

of



Industria y Comercio

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División de Nuevas Creaciones

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54. TÍTULO Métodos y composiciones para la cicatrización de Heridas.

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A61P 17/02

71. SOLICITANTE The Ohio State University Research Foundation.

DOMICILIO Columbus, Ohio.

74. APODERADO Dilia Maria Rodriguez D' Aleman

22. BOGOTÁ, D. C., Enero 10/04

**FORMULARIO ÚNICO DE SOLICITUD DE PATENTE
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ENTRADA EN FASE NACIONAL**

1) SOLICITUD DE:			
☒ Patente de invención		☐ Patente de Modelo de Utilidad	
☐ Capítulo I.		☒ Capítulo II.	
2) SOLICITANTE (71)	Nombre: THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION Dirección: 1960 Kenny Road, Columbus, OH 43210 USA Nacionalidad o domicilio: Columbus, OH, USA Lugar de constitución: Ohio, USA		IDENTIFICACIÓN C.C. ☐ NIT: ☐ C.E. ☐ OTRO ☐ Cual _____ NÚMERO _____
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3) REPRESENTANTE O APODERADO	Nombre: DILIA MARIA RODRIGUEZ D'ALEMAN Dirección: Carrera 11 N°. 86-53 Piso 8 Bogotá D.C. Teléfono: 6181088 Fax: 8360824 E. Mail: info@clarkemodet.com.co		IDENTIFICACIÓN C.C. ☒ NIT: ☐ C.E. ☐ OTRO ☐ NÚMERO: 41.654.892 TP: 20824 CSJ
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Instrucciones para el diligenciamiento del presente formulario. Ver reverso de esta página			

10)

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☐ Comprobante de pago por reivindicación de prioridad
- ☐ Documento que acredite la existencia y representación legal cuando el solicitante sea persona Jurídica
- ☐ Poderes, si fuera el caso
- ☐ Documento de cesión del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☒ Copia de la solicitud Internacional. (Resumen, descripción, reivindicación, Dibujos).
- ☒ Traducción de la solicitud internacional al castellano. (Resumen, descripción, reivindicación, Dibujos)
- ☐ Copia del examen preliminar efectuado por la IPEA
- ☐ De ser el caso, copia del contrato de acceso
- ☐ De ser el caso, documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ De ser el caso, certificado de depósito del material biológico
- ☐ Arte final 12 x 12 y 6 x 6 cm
- ☐ De ser el caso, información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante, relacionados parcial o totalmente con la invención de esta solicitud.
- ☒ Otros:
 1. Reporte Internacional de búsqueda
 2. Opinión Escrita

11) FIGURA CARACTERÍSTICA:

12) Solicitud concesión de la patente,

NOMBRE: DILIA RODRÍGUEZ D'ALEMAN

FIRMA:

C.C. 41.654.882 de Bogotá TP 20824 CSJ

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CLARKE ROBERT Y CA. COLOMBIA	CONSIGNACION	BANCO POPULAR	050-00110-6	55053479	28/12/2006	3,705,000.00

CONCEPTO

CANT. HISTORICO	CONCEPTO	TOTAL CONCEPTO
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Publications

- with international search report
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For two-letter codes and other abbreviations, refer to the "Guide
to the Search" in Codes and Abbreviations appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: METHODE AND COMPOSITIONS FOR WOUND HEALING

(57) Abstract: Methods, devices, and compositions for treating lacerations in animals, comprising hydrogen peroxide in concentrations that are less than conventionally used. The methods, devices, and compositions provide an increased rate of wound healing.

WO 2006/009853 A1

METHODS AND COMPOSITIONS FOR WOUND HEALING

DESCRIPTION OF THE INVENTION

Field of the Invention

[001] This application claims priority to U.S. Utility Application No. 10/871,158, filed June 18, 2004, the entire disclosure of which is incorporated herein by reference.

[002] This invention generally relates to methods and compositions for treating wounds.

Background of the Invention

[003] For years it has been believed that reactive oxygen species (ROS) are primarily damaging to living cells. In fact, ROS such as hydrogen peroxide and ozone have been used for their powerful oxidizing effects as disinfectants. These oxidizing effects are non-specific; in addition to destroying unwanted microorganisms, considerable collateral damage is produced. Thus, in the doses traditionally used in disinfecting, hydrogen peroxide is destructive to living tissue.

[004] However, it has been discovered that at lower doses, hydrogen peroxide has surprising effects on the healing process. The present invention relates to the use of low-dose hydrogen peroxide for its wound healing effects.

SUMMARY OF THE INVENTION

[005] The present invention provides methods of increasing the rate of lesion healing in mammals comprising applying to the lesion about 500 nanomoles to about 50 micromoles of hydrogen peroxide per square centimeter of lesion. In some embodiments, the methods comprise applying about 1 to about 50, or about 1 to about 10, or about 1 to about 2 micromoles of hydrogen peroxide per square

centimeter of lesion. The hydrogen peroxide can be applied to the lesion in a source chosen from, for example, enzymatic and chemical sources. In some embodiments, the source of hydrogen peroxide is chemical, and the source is hydrogen peroxide.

[006] The invention also provides methods of increasing the rate of lesion healing in a mammal comprising applying hydrogen peroxide to the lesion at a rate of about 500 nanomoles to about 50 micromoles of hydrogen peroxide per square centimeter of lesion over a period of from about 12 hours to about 24 hours. In some embodiments, the hydrogen peroxide is applied at a rate of from about 1 to about 10 micromoles. The hydrogen peroxide can be applied in a pharmaceutically acceptable composition, which may be in a form chosen from, for example, gels, lotions, ointments, creams, pastes, and liquids. The hydrogen peroxide can be applied in a pharmaceutically acceptable device, including but not limited to, bandages, surgical dressings, gauzes, adhesive strips, surgical staples, clips, hemostats, intrauterine devices, sutures, trocars, catheters, tubes, and implants. Implant include, but are not limited to, pills, pellets, rods, wafers, discs, and tablets.

[007] The device can comprise a polymeric material, which can comprise an absorbable material. In some embodiments, the absorbable material comprises a synthetic material. Synthetic materials can be chosen from cellulosic polymers, glycolic acid polymers, methacrylate polymers, ethylene vinyl acetate polymers, ethylene vinyl alcohol copolymers, polycaprolactam, polyacetate, copolymers of lactide and glycolide, polydioxanone, polyglactin, polylactide, polyglyconate, polygluconate, and combinations thereof. In some embodiments, the absorbable material comprises a non-synthetic material. Non-synthetic material can be chosen from catgut, corgile membrane, fascia lata, gelatin, collagen, and combinations thereof.

[008] The device can comprise a polymeric material, which can comprise a nonabsorbable material. In some embodiments, the nonabsorbable material comprises a synthetic material. Synthetic materials can be chosen from nylons, rayons, polyesters, polyolefins, and combinations thereof. In some embodiments, the nonabsorbable material comprises a non-synthetic material. Non-synthetic materials can be chosen from silk, dermal silk, cotton, linen, and combinations thereof.

[009] The method can be used to treat lesions chosen from wounds, ulcers, and burns. Wounds can be chosen from acute wounds and chronic wounds. The wounds can be chosen from full thickness wounds and partial thickness wounds. Acute wounds can be chosen from, for example, surgical wounds, penetrating wounds, avulsion injury, crushing injury, shearing injury, burn injury, laceration, and bite wound. Chronic wounds can be chosen from, for example, arterial ulcers, venous ulcers, pressure ulcers, and diabetic ulcers.

[010] The present invention also provides a hydrogen peroxide delivery device for administration to a lesion, comprising hydrogen peroxide and a carrier material, which device releases said hydrogen peroxide for a period of time which is at least about 12 hours, wherein the hydrogen peroxide released from the device is in insufficient concentration to produce necrosis of the lesion. In some embodiments, the device releases from about 0.6 to 50 μmol hydrogen peroxide/ cm^2 wound/12 hr to about 0.5 to 50 μmol hydrogen peroxide/ cm^2 wound /24 hr. The carrier material can comprise a polymeric material. In some embodiments, the polymeric material comprises an absorbable material. In some embodiments, the polymeric material comprises a synthetic material.

