(CONVENTION. Company.) Form 8

COMMONWEALTH OF AUSTRALIA

Patents Act 1952-1969

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION FOR A PATENT OR PATENT OF ADDITION

(1) Here insert (in full) Name of Company.

In support of the Convention Application made by(1) CuraTech, Inc.

(hereinafter referred to as the applicant) for a Patent

(2) Here insert title of Invention.

for an invention entitled:(2) WOUND HEALING AGENTS

(3) Here insert full Name and Address, of Company official authorized to make declaration.

I,(3) Timothy I. Maudlin

of 1201 Marquette Avenue, Suite 400, Minneapolis, Minnesota 55403 United States of America

do solemnly and sincerely declare as follows:

1. I am authorised by the applicant for the patent to make this declaration on its behalf.

2. The basic application s as defined by Section 141 of the Act ~~was~~ were made in(4) United States of America on the 29th day of November 19 84 ~~by~~ and on the 10th day of October 19 85 ~~by~~ both by DAVID R. KNIGHTON, Route 3, Box 157, Hudson, Wisconsin, 54016, United States of America

(4) Here insert basic Country or Countries followed by date or dates and basic Applicant or Applicants.

(5) Here insert (in full) Name and Address of Actual Inventor or Inventors.

3.(5) David R. Knighton
Route 3, Box 157, Hudson, Wisconsin 54016
United States of America

~~is~~/are the actual inventor s of the invention and the facts upon which the applicant is entitled to make the application are as follow:

The applicant is the assignee of the said DAVID R. KNIGHTON

4. The basic application s referred to in paragraph 2 of this Declaration ~~was~~ were the first application s made in a Convention country in respect of the invention the subject of the application.

DECLARED at Minneapolis, Minnesota, U.S.A.

this 11th day of August 19 86

(6) Signature

(12) PATENT ABRIDGMENT (11) Document No. AU-B-50949/85
(19) AUSTRALIAN PATENT OFFICE (10) Acceptance No. 596954

(54) Title
WOUND HEALING AGENTS

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(56) Prior Art Documents
US 4479896
US 3628974

(57) Claim

1. A method for enhancing wound repair which comprises treatment of a wound with a substance comprising platelet-derived angiogenic factor and platelet-derived growth factors.

15. A method of treatment of tissue comprising :

applying a composition topically onto said tissue, said composition comprising a substance which is both chemotactic and non-mitogenic for capillary endothelial cells, said composition being applied in an amount sufficient to cause directed growth of capillary endothelium.

.../2

22. A therapeutic composition for application to tissue for the purpose of forming granulation tissue and/or capillaries and/or epithelial tissue, said composition comprising :

(i) the material released from human platelets; and

(ii) a pharmaceutically acceptable carrier or diluent therefore

wherein said composition is substantially free of (i) blood or plasma contaminants and (ii) platelet ghosts or other material found in human platelets but not released by said platelets.

PCT

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(54) Title: WOUND HEALING AGENTS

(57) Abstract

Platelet enriched plasma is produced from blood. The platelets are activated by thrombin which causes the release of platelet derived growth and angiogenesis factors. A carrier such as a microcrystalline collagen is added to produce a wound treating salve. The compound is applied directly to wounds and initiates healing in non-healing wounds as well as accelerating normal wound healing by increasing vascularization, stimulating fibroblast mitosis and migration and increasing collagen synthesis by fibroblasts.

WOUND HEALING AGENTS

This application is a continuation-in-part of co-pending application Serial No. 676,471, filed November 29, 1984.

Field of the Invention

This invention relates to wound healing agents, specifically angiogenic and growth factors, their production from blood and their use to facilitate the healing of wounds.

Background of the Invention

Angiogenesis, which is the proliferation and directed growth of capillary endothelium, along with fibroplasia and collagen synthesis are integral components of a host's response to wounding. The activation of platelets and the clotting cascade are among the first reactions to injury.

Platelets activated by thrombin release a mitogen, or growth factor, for fibroblasts and smooth muscle cells and stimulate increased collagen synthesis by smooth muscle cells in vitro. The mitogen, (platelet-derived growth factor, hereinafter PDGF) is composed of two polypeptides. An article describing PDGF was published in 1982 by G.R. Grotendorst, T. Chang, H.E.J. Seppa, H.K. Kleinman and G.R. Martin in the Journal of Cellular Physiology entitled "Platelet-Derived Growth Factor is a Chemoattractant for Vascular Smooth Muscle Cells", Vol. 113, pp. 261-266. The article is incorporated herein by reference.

