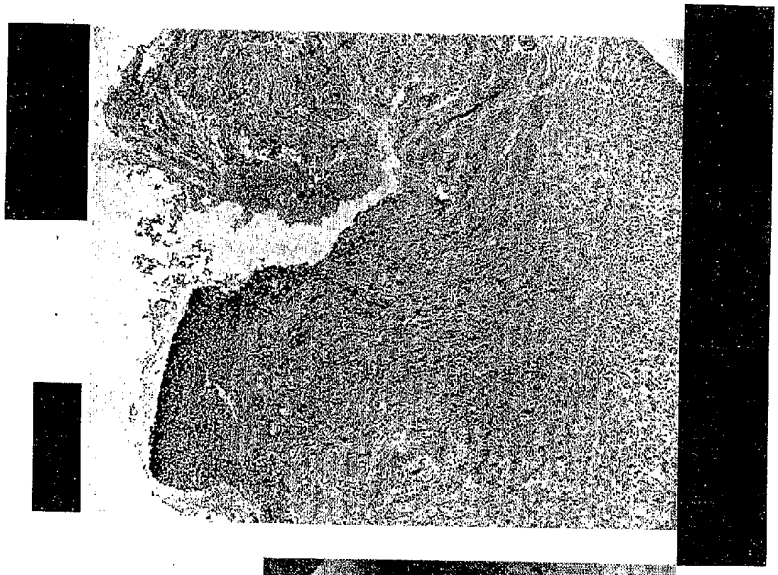


FIG 27

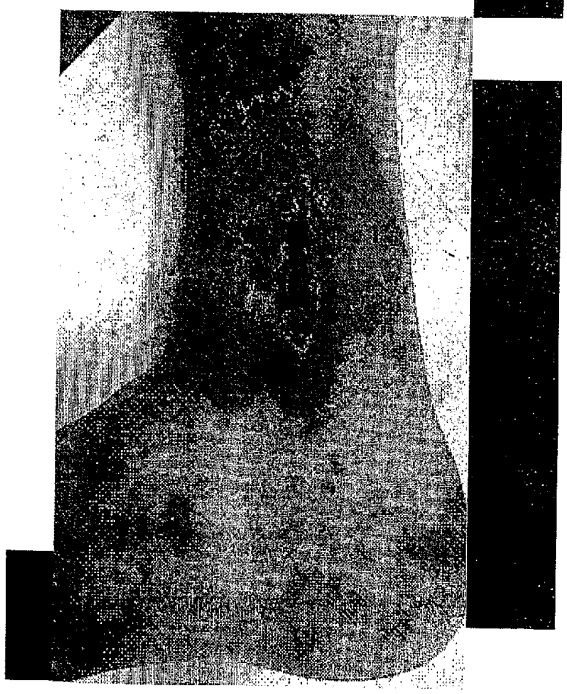


FIG 26

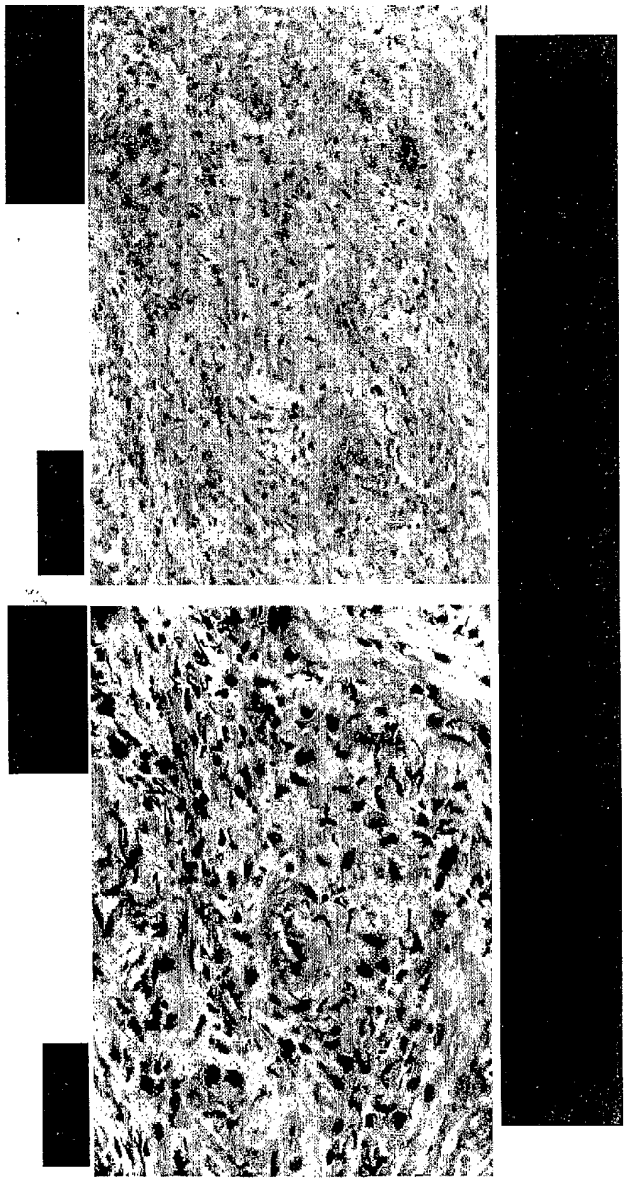


Fig 29

Fig 28