[011] The invention also provides a composition for treating lesions in mammals comprising: hydrogen peroxide and a pharmaceutically acceptable carrier, wherein a unit dose of the composition comprises from about 0.5 to about 50 μmol hydrogen peroxide/ cm^2 wound. In some embodiments, the carrier comprises a gel material, and in some embodiments, the carrier comprises a liquid material.

[012] Additional objects and advantages of the invention will be set forth in part in the description which follows, and in part will be obvious from the description, or may be learned by practice of the invention. The objects and advantages of the invention will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[013] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWINGS

[014] Figure 1. Presence of ROS at wound-site. A. H_2O_2 concentration in wound fluid. Hunt/Schilling wire mesh cylinders were subcutaneously implanted on the back of 5 week old C57BL/6 mice via incisional wounding. After 5d, the wound fluid was collected and the steady-state H_2O_2 concentration in the fluid was measured using a real-time electrochemical technique as described herein below. The baseline was collected in PBS. Wound fluid (0.15 ml) was added to DPBS (1 ml) at time indicated with an arrow. Using a standard curve, the concentration of H_2O_2 determined in the wound fluid was 1.1 μM . B. EPR spectra of DMPO adduct measured from wound rinsate. The spectra were acquired from DMPO (100 mM, 0.1 ml) effluents collected from wound cavity at 0h (sham control, upper panel) 12h post-wounding (lower panel). The spectra in lower panel was identified as that of

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DMPO-OH with the following coupling constants: $a_N=14.90$ G, $a_H=14.90$. The data acquisition parameters were: microwave frequency, 9.8682 GHz; sweep width, 100 G; microwave power, 20 mW; modulation amplitude, 0.5 G; modulation frequency, 100 kHz; time constant 80 msec. C. *Superoxide production in normal skin and wound edge tissue*. The wound edge samples were harvested at 12 h after wounding and immediately frozen in OCT. Fresh 30-micron sections were incubated with DHE (0.01 mM, 20 min, 200x) to detect O_2^- and visualized by confocal microscopy.

[015] **Figure 2. Catalase over-expression attenuates healing.** The skin to be wounded was subcutaneously injected once with either catalase and LacZ (control) adenoviruses (10^{11} pfu) 5 days before wounding to allow for maximum over-expression of catalase at the wound site. Two 8x16 mm full-thickness secondary intention wound were placed on the dorsal skin of 8 wk old C57BL/6 mice (Figure 2). A. Western blot of infected skin showing catalase over-expression in the side treated with AdCatalase (AdCat) virus compared to the side treated with Control Ad LacZ virus. Blots were reprobed with β -actin to show equal loading of samples. B. Wound closures are shown as percentage of area of initial wound determined on the indicated day after wounding. Dotted line represents standard healing curve of saline treated C57BL/6 mice (open circles, O) without viral infection. AdCat Treatment (closed triangles, $\blacktriangledown$); AdlacZ treatment (closed circles, $\bullet$); * $p < 0.05$, compared to LacZ treated side. C. Masson trichrome staining was performed on formalin fixed paraffin sections of regenerated skin at the wound-site sampled on the day both wounds closed. AdCat side shows broader HE region indicative of incomplete (vs. control) regeneration of skin, consistent with slower closure. Es, eschar; G, granulation tissue; HE.

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11**[016] Figure 3. Topical H₂O₂ on wound closure: dosage a key issue.**

Two 8 x 16 mm full-thickness excisional wounds (*inset*) were placed on the dorsal skin of C57BL/6 male mice (8 wk old). Each of the two wounds was topically treated either with H₂O₂ or saline. A. Low-dose of H₂O₂ (1.25 micromoles/wound; or 0.025 ml of 0.15% solution/wound; once daily, days 0-4, open circles, O) treatment facilitated closure moderately compared to placebo treated (closed circles, ●) side. *, p < 0.05. B. Low dose H₂O₂ treatment is not toxic to wound microflora. For determination of surface microflora, wounds (treated with either 1.25 micromole H₂O₂/wound, open bar, or saline, closed bar) were swabbed (24-48 h post wounding) for 20 sec with an alginate-tipped applicator. Quantitative assessment of surface bacterial load was performed. For deep tissue wound microflora, 48h after wounding eschar tissue was removed, wound bed tissue underneath eschar was sampled and quantitative assessment of bacterial load was performed. Values shown represent mean ± SD of CFU of four observations. C. High dose (high, 25 micromoles/wound; closed circles, ●, 0.025 ml of 3% solution versus low, 1.25 micromoles/wound or 0.025 ml of 0.15%; open circles, O, once daily days 0-4, of H₂O₂ adversely affected closure. *, p < 0.05; compared to low dose H₂O₂ treatment. A higher concentration (*inset*) of H₂O₂ (82.5 micromoles/wound, left side treated; 0.025 ml of 7.5% solution/wound, once on day 0) treatment causes necrotic tissue damage and severe injury leading to death of mice.

[017] Figure 4. Wound and H₂O₂-induced changes in angiogenesis related genes, vascularization and wound-edge blood flow. Paired excisional wounds (Figure 2) were either treated with placebo saline or H₂O₂ (1.25 micromole/wound, days 0-4, once daily). Wound-edge tissue was collected at indicated times after wounding. A. Ribonuclease protection assay (RPA) showing

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kinetics of angiogenesis-related mRNA expression in a placebo-treated wound. B. Low dose H_2O_2 treatment (1.25 micromole/wound, once daily, days 0-4) to wounds further augmented wound-induced Flt-1 and VEGF mRNA expressions as determined using RPA. C. Blood flow imaging of wounds was performed non-invasively using a Laser Doppler blood flow imaging device. Images reflecting the blood flow (right panel) and a digital photo (region of interest; left panel) from post-heal tissue are shown. Data i.e., mean $\pm$ SD of the blood flow is presented (bar graph). The mean represents the arithmetic mean of all valid blood flow values for pixels within the region of interest. The results show that the treatment resulted in increased blood flow, a functional outcome of enhanced angiogenesis. D. Day 8 post-wounding, wound-edge was cryosectioned and vascularization was estimated by staining for CD31 (red, rhodamine) and DAPI (blue, nuclei); higher abundance of CD31 red stain in section obtained from H_2O_2 treated side (bottom) reflect better vascularization vs control (top).

[018] Figure 5. H_2O_2 -induced phosphorylation of focal adhesion kinase (FAK) in microvascular endothelial cells and wound edge tissue. Human microvascular endothelial cells (HMEC-1) were treated with H_2O_2 for indicated dose and duration. Phosphorylation of FAK was detected using Western blot and phosphorylation site-specific antibodies against FAK. Native FAK or β -actin was blotted to show equal loading. A. Effect of various doses of H_2O_2 treatment on phosphorylation (Ty 825) state of FAK. B. Kinetics of site-specific activation phosphorylation of FAK in HMEC cells following H_2O_2 (0.1 mM) treatment. C. Paired excisional wounds (Figure 2) were either treated with placebo saline or H_2O_2 (1.25 micromole/wound). Wound-edge tissue was collected 30 min after wounding. FAK

phosphorylation in wound edge tissue was determined using Western blot. Data from three animals (#1-#3) are shown.

[019] **Figure 6. MCP-1 and p47phox deficiency impairs dermal healing.** Two excisional wounds (Figure 2) were placed on the dorsal skin of 8 wk old C57BL/6, MCP-1 or p47phox KO mice. Each of the two wounds was treated with either saline or H₂O₂ (1.25 micromoles/wound; days 0-4). A. RPA showing kinetics of monocyte/macrophage chemotactic protein related mRNA expression in placebo-treated wounds of wild-type (C57 BL/6) mice. B. Wound closures in saline (closed circles, ●) treated wounds of C57BL/6 and H₂O₂ (closed triangles, ▼) or saline (open circles, ○) treated MCP-1 KO mice are shown as percentage of area of initial wound. * p< 0.05; compared to C57BL/6 saline treatment. #, p< 0.05; compared to KO saline treatment. C. Wound closures in saline (closed circles, ●) treated wounds of C57BL/6 and H₂O₂ (closed triangles, ▼) or saline (open circles, ○) or p47 Phox KO mice are shown as percentage of area of initial wound. *p< 0.05; compared to C57BL/6 saline treatment. #, p< 0.05; compared to KO saline treatment. D. Keratin 14 (green fluorescence) expression in skin of p47phox KO mice harvested from wound sites after closure on day 18 post-wounding. Note higher expression of keratin 14 in control side compared to H₂O₂-treated side indicating healing is ongoing on the control side, while H₂O₂ treated side shows keratin 14 expression comparable to normal skin.

DESCRIPTION OF THE EMBODIMENTS

[020] The present invention will now be described by reference to more detailed embodiments. This invention may, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather,

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these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

[021] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. The terminology used in the description of the invention herein is for describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the invention and the appended claims, the singular forms "a," "an," and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

[022] Unless otherwise indicated, all numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[023] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements. Every numerical

range given throughout this specification will include every narrower numerical range that falls within such broader numerical range, as if such narrower numerical ranges were all expressly written herein.

[024] The present invention generally relates to the use of hydrogen peroxide or its sources in lesion healing. In some embodiments, the rate of healing is increased, and in some embodiments, there is a reduction in scarring. The invention can generally be used to treat any damage to a living body in which the body's natural repair process will occur. The invention can be used to treat lesions in animals, such as mammals, and specifically including humans.

[025] The term "lesion" is used herein in its generic sense, meaning that it encompasses all sorts of wounds and injuries. "Wound" can also be used in its generic sense, meaning that it encompasses wounds, burns, ulcers, etc. "Wound" and "lesion" may be used interchangeably herein, and unless the context specifically dictates otherwise, no distinction is intended. Lesions can be wounds, burns, ulcers, etc. Lesions/wounds can be acute or chronic. Wounds can be full thickness, i.e., penetrating all layers of skin, or partial thickness, i.e., penetrating less than all layers of skin. Examples of acute wounds include, but are not limited to, surgical wounds, penetrating wounds, avulsion injuries, crushing injuries, shearing injuries, burn injuries, lacerations, and bite wounds. Examples of chronic wounds include, but are not limited to, ulcers, such as arterial ulcers, venous ulcers, pressure ulcers, and diabetic ulcers. Of course, acute wounds can become chronic wounds.

[026] The composition that is applied to the lesion to be treated contains hydrogen peroxide, or a source of hydrogen peroxide. The concentration of hydrogen peroxide applied to the lesion is less than that amount that is conventionally used, and in some embodiments is less than that amount that

produces an oxidizing effect on microbes or other living cells, and in some embodiments is less than that amount that produces a necrotic effect on contacted tissue. In some embodiments, the amount of hydrogen peroxide applied to a lesion is from about 500 nanomoles (nmol) to about 50 micromoles (μmol) per square centimeter (cm^2) of lesion. In some embodiments, the amount of hydrogen peroxide applied to a lesion is from about 5 μmol to about 500 μmol per cubic centimeter (cm^3) of lesion.

[027] The amount of hydrogen peroxide applied to a lesion can range from about 500, 600, 700, 800, or 900 nmol, or 1, 2, 3, 4, 5, 6, 7, 8, 9, or about 10 μmol or higher, to about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, or about 50 μmol , per square centimeter (cm^2) of lesion. The amount can range, for example, from 1-50, 1-25, 1-10, or 1-2 μmol , per square centimeter (cm^2) of lesion. The amount of hydrogen peroxide applied to a lesion can range from about 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, or about 100 μmol or higher, to about 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 400, or about 500 μmol , per cubic centimeter (cm^3) of lesion. The amount can range, for example, from 10-500, 10-250, 10-100, or 10-20 μmol , per cubic centimeter (cm^3) of lesion.

[028] The concentration of hydrogen peroxide applied to a lesion can range from about 10, 15, 20, 25, 30, 35, 40, 45, 50, or about 75 mM to about 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 mM, or higher. Thus, the concentration of hydrogen peroxide can range from about 10 to about 100 mM, or from about 25 to about 75 mM, or from about 40 to about 60 mM.

[029] The hydrogen peroxide can be applied in any form of vehicle or carrier, including but not limited to, liquids, gels, lotions, creams, pastes, and ointments. The means of application will depend upon what form the hydrogen

peroxide takes: liquids can be sprayed or poured, for example; gels, lotions, creams, pastes, and ointments can be rubbed or massaged, for example. These and other forms, and/or carriers/vehicles, for hydrogen peroxide delivery, are described in publications such as Remington's Pharmaceutical Science, and other similar publications.

[030] The delivery forms can be homogeneous, e.g., forms in which the hydrogen peroxide is in solution, or heterogeneous, e.g., forms in which hydrogen peroxide is contained within liposomes or microspheres. The forms can produce an immediate effect, and can alternatively, or additionally, produce an extended effect. For example, liposomes, or microspheres, or other similar means of providing an extended release of hydrogen peroxide, can be used to extend the period during which the hydrogen peroxide is exposed to the lesion; non-encapsulated hydrogen peroxide can also be provided for an immediate effect.

[031] The delivery forms can also take the form of devices, which can deliver hydrogen peroxide to a lesion for a desired period of time. Devices include, but are not limited to, bandages, surgical dressings, gauzes, adhesive strips, surgical staples, clips, hemostats, intrauterine devices, sutures, trocars, catheters, tubes, and implants. Implants include, but are not limited to, pills, pellets, rods, wafers, discs, and tablets.

[032] Devices according to the invention can be prepared according to known methods, and can include, or be made from, polymeric material. In some instances, the polymeric material will be an absorbable material and in other instances, a non-absorbable material. Devices can, of course, include both absorbable and non-absorbable materials.

[033] Absorbable materials can be synthetic materials and non-synthetic materials. Absorbable synthetic materials include, but are not limited to, cellulosic polymers, glycolic acid polymers, methacrylate polymers, ethylene vinyl acetate polymers, ethylene vinyl alcohol copolymers, polycaprolactam, polyacetate, copolymers of lactide and glycolide, polydioxanone, polyglactin, poliglecaprone, polyglyconate, polygluconate, and combinations thereof. Absorbable non-synthetic materials include, but are not limited to, catgut, carilla membrane, fascia lata, gelatin, collagen, and combinations thereof.

[034] Nonabsorbable synthetic materials include, but are not limited to nylons, rayons, polyesters, polyolefins, and combinations thereof. Non-absorbable non-synthetic materials include, but are not limited to, silk, dermal silk, cotton, linen, and combinations thereof.

[035] Combinations of the foregoing devices and carriers/vehicles are also envisioned. For example, a hydrogen peroxide gel or ointment can be impregnated into a bandage or wound dressing for delivery of the hydrogen peroxide to the desired location. As another example, an implantable absorbable device can be loaded with a hydrogen peroxide solution and release the solution from the device over a period as desired. The physical form used to deliver the hydrogen peroxide is not critical and the choice or design of such devices is well within the level of skill of one in the art.

[036] Hydrogen peroxide can be delivered to the desired target site as hydrogen peroxide, per se, or it can be delivered as a precursor. For example, superoxide is transformed into hydrogen peroxide by superoxide dismutase, which is naturally present in animals. Thus, hydrogen peroxide can be delivered to a target site by administering superoxide, which is transformed into hydrogen peroxide.

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Hydrogen peroxide-like peroxides can be delivered by delivering, for example, tert-butyl hydroperoxide. All of these types of sources can be considered chemical sources of hydrogen peroxide.

[037] Hydrogen peroxide also is formed naturally in the body by a reaction between hemoglobin and oxygen to produce superoxide, which is then converted to hydrogen peroxide by superoxide dismutase. The hydrogen peroxide is naturally degraded in the body by an enzyme called catalase. Hydrogen peroxide can be allowed to accumulate at a lesion site by administering a catalase inhibitor to the target site. Hydrogen peroxide can also be caused to accumulate by administering additional superoxide dismutase. Delivery of hydrogen peroxide to a lesion site in this manner is considered to have been performed by enzymatic source. This can also be considered a natural source of hydrogen peroxide, as opposed to an extraneous source.

[038] Hydrogen peroxide can also be generated as a byproduct of a number of reactions, including, for example: 1) glucose + glucose oxidase; 2) xanthine + xanthine oxidase; 3) hypoxanthine + xanthine oxidase; and 4) ascorbate + ascorbate oxidase. Hydrogen peroxide concentration can also be increased in a body by the overexpression of rac1 and rac2, NADPH oxidase, and superoxide dismutase. All of these are considered enzymatic sources of hydrogen peroxide and are within the scope of the invention.

[039] The hydrogen peroxide is delivered to the desired target site at least once. In some embodiments the hydrogen peroxide is delivered to the target site two, three, four, five, six, seven, eight, nine, ten, or more times. The delivery can be as often as every two, four, six, eight, ten, twelve, fourteen, sixteen, eighteen, twenty, twenty-two, or twenty-four hours, or more. Where repeated doses are

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desired, devices or other carriers can be "programmed" to release doses of hydrogen peroxide at desired times. For example, a microsphere formulation can include unencapsulated hydrogen peroxide for an immediate effect on administration; an encapsulated component that delivers a second dose at twenty-four hours; and an encapsulated component that delivers a third dose at forty-eight hours. The treatment strategy is left to the practitioner; the design of devices or carriers, etc., is within the level of skill in the art.

[040] It may be desirable to provide for other conditions in the practice of the present invention. For example, it may be desirable to ensure that the target region of the lesion is sufficiently oxygenated; generally, it is sufficient that atmospheric oxygen be present. It also may be desirable to maintain a desired level of moisture and a particular temperature; in some embodiments, a warm, moist environment is desirable. While not required, it may also be desirable to establish or maintain a sterile environment.

[041] Additionally, it may be desirable to include other therapeutically beneficial agents in the formulation. For example, the vehicles or carriers may also include humectants or moisturizers to maintain a desired moisture level in the treated area. Other possibilities include drugs such as anesthetics or antibiotics, which provide other desired effects. Again, the possibilities are unlimited and are left to the practitioner.

[042] The following Examples are provided to even more clearly describe and explain the invention.

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[043] **EXAMPLES**

[044] **Example 1: Presence of ROS at the wound-site.**

[045] *H₂O₂ concentration in wound fluid.* Hunt/Schilling wire mesh cylinders were subcutaneously implanted on the back of 5-week-old C57BL/6 mice via incisional wounding. After five days, the wound fluid was collected and the steady-state H₂O₂ concentration in the fluid measured using a real-time electrochemical technique as described in Liu and Zweier (Free Radic Biol Med. 2001 Oct 1;31(7):894-901). The baseline was collected in PBS. The results are shown in Figure 1A.

[046] Wound fluid (0.15 ml) was added to DPBS (1 ml) at time indicated with an arrow. Using a standard curve, the concentration of H₂O₂ determined in the wound fluid was 1.1 μ M. This result is in contrast to measurements of blood plasma, which do not show measurable hydrogen peroxide.

[047] *H₂O₂ detection at wound edge.*

[048] Hydrogen peroxide was tested at the wound edge by detection of radical footprints of hydrogen peroxide in the wound cavity using a spin-trap solution to rinse the exposed wound. Twelve hours after wounding, the site was treated with the spin trap, DMPO (5,5-dimethyl-pyrroline-1-oxyl). To obtain control data, a freshly inflicted wound was subjected to the same spin-trap treatment. After 15 minutes, the spin trap solution was withdrawn from the wound cavity and subjected to electron paramagnetic resonance (EPR) assay.

[048] EPR measurements were performed using a Bruker ER 300 EPR spectrometer operating at X-band with a TM 110 cavity. Figure 1B shows EPR spectra of DMPO adduct measured from wound rinsate. The spectra were acquired from DMPO (100 mM, 0.1 ml) effluents collected from wound cavity at 0h (sham control, upper panel) 12h post-wounding (lower panel). The spectra in the lower panel was identified as that of DMPO-OH with the following coupling constants: $a_N=14.90$ G, $a_H=14.90$. The data acquisition parameters were: microwave frequency, 9.8682 GHz; sweep width, 100 G; microwave power, 20 mW; modulation amplitude, 0.5 G; modulation frequency, 100 kHz; time constant 80 msec.

[050] While the spectrum from the sham-treated spin trap solution did not shown any prominent spin adduct, the spectrum obtained from wound rinsate at 12-hour post-wound period showed a clear 1:2:2:1 quartet pattern. The individual components were identified as DMPO-OH (hydroxyl radical adduct) by simulation.

[051] *Superoxide production in normal skin and wound edge tissue.* As a functional outcome of NADPH oxidase activity, $O_2^{\cdot -}$ generation is often measured from tissue cryosections using dihydroethidium (DHE) as the ROS-sensitive fluorescent dye. Briefly, the wound edge samples were harvested at 12 hours after wounding and immediately frozen in OCT. Fresh 30-micron sections were incubated with DHE (0.01 mM, 20 min, 200x) to detect $O_2^{\cdot -}$ and visualized by confocal microscopy. Results are shown in Figure 1C.

[052] Employing this approach, it was clearly observed that the edge of wound-tissue stained much more prominently compared to freshly sliced normal skin. This finding lends further support that the wound site is enriched in ROS.

[053] Together, these experiments clearly demonstrate that ROS, including hydrogen peroxide, are present in wound-healing tissue.

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[054] **Example 2: Effect of excess catalase on wound-healing.**

[055] Because hydrogen peroxide was shown to be present in wound-healing tissue, experiments were performed to determine whether decreasing its concentration would hinder wound healing. Catalase is the natural enzyme that hydrolyzes hydrogen peroxide, so it was introduced into wounds to be tested.

[056] Briefly, catalase was introduced into wounds by its overexpression using an adenoviral vector. This vector allowed for high efficiency of over-expression in the murine skin. The skin to be wounded was subcutaneously injected once with either catalase or LacZ (control) adenoviruses (10^{11} pfu) five days before wounding to allow for maximum over-expression of catalase at the wound site. Two 8x16 mm full-thickness secondary intention wounds were placed on the dorsal skin of eight-week-old C57BL/6 mice.

[057] Figure 2A shows a Western blot of infected skin showing catalase over-expression in the skin treated with Ad-catalase (AdCat) virus compared to the skin treated with Control Ad-LacZ virus. Blots were re-probed with β -actin to show equal loading of samples.

[058] Figure 2B shows wound closures as a percentage of area of initial wound determined on the indicated day after wounding. The dotted line represents a standard healing curve of saline treated C57BL/6 mice (open circles, O) without viral infection. AdCat Treatment (closed triangles, $\blacktriangledown$); AdlacZ treatment (closed circles, $\bullet$); * $p < 0.05$, compared to LacZ treated side.

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[059] Figure 2C shows Masson trichrome staining performed on formalin-fixed paraffin sections of regenerated skin at the wound-site sampled on the day both wounds closed. The AdCat slide shows broader HE region, indicative of incomplete (vs. control) regeneration of skin, consistent with slower closure. Es, eschar; G, granulation tissue; HE, hyperproliferative epithelium.

[060] Example 3: Effect of hydrogen peroxide on wound closure.

[061] As it was clearly demonstrated that hydrogen peroxide is present in healing wounds (Example 1), and that its absence from healing wounds attenuates the healing process (Example 2), experiments were performed to examine the effect of added hydrogen peroxide.

[062] Briefly, two 8x16-mm full-thickness excisional wounds (Figure 3, insets) were placed on the dorsal skin of C57BL/6 male mice (8 weeks old). Each of the two wounds was topically treated either with H_2O_2 or saline.

[063] Figure 3A shows low-dose of H_2O_2 (1.25 micromoles/wound; or 0.025 ml of 0.15% solution/wound; once daily, days 0-4, open circles, ○) treatment facilitated closure moderately compared to placebo treated (closed circles, ●) side. [†], $p < 0.05$.

[064] Figure 3B shows that low dose H_2O_2 treatment does not influence wound microflora. For determination of surface microflora, wounds (treated with either 1.25 micromoles H_2O_2 /wound, open bar, or saline, closed bar) were swabbed (24-48 h post wounding) for 20 sec with an alginate-tipped applicator. Quantitative assessment of surface bacterial load was performed. For deep tissue wound microflora, 48 hours after wounding eschar tissue was removed, wound bed tissue underneath eschar was sampled, and quantitative assessment of bacterial load was performed. Values shown represent mean $\pm$ SD of CFU of four observations.

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[065] Figure 3C shows high dose (high, 25 micromoles/wound; closed circles, ●, 0.025 ml of 3% solution, versus low, 1.25 micromoles/wound or 0.025 ml of 0.15%; open circles, ○, once daily days 0-4) of H_2O_2 adversely affected closure. (*, $p < 0.05$; compared to low dose H_2O_2 treatment.) A higher concentration (*inset*) of H_2O_2 (62.5 micromoles/wound, left side treated; 0.025 ml of 7.5% solution/wound, once on day 0) treatment caused necrotic tissue damage and severe injury, leading to death of the mice.

[066] Example 4: Wound and H_2O_2 -induced changes in angiogenesis related genes, vascularization, and wound-edge blood flow.

[067] Additional experiments were performed to study the mechanism by which low doses of hydrogen peroxide increased the rate of wound healing.

[068] Paired excisional wounds were either treated with placebo saline or H_2O_2 (1.25 micromole/wound, days 0-4, once daily). Wound-edge tissue was collected at indicated times after wounding. Figure 4A shows a ribonuclease protection assay (RPA) showing kinetics of angiogenesis-related mRNA expression in a placebo-treated wound. Figure 4B shows how low-dose H_2O_2 treatment (1.25 micromole/wound, once daily, days 0-4) to wounds further augmented wound-induced Flt-1 and VEGF mRNA expressions as determined using RPA.

[069] Figure 4C shows blood-flow imaging of wounds performed non-invasively using a Laser Doppler blood-flow imaging device. Images reflecting the blood flow (right panel) and a digital photo (region of interest; left panel) from post-heal tissue are shown. Data of the blood flow is presented as mean $\pm$ SD (bar graph). The mean represents the arithmetic mean of all valid blood flow values for pixels within the region of interest. The results show that the treatment resulted in increased blood flow, a functional outcome of enhanced angiogenesis.

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[070] Figure 4D shows the results from day 8 post-wounding. Wound-edge was cryosectioned and vascularization was estimated by staining for CD31 (red, rhodamine) and DAPI (blue, nuclei); higher abundance of CD31 red stain in section obtained from H_2O_2 treated side (bottom) reflect better vascularization vs control (top).

[071] Example 5: H_2O_2 -induced phosphorylation of focal adhesion kinase (FAK) in microvascular endothelial cells and wound-edge tissue.

[072] Human microvascular endothelial cells (HMEC-1) were treated with H_2O_2 for indicated dose and duration. Phosphorylation of FAK was detected using Western blot and phosphorylation site-specific antibodies against FAK. Native FAK or β -actin was blotted to show equal loading.

[073] Figure 5A shows the effect of various doses of H_2O_2 treatment on the phosphorylation (Ty 925) state of FAK. Figure 5B shows the kinetics of site-specific activation phosphorylation of FAK in HMEC cells following H_2O_2 (0.1 mM) treatment.

[074] In Figure 5C, paired excisional wounds were either treated with placebo saline or H_2O_2 (1.25 micromole/wound). Wound-edge tissue was collected 30 minutes after wounding. FAK phosphorylation in wound edge tissue was determined using Western blot. Data from three animals (#1-#3) are shown.

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[075] **Example 6: MCP-1 and p47phox deficiency impairs dermal healing.**

[076] By attracting hydrogen-peroxide producing macrophages, monocyte/macrophage chemoattractant/chemotactic protein-1 (MCP-1) plays a key role in the development and resolution of the acute inflammatory response to wounding. P47^{phox} is a regulatory subunit of NADPH oxidase, which is involved in ROS production. Because of the importance of these factors in ROS production and in wound healing, tests were performed to examine how hydrogen peroxide affects wounds in animals lacking these factors.

[077] Briefly, two excisional wounds were placed on the dorsal skin of eight-week-old C57BL/6, MCP-1 or p47^{phox} knockout mice. Each of the two wounds was treated with either saline or H₂O₂ (1.25 micromoles/wound; days 0-4).

[078] Figure 6A shows an RNase protection assay showing the kinetics of monocyte/macrophage chemotactic protein related mRNA expression in placebo-treated wounds of wild-type (C57 BL/6) mice. Figure 6B shows wound closures in saline (closed circles, ●) treated wounds of C57BL/6, and H₂O₂ (closed triangles, ▼) or saline (open circles, ○) treated MCP-1 knockout mice are shown as percentage of area of initial wound. (* p< 0.05; compared to C57BL/6 saline treatment. #, p< 0.05; compared to knockout saline treatment.) Figure 6C shows wound closures in saline (closed circles, ●) treated wounds of C57BL/6 and H₂O₂ (closed triangles, ▼) or saline (open circles, ○) or p47^{phox} knockout mice are shown as percentage of area of initial wound. *p< 0.05; compared to C57BL/6 saline treatment. #, p< 0.05; compared to KO saline treatment.

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[079] Keratin 14 supports epidermal differentiation and regeneration and its expression is triggered by dermal wounding. Figure 6D shows keratin 14 (green fluorescence) expression in skin of p47^{max} knockout mice harvested from wound sites after closure on day 18 post-wounding. Note higher expression of keratin 14 in control side compared to H₂O₂-treated side indicating that in the control side healing is ongoing and incomplete, while H₂O₂-treated side shows that keratin 14 expression is comparable to normal skin indicating complete healing.

[080] Other embodiments of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

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WHAT IS CLAIMED IS:

1. A method of increasing the rate of lesion healing in mammals comprising applying to the lesion about 500 nanomoles to about 50 micromoles of hydrogen peroxide per square centimeter of lesion.
2. The method according to claim 1, comprising applying about 1 to about 50 micromoles of hydrogen peroxide per square centimeter of lesion.
3. The method according to claim 2, comprising applying about 1 to about 10 micromoles of hydrogen peroxide per square centimeter of lesion.
4. The method according to claim 3, comprising applying about 1 to about 2 micromoles of hydrogen peroxide per square centimeter of lesion.
5. The method according to claim 1, wherein the hydrogen peroxide applied to the lesion is in a source chosen from enzymatic and chemical sources.
6. The method according to claim 5, wherein the source of hydrogen peroxide is chemical, and the source is hydrogen peroxide.
7. A method of increasing the rate of lesion healing in a mammal comprising applying hydrogen peroxide to the lesion at a rate of about 500 nanomoles to about 50 micromoles of hydrogen peroxide per square centimeter of lesion over a period of from about 12 hours to about 24 hours.
8. The method according to claim 7, wherein the hydrogen peroxide is applied at a rate of from about 1 to about 10 micromoles.
9. The method according to claim 7, wherein the hydrogen peroxide is applied in a pharmaceutically acceptable composition.
10. The method according to claim 9, wherein the pharmaceutically acceptable composition is in a form chosen from gels, lotions, ointments, creams, pastes, and liquids.

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11. The method according to claim 7, wherein the hydrogen peroxide is applied in a pharmaceutically acceptable device.
12. The method according to claim 11, wherein the pharmaceutically acceptable device is chosen from bandages, surgical dressings, gauzes, adhesive strips, surgical staples, clips, hemostats, intrauterine devices, sutures, locars, catheters, tubes, and implants.
13. The method according to claim 12, wherein the implant is chosen from pills, pellets, rods, wafers, discs, and tablets.
14. The method according to claim 11, wherein the device comprises a polymeric material.
15. The method according to claim 14, wherein the polymeric material comprises an absorbable material.
16. The method according to claim 15, wherein the absorbable material comprises a synthetic material.
17. The method according to claim 16, wherein the synthetic material is chosen from cellulosic polymers, glycolic acid polymers, methacrylate polymers, ethylene vinyl acetate polymers, ethylene vinyl alcohol copolymers, polycaprolactam, polyacetate, copolymers of lactide and glycolide, polylactone, polyglactin, poliglecaprone, polyglyconate, polygluconate, and combinations thereof.
18. The method according to claim 15, wherein said absorbable material comprises a non-synthetic material.
19. The method according to claim 18, wherein said non-synthetic material is chosen from catgut, corgile membrane, fascia lata, gelatin, collagen, and combinations thereof.

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20. The method according to claim 14, wherein the polymeric material comprises a nonabsorbable material.
21. The method according to claim 20, wherein the nonabsorbable material comprises a synthetic material.
22. The method according to claim 21, wherein the synthetic material is chosen from nylons, rayons, polyesters, polyolefins, and combinations thereof.
23. The method according to claim 20, wherein the nonabsorbable material comprises a non-synthetic material.
24. The method according to claim 23, wherein said non-synthetic material is chosen from silk, dermal silk, cotton, linen, and combinations thereof.
25. The method according to claim 7, wherein the lesion is chosen from wounds, ulcers, and burns.
26. The method according to claim 25, wherein the wounds are chosen from acute wounds and chronic wounds.
27. The method according to claim 25, wherein the wounds are chosen from full thickness wounds and partial thickness wounds.
28. The method according to claim 26, wherein the acute wounds are chosen from surgical wounds, penetrating wounds, avulsion injury, crushing injury, shearing injury, burn injury, laceration, and bite wound.
29. The method according to claim 26, wherein the chronic wounds are chosen from arterial ulcers, venous ulcers, pressure ulcers, and diabetic ulcers.
30. A hydrogen peroxide delivery device for administration to a lesion, comprising hydrogen peroxide and a carrier material, which device releases said hydrogen peroxide for a period of time which is at least about 12 hours, wherein the

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hydrogen peroxide released from the device is in insufficient concentration to produce necrosis of the lesion.

31. The hydrogen peroxide delivery device according to claim 30, wherein the device releases from about 0.5 to 50 μmol hydrogen peroxide/ cm^2 wound/12 hr to about 0.5 to 50 μmol hydrogen peroxide/ cm^2 wound /24 hr.

32. The hydrogen peroxide delivery device according to claim 31, wherein the carrier material comprises a polymeric material.

33. The hydrogen peroxide delivery device according to claim 32, wherein the polymeric material comprises an absorbable material.

34. The hydrogen peroxide delivery device according to claim 32, wherein the polymeric material comprises a synthetic material.

35. A composition for treating lesions in mammals comprising: hydrogen peroxide and a pharmaceutically acceptable carrier, wherein a unit dose of the composition comprises from about 0.5 to about 50 μmol hydrogen peroxide/ cm^2 wound.

36. The composition according to claim 35, wherein the carrier comprises a gel material.

37. The composition according to claim 35, wherein the carrier comprises a liquid material.

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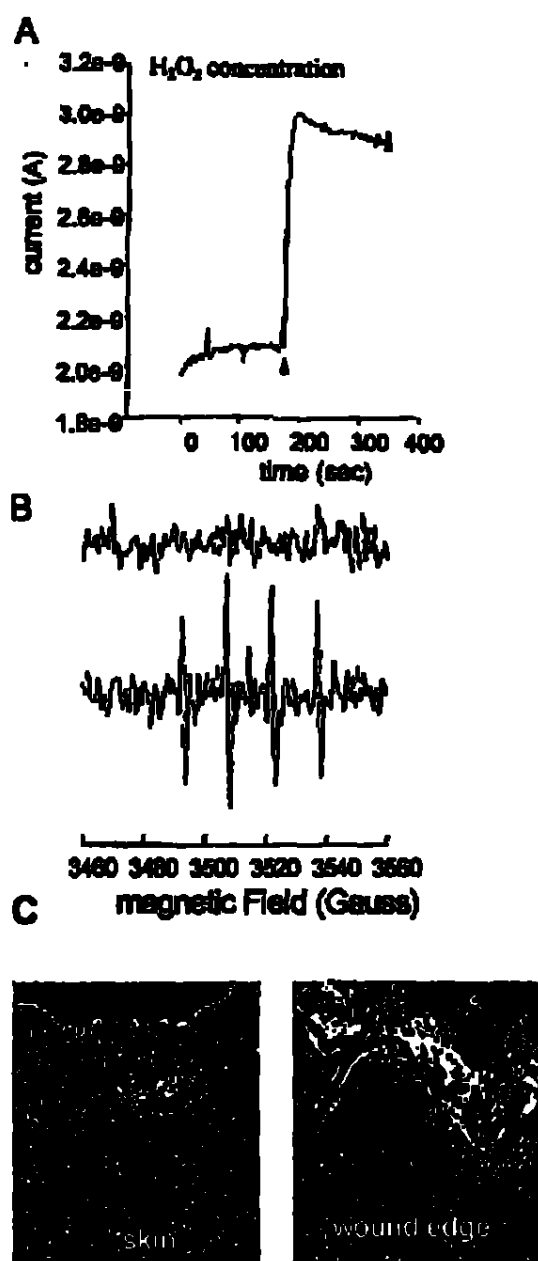


Figure 1

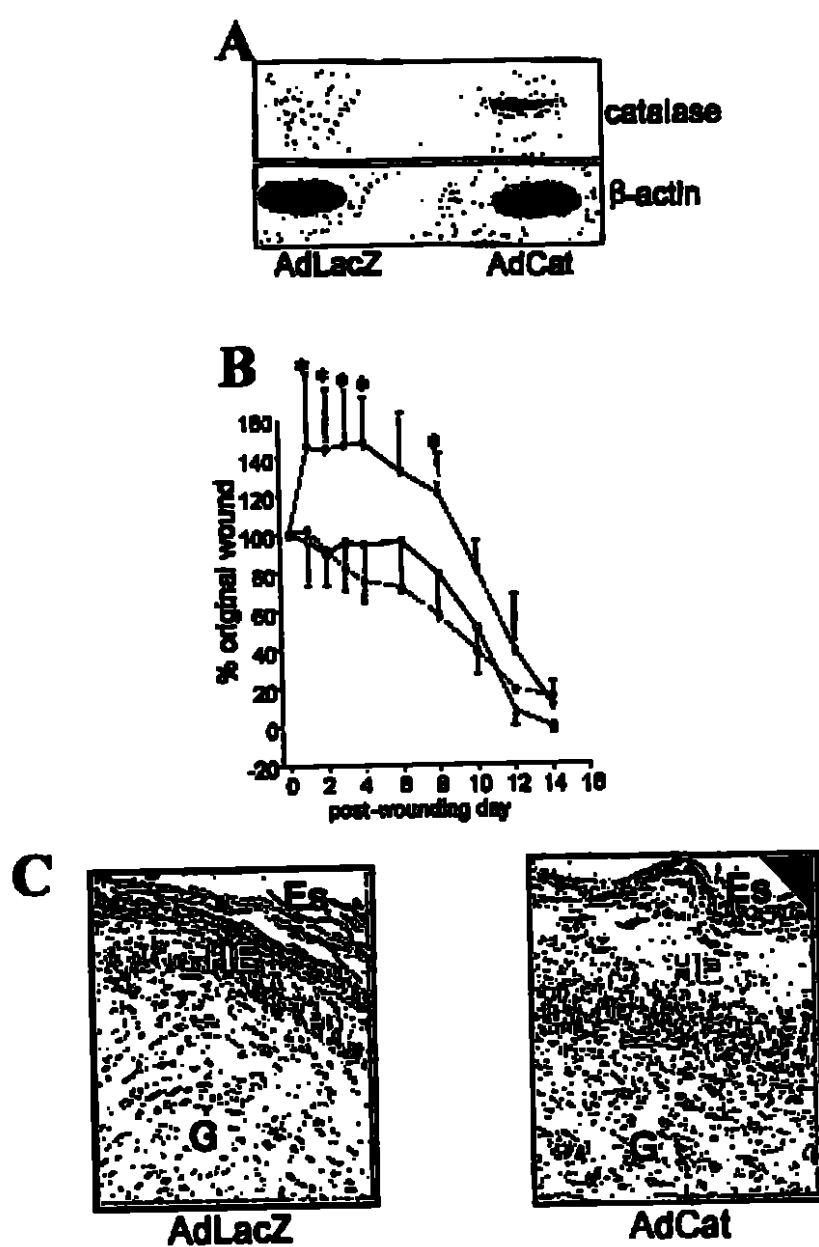


Figure 2

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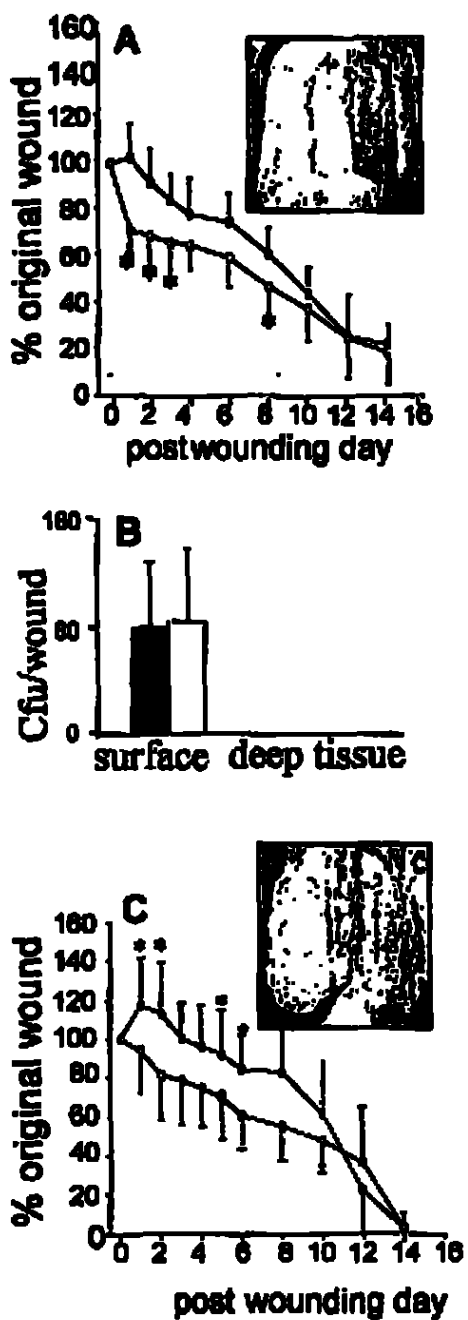


Figure 3

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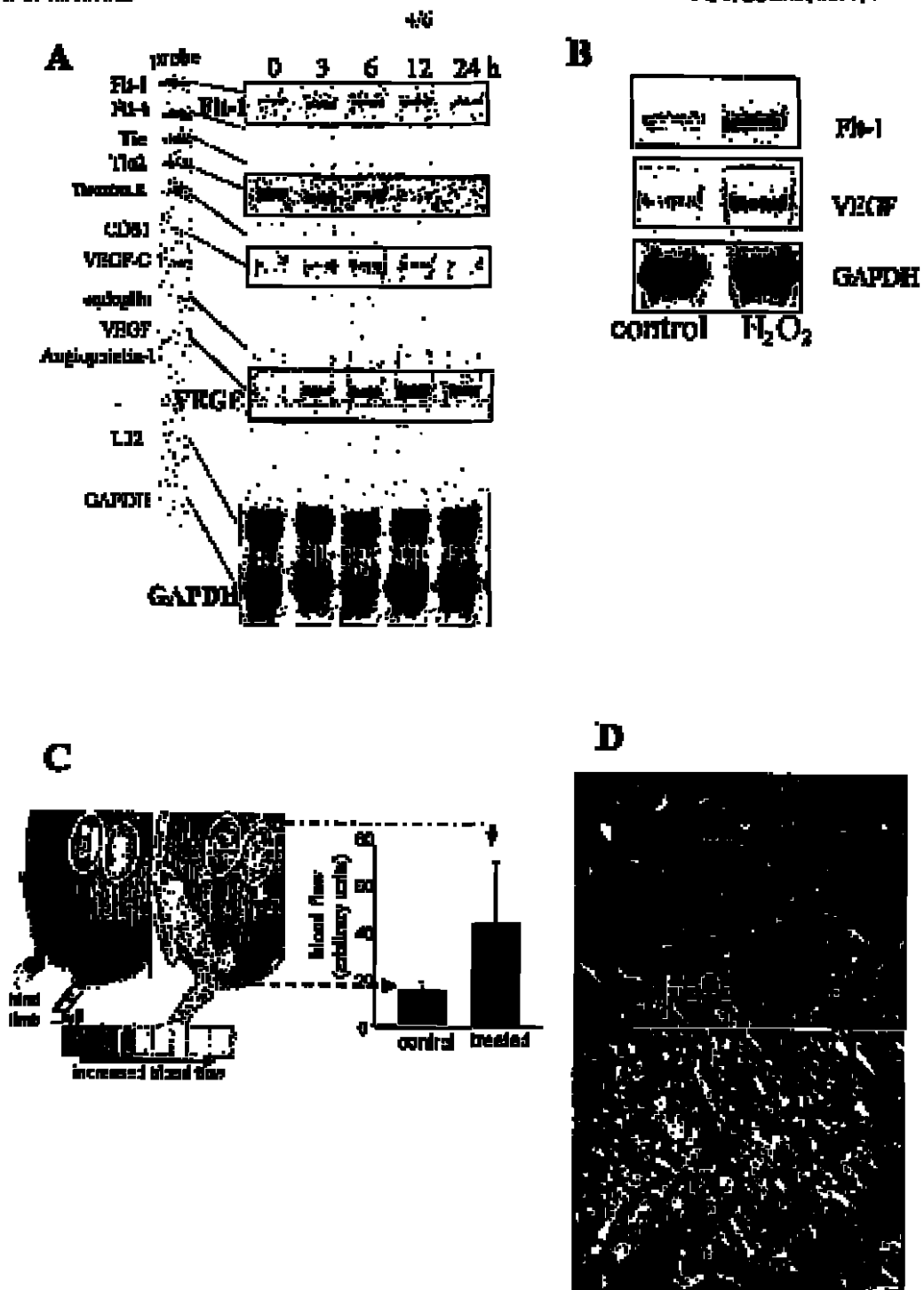


Figure 4

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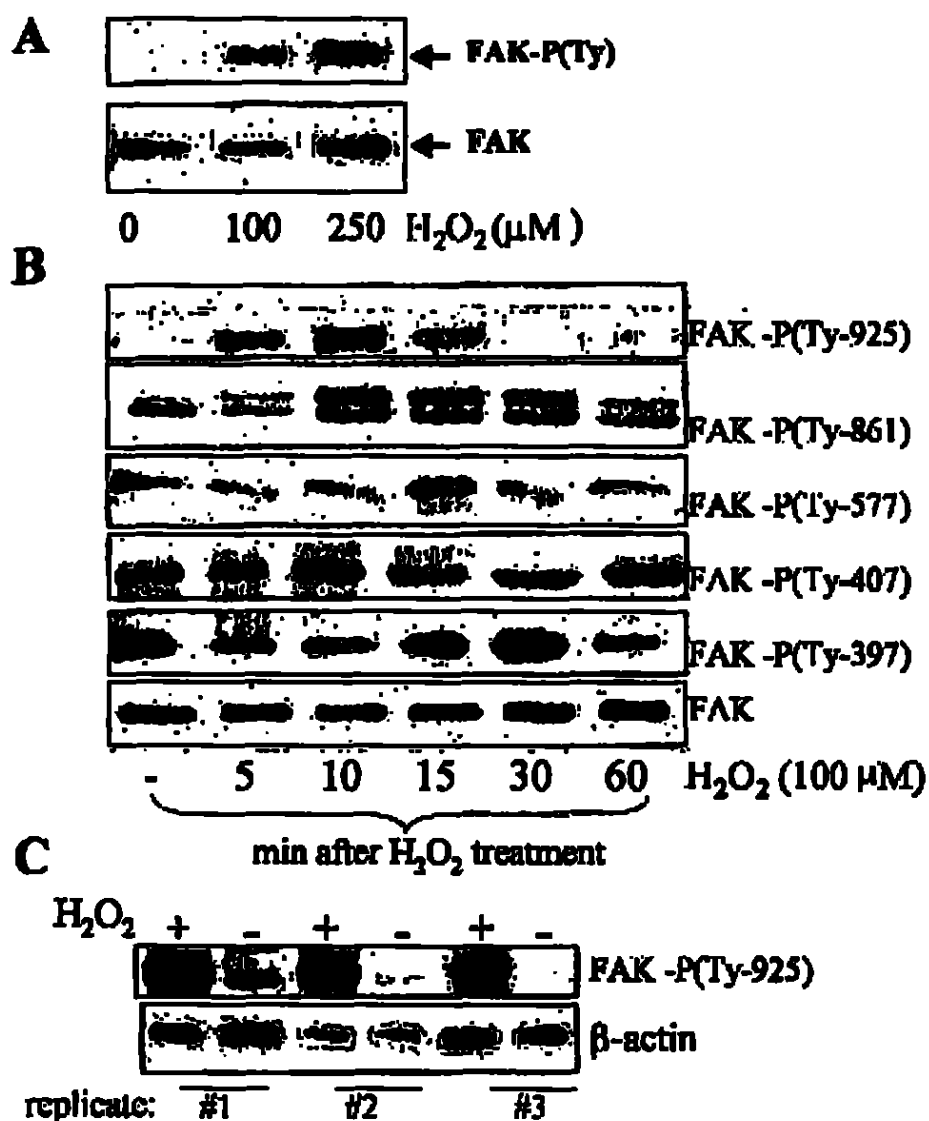


Figure 5

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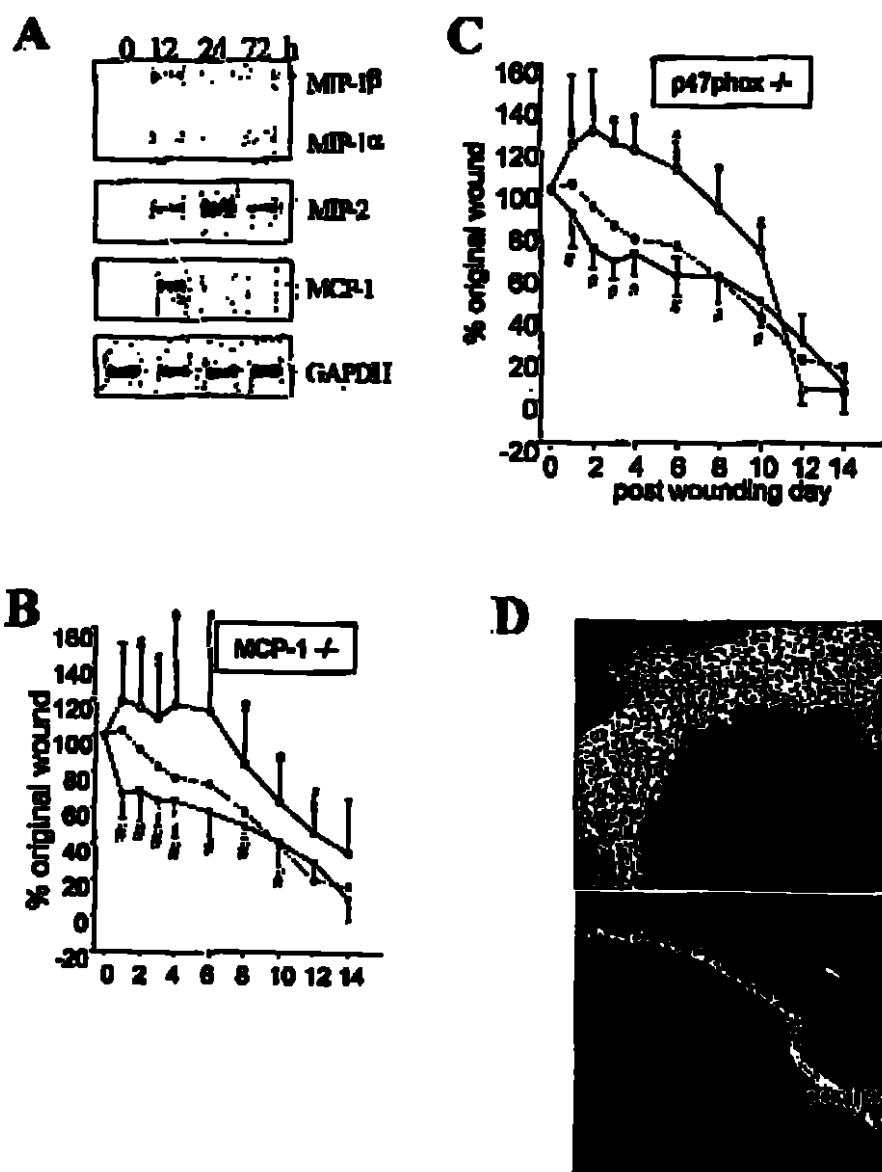


Figure 6

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INTERNATIONAL SEARCH REPORT

International Classification No.
PCT/US85/21437

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) : A61K38/00 USC : 424/613,616 According to International Patent Classification (IPC) or to related classification and ICS		
B. FIELD OF SEARCHING Minimum documentation searched (classification system followed by classification symbols) 123 : 424*613,616 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Protein Data Bank		
C. DOCUMENTS CONSULTED (OTHER THAN RELEVANT)		
Category	Character of document, with indication, where appropriate, of the relevant passages	Reference to claims
X	US 2003/0215389 A (SANTANA et al.) 27 November 2003 (27.11.2003), claims 1, 3, 5, 14, 15.	35-37
X	US 4,343,901 A (WICKERTT et al.) 20 August 1979 (27.08.1979), column 1, lines 29-33, column 4, lines 27-31, column 5, lines 4-13, column 6, lines 50-53, column 7, lines 1-5, column 8, lines 15-34.	1, 2, 3, 7, 9-11, 30, 31 3, 13, 17-20, 31-36
X	US 2004/0185277 A (WILLSON et al.) 04 January 2004 (04.01.2004), paragraphs 01-07, 01-05-01, 02, 07-0179.	20-27
X	US 2004/0185277 A (WILLSON et al.) 04 January 2004 (04.01.2004), paragraphs 01-07, 01-05-01, 02, 07-0179.	1-37
X	US 2003/0119905 A (SANTANA et