A non-mitogenic substance, called angiogenic factor, is also produced by thrombin activated platelets and stimulates capillary growth. Various angiogenesis factors are known including tumor, retinal and wound fluid angiogenesis factors. It is unknown whether all angiogenesis factors share a common mechanism of action upon capillary endothelial cells.

Angiogenesis factors were isolated and described

by M.S. Banda, D.R. Knighton, T.K. Hunt and Z. Werb in Proc. Nat'l. Acad. Sci. U.S.A. (7773 - 7777, Dec. 1982), in an article entitled "Isolation of a nonmitogenic angiogenesis factor from wound fluid", the disclosure of which is incorporated herein by reference.

Angiogenesis and platelet derived growth factors are described by D.R. Knighton, T.K. Hunt, K.K. Thakral and W.H. Goodson III, in "Role of Platelets and Fibrin in the Healing Sequence," Annals of Surgery 196: 379-388 (1982), the disclosure of which is incorporated by reference. In this article, the successful treatment of a non-healing wound in a patient is described in which a single, ten-unit platelet transfusion was given. The wound healed in three weeks.

A recent study has indicated that when the body's normal healing process works, it is only at about a 50% effectiveness level.

A human angiogenic factor is produced from human foreskin fibroblasts in United States Patent 4,273,871 to Tolbert et al. A publically available foreskin fibroblast cell line is utilized to produce an angiogenic factor.

In United States Patent 4,479,896 to Antoniades the disclosure of which is incorporated herein by reference, platelet-derived growth factors are characterized and extracted for study by gel electrophoresis means.

~~Brief Summary of the Invention~~

Thrombin activated platelets have the capacity to stimulate angiogenesis, increased collagen synthesis and cell division and growth. It has been found that samples of whole blood may be utilized to prepare a platelet-enriched plasma, which when activated by thrombin, contains angiogenic and growth factors which may be used to speed the healing process of wounds.

~~Blood is stabilized and centrifuged to obtain a~~

The present invention therefore provides a method for enhancing wound repair which comprises treatment of a wound with a substance comprising platelet-derived angiogenic factors and platelet-derived growth factors. Preferably the treatment is a topical treatment. Preferably the substance comprises platelet-derived angiogenic and growth factors are derived from mammalian blood. The invention also provides a method for treatment of tissue, said method comprising :

applying material released from platelets topically onto said tissue. Preferably the material is applied topically in an amount sufficient to cause migration and/or division of fibroblast cells, capillary endothelial cells and/or epithelial cells, the platelets are isolated from blood prior to release of said material, the tissue is mammalian tissue, and the tissue is human tissue. Preferably the platelets are mammalian platelets, the said platelets are human platelets. Preferably prior to release of said material said platelets were removed from the person whose tissue is being treated, or prior to release of said material said platelets were removed from a person or persons other than the person whose tissue is being treated. Preferably the material is released from said platelets by use of an activator selected from the group consisting of thrombin, adenosine diphosphate and collagen and most preferably thrombin.

The invention further provides a method of treatment of tissue comprising :

applying a composition topically onto said tissue, said composition comprising a substance which is both chemotactic and non-mitogenic for capillary endothelial cells, said composition being applied in an amount sufficient to cause directed growth of capillary endothelium. Preferably the substance is platelet derived angiogenesis factor, the composition further comprises a second substance which is mitogenic for cells selected from

the group consisting of fibroblast cells, capillary endothelial cells and epithelial cells, the second substance is a platelet derived growth factor. Preferably the composition is applied topically to tissue which is a wound and such application causes substantial formation of granulation tissue in said wound, the tissue is mammalian tissue, and more preferably the tissue is human tissue.

The present invention also provides a therapeutic composition for application to tissue for the purpose of forming granulation tissue and/or capillaries and/or epithelial tissue, said composition comprising :

(i) the material released from human platelets; and

(ii) a pharmaceutically acceptable carrier or diluent therefore

wherein said composition is substantially free of (i) blood or plasma contaminants and (ii) platelet ghosts or other material found in human platelets but not released by said platelets. Preferably the concentration of the material in said composition is within the range of concentration equivalent to the amount of material released from about 10^6 to 10^9 platelets per one mililiter of said composition.

Brief Summary of the Invention

Thrombin activated platelets have the capacity to stimulate angiogenesis, increased collagen synthesis and cell division and growth. It has been found that samples of whole blood may be utilized to prepare a platelet-enriched plasma, which when activated by thrombin, contains angiogenic and growth factors which may be used to speed the healing process of wounds.

Blood is stabilized and centrifuged to obtain a

platelet-rich plasma. The blood is stabilized by mixing with citrate-phosphate-dextrose in a ratio of 1:5 (20% solution). The platelet-rich plasma (hereinafter PRP) is preferably centrifuged again until a high concentration of platelets is obtained. The platelets are then placed in a platelet buffer. The concentration of platelets should be at least 1,000,000 platelets per milliliter. Preferably, the concentration should be on the order of 1,000,000,000 platelets per milliliter.

Thrombin is added to the PRP in order to activate the platelets. Preferably, about 1 to about 10 units of thrombin are utilized per milliliter of PRP. The thrombin-activated platelets release platelet derived growth factors (hereinafter PDGF) and platelet derived angiogenesis factors (hereinafter PDAF). The platlets and thrombin are allowed to incubate at room temperature for about 5 to 10 minutes.

The activated PRP containing PDGF and PDAF is preferably added to a biologically compatible macromolecular substance which acts as a carrier. First the platlets are centrifuged at about 950 x g and the platelet free supernatant is mixed with the carrier. Preferably, a microcrystalline collagen such as Avitene® brand collagen as sold by FMC Corp., Avicel Dept., Marcus Hook, PA 19061 is utilized as the biologically compatible carrier. Microcrystalline collagens are biologically compatible in the body. Enough carrier is added to soak up all the platelet rich plasma that is obtained from the blood. For example, a 40ml blood sample would typically require about 25ml of carrier after enrichment. The paste so obtained is preferably stored on ice or in the refrigerator.

The pharmaceutical preparations for use as a wound dressing sold by Pharmacia Fine Chemicals, Inc. of Piscataway, New Jersey under the trademark Debrisan is a suitable carrier.

The activated PRP within the carrier may then be applied to a wound. The highly enriched and active PDGF and PDAF therewithin assists in healing by proliferating and directing the growth of capillary endothelium, doubling the rate of collagen synthesis and by producing leukocyte chemotaxis. Mitogenic activity results in cellular division and growth to replace the lost tissue.

Daily application of the activated PRP to wounds stimulates and bolsters the healing sequence. The amount of PRP processed from 40ml of blood is enough to produce applications for seven days. The material is placed over the entire wound at a relatively uniform thickness, approximately two millimeters thick. Granulation, contraction and epithelization may be initiated through the use of activated PRP where the body's own repair signals are inadequate to stimulate good healing.

Whenever thrombin is used herein, it is referring to thrombin as a biologic release agent for platelet release. Other biologic release agents known in the art, including collagen, ADP and serotonin, may be utilized instead of or in addition to thrombin to activate the platelets, although thrombin is preferred.

Detailed Description of the Invention

Blood obtained from the individual to be treated with the wound healing factors of the invention is stabilized in siliconized tubes containing acid-citrate dextrose (0.15M citrate, 2% glucose, pH 4.2) (hereinafter CPD) and is centrifuged in order to separate out the platelet-rich plasma therefrom. Forty to sixth milliliters of blood combined with 4-6ml of CPD is then centrifuged at about 135 x g for 20 minutes at about 4°C to obtain platelet-rich plasma. The platelet rich plasma is removed and placed into another sterile, 50ml tube. A platelet count is then taken. The CDP is utilized to prevent activation of the clotting sequence by contact of the blood

with the plastic in the syringe. The CPD is present in the syringe while the blood is withdrawn from the patient. The blood is continuously mixed with the CPD to prevent coagulation. The platelet-rich plasma in the tube is then centrifuged at 750 x g for 10 minutes at 4°C.

The platelet-free plasma is removed and discarded. The platelet pellet is resuspended in a quantity of platelet buffer to produce a final ml. A lower concentration of about a million platelets per ml is useful, but is less preferred. The platelet buffer utilized contains .05 M HEPES (N-2-hydroxyethylpiperazine-n-2-ethanesulfonic acid), 0.03 M glucose, 0.004 M KCl, 0.1 M NaCl and about 0.35% human serum albumin adjusted to a pH of about 6.5. A sample is frozen at about -20°C for later testing of mitogenic activity. Another sample is streaked onto blood agar as a sterility test.

The platelet-rich plasma is the only blood fraction utilized in the processes and compositions of the invention. The PRP is then activated with purified thrombin at a rate of about 1 to about 10 units of thrombin per milliliter of PRP. Preferably, about 1 unit of thrombin per ml of platelet-rich plasma is utilized. The activity of the thrombin coagulates the fibrinogen and activates platelets causing them to release alpha granules containing platelet-derived growth factor and platelet-derived angiogenesis factor. The thrombin used was Thrombinar™ brand from Armour Pharmaceutical Co. of Kankakee, Illinois. The platelets and thrombin are allowed to incubate at room temperature for about 5-10 minutes.

The PRP is then subjected to a removal of platelets and fibrin by centrifugation. The resulting supernatant contains both PDAF and PDGF after centrifuging at 950 x g for about 5 minutes at 4°C. The pellet is discarded since the PDAF & PDGF have been extracted into the supernatant. PDGF has been isolated and characterized.

It is a protein of 30,000 molecular weight which breaks down into two molecular weight species of 15,000 and 14,000 molecular weight.

In order to apply the PDAF and PDGF in the platelet-free supernatant thus obtained to a wound, it is desirable to utilize a carrier substance which is biologically compatible and acts as a temporary "depot". A macromolecular substance such as microcrystalline collagen provides a suitable carrier. An especially preferred carrier is Avitene® brand microcrystalline collagen from FMC Corp., Avicel Dept., Marcus Hook, PA 19061. The resultant composition is thicker and will tend to remain in position in contact with the wound. Debrisan™ brand wound dressing which contains Sepharose™ brand beads, trademarks of Pharmacia Fine Chemicals, Inc. of Piscataway, New Jersey, may be utilized as an alternative carrier. Preferably, about 8-10ml of supernatant per gram of carrier is used to produce a paste.

Application of the wound treating composition is by physically applying the material over an into the wound as in applying a medicated salve. Treatments should be repeated on a daily basis as long as the wound remains open. A preferred treatment is to apply an approximately one mm thick dressing of the platelet factor/carrier complex to the wound in the morning. It is then dressed with a sterile, dry dressing. In the evening, the dressing is removed and the substance is removed by washing with sterile saline.

Although the clinical testing involving the wound treating compositions of the invention have been directed to wounds on the body exterior, the compositions may treat internal wounds as well. Sutures may be impregnated with the wound treating compositions to speed internal healing. The wound treating compositions may also be used in conjunction with biodegradable dressings, as a coating over

implantable devices and biodegradable devices utilized in surgical procedures. Generally, any foreign body to be inserted into a patient may be coated with the composition to speed the healing process. Alternatively, the composition may be applied over the damaged tissue directly.

Initial clinical trials have been performed on eight patients, all with nonhealing wounds from periods of one to five years. All patients had maximal standardized care in attempts to heal the wounds. That therapy had failed. In all cases, administration of platelet-derived factors initiated a healing response as evidenced by granulation tissue formation (granulation tissue contains fibroblasts, endothelial cells and collagen). The wounds closed by contraction and epithelialization or by skin grafting. Stimulation of healing and eventual repair occurred in all applications.

While it is preferred to prepare activated PRP for wound treatment purposes directly from the injured animal's own blood, the advantages of the invention may be achieved by using blood or outdated platelets from animals of the same species. Utilization of blood from the injured individual to be treated is especially preferred since it avoids exposure to possible hepatitis or other contaminants from banked blood. The use of a patient's own blood would also eliminate any possible allergic reactions. A consistent source of the material may be obtained from washed, outdated human platelets. The substances may also be utilized in veterinary applications by utilizing platelets derived from the animal itself or another animal within the same species.

Example I

A patient having an open wound on the left foot following debridement of dead tissue and transmetatarsal amputation was started on PDGF and PDAF obtained as described above from his own blood. After the treatment

protocol, the wound was filled with new granulation tissue. A subsequent debridement showed completely covered metatarsal bones and contracture of the sizable wound.

Example II

A patient underwent amputation of his right great toe and was treated with standard therapy for three weeks without any granulation tissue accumulating within the wound. He was then started on the platelet factor therapy of the invention. After three weeks of treatment, the wound contracted approximately 30-40% and was healing rapidly.

Example III

A patient having two large wounds on the medial and lateral aspect of his transmatatarsal amputation stump had been treated for four months without healing using conventional therapy. Within two weeks of treatment with PDAF and PDGF as described above, the wound had cleared of an apparent infection and started producing granulation tissue.

Thirty-eight nonhealing ulcers from 28 diabetic patients were treated with the PRP paste. The average duration of the ulcers before treatment was 6-1/2 years. A paste prepared from PRP at a concentration of about 10^9 platelets/ml was combined with Avitene brand collagen. The patients applied the PDGF and PDAF containing paste daily for 12 hour periods for an average of 8 weeks. Each day, the wounds were debrided of dead tissue. All of the wounds produced granulation tissue and closed an average of 83% when compared to starting wound area. Ninety-five percent of the ulcers were successfully treated resulting in either total wound epithlialization or successful skin grafting. Only two of these nonhealing wounds did not heal. The healed ulcers remain closed with no evidence of hypertrophic scar formation or neoplastic formation.

In considering this invention, it should be

remembered that the disclosure is illustrative only, and that the scope of the invention should be determined by the appended claims.

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A method for enhancing wound repair which comprises treatment of a wound with a substance comprising platelet-derived angiogenic factor and platelet-derived growth factors.

2. A method as claimed in Claim 1 in which the treatment comprises topical application of the substance.

3. A method as claimed in Claim 1 or 2 in which the substance comprises platelet-derived angiogenic and growth factors are derived from mammalian blood.

4. A method for treatment of tissue, said method comprising :

applying material released from platelets topically onto said tissue.

5. The method as claimed in Claim 3 or 4 wherein said material is applied topically in an amount sufficient to cause migration and/or division of fibroblast cells, capillary endothelial cells and/or epithelial cells.

6. The method as claimed in Claim 4 wherein said platelets are isolated from blood prior to release of said material.

7. The method as claimed in Claim 4 or 5 wherein said tissue is mammalian tissue.

8. The method as claimed in any one of Claims 4 to 7 wherein said tissue is human tissue.

9. The method as claimed in any one of Claims 4 to 8 wherein said platelets are mammalian platelets.

10. The method as claimed in any one of Claims 4 to 9 wherein said platelets are human platelets.

11. The method as claimed in Claim 10 wherein prior to release of said material said platelets were removed from the person whose tissue is being treated.

12. The method as claimed in Claim 10 wherein prior to release of said material said platelets were removed from a person or persons other than the person whose tissue is being treated.

13. The method as claimed in Claim 10 wherein said material is released from said platelets by use of an activator selected from the group consisting of thrombin, adenosine diphosphate and collagen.

14. The method as claimed in Claim 13 wherein said activator is thrombin.

15. A method of treatment of tissue comprising :

applying a composition topically onto said tissue, said composition comprising a substance which is both chemotactic and non-mitogenic for capillary endothelial cells, said composition being applied in an amount sufficient to cause directed growth of capillary endothelium.

16. The method as claimed in Claim 15 wherein said substance is platelet derived angiogenesis factor.

17. The method as claimed in Claim 15 wherein said composition further comprises a second substance which is mitogenic for cells selected from the group consisting of fibroblast cells, capillary endothelial cells and epithelial cells.

18. The method as claimed in Claim 17 wherein the second substance is platelet derived growth factor.

19. The method as claimed in Claim 17 wherein said composition is applied topically to tissue which is a wound and such application causes substantial formation of granulation tissue in said wound.

20. The method as claimed in Claim 15 wherein the tissue is mammalian tissue.

21. The method as claimed in Claim 20 wherein said tissue is human tissue.

22. A therapeutic composition for application to tissue for the purpose of forming granulation tissue and/or capillaries and/or epithelial tissue, said composition comprising :

(i) the material released from human platelets; and

(ii) a pharmaceutically acceptable carrier or diluent therefore

wherein said composition is substantially free of (i) blood or plasma contaminants and (ii) platelet ghosts or other material found in human platelets but not released by said platelets.

23. The therapeutic composition as claimed in Claim 22 wherein the concentration of the material in said composition is within the range of concentration equivalent to the amount of material released from about 10^6 to 10^9 platelets per one mililiter of said composition.

24. A pharmaceutical substance substantially as hereinbefore described with reference to any one of the Examples or substantially as hereinbefore described with reference to any one of the Examples as applied to other pharmaceutical substance or substances in substance disclosed in the specification.

DATED THIS 2ND DAY OF MARCH, 1990
CURATECH, INC.

WATERMARK PATENT & TRADEMARK ATTORNEYS
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IAS:JC (15.8)

INTERNATIONAL SEARCH REPORT

International Application No PCT/US85/02205

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. 424/101 IPC[4] A61K 35/14

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
US	424/101 514/2, 773 & 774

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

CHEMICAL ABSTRACTS 10TH COLLECTIVE TO DATE VOL. 86-101 1977-1985
"BLOOD-PLATELET" "COLLAGEN" "ANIMAL GROWTH FACTOR"

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	US, A, 4,479,896 Published Oct. 30, 1984 Antoniades	1-19
Y	US, A, 3,628,974 Published 21 December 1971 Battista	1-19
Y	N, Annals of Surgery Vol. 196 No. 4 (Oct. 1982) Knighton et al, Role of Platelets and Fibrin in the Healing Sequence, pages 379-388	1-19

* Special categories of cited documents: [15]

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

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
23 January 1986	31 JAN 1986
International Searching Authority [1]	**Signature of Authorized Officer [20]**
ISA/US	SAM ROSEN