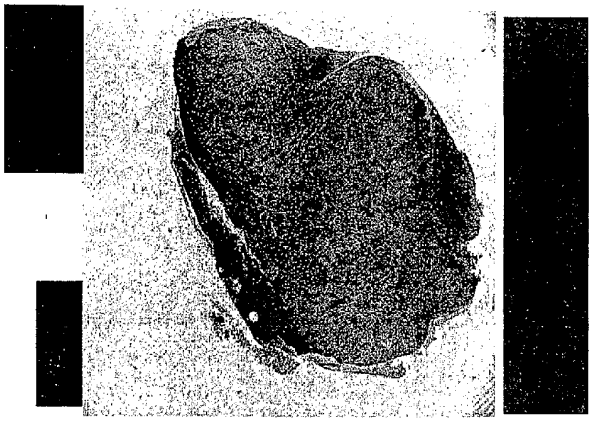


Fig 31

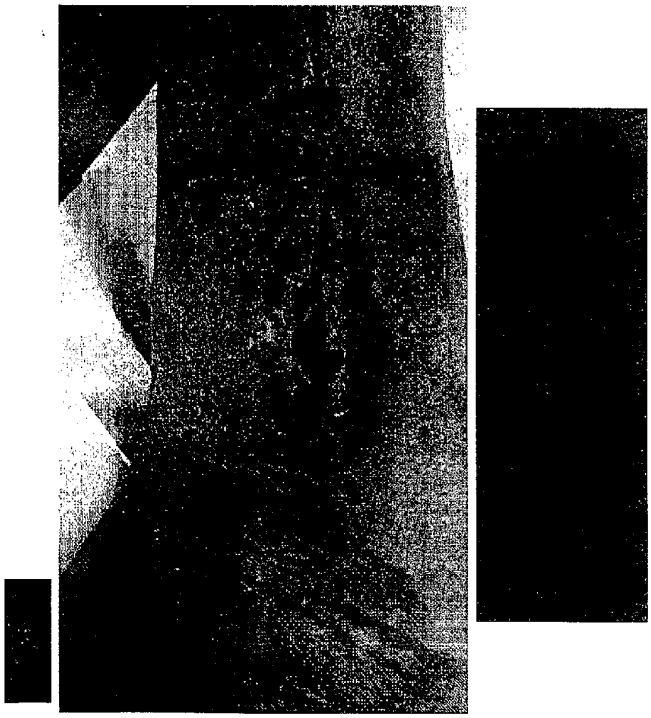


Fig 30

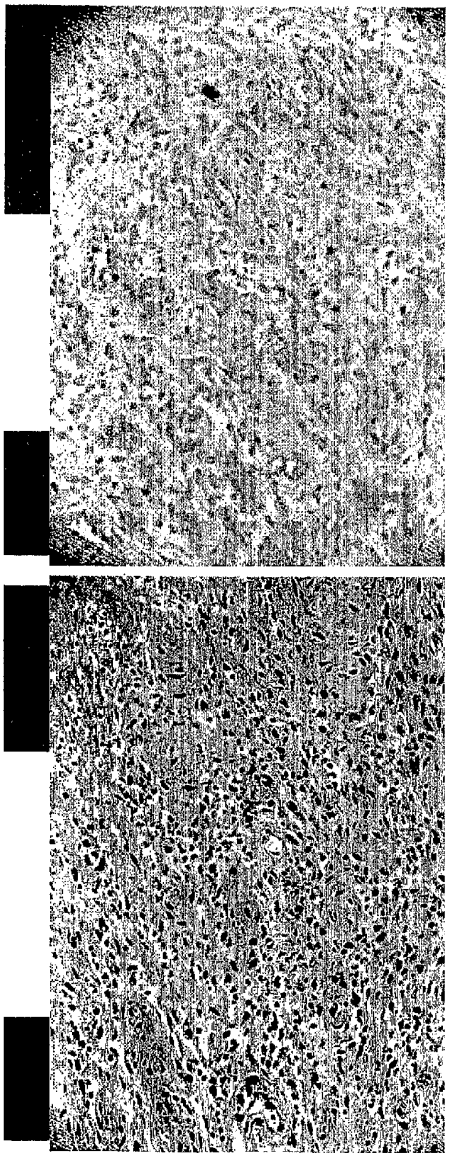
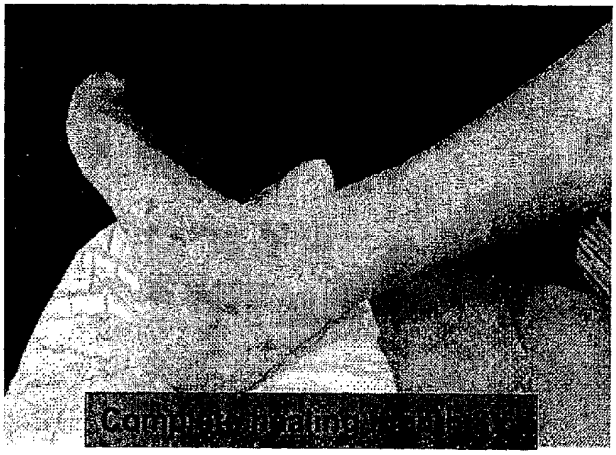


FIG 33

FIG 32

FIG 34

FIG 35



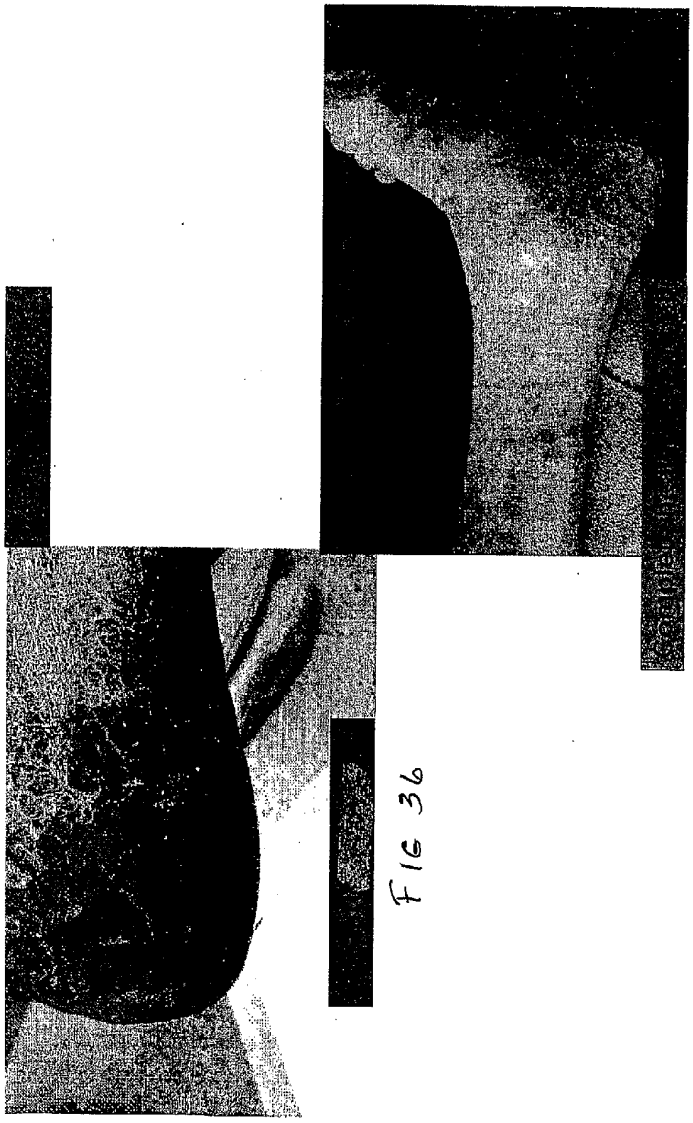


FIG 36

FIG 37

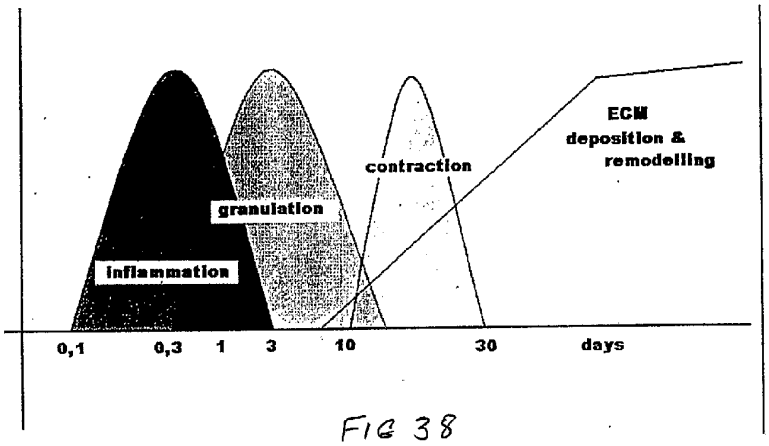

ECM
deposition &
remodelling
contraction
granulation
inflammation
0,1
0,3
1
3
10
30
days

FIG 38

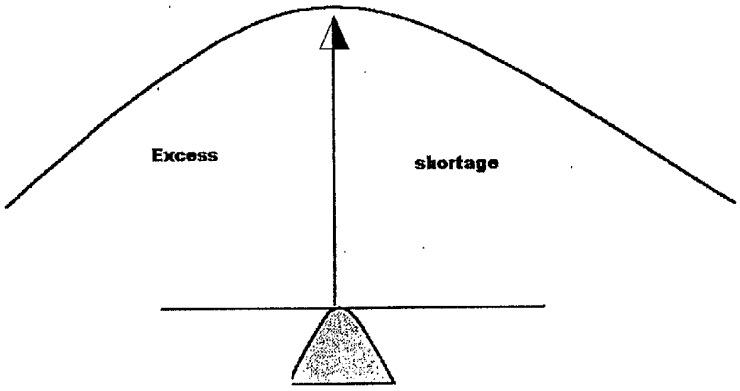

Excess
skortage

FIG 39

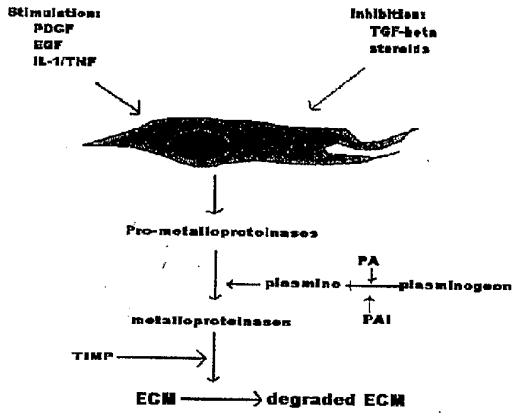

Stimulation:
PDGF
EGF
IL-1/TNF
Inhibition:
TGF-beta
steroids
Pro-metalloproteinases
PA
plasmin
plasminogeon
metalloproteinases
PAI
TIMP
ECM
degraded ECM

FIG 40

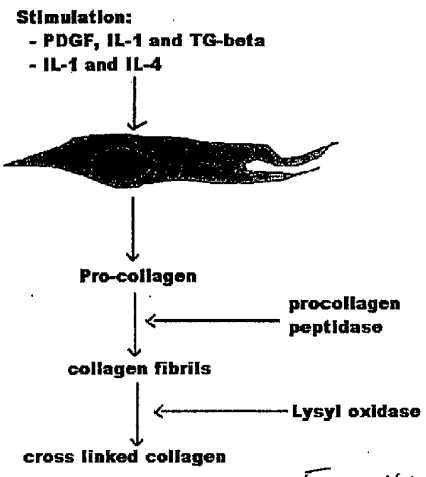

Stimulation:
- PDGF, IL-1 and TG-beta
- IL-1 and IL-4
Pro-collagen
procollagen
peptidase
collagen fibrils
Lysyl oxidase
cross linked collagen

FIG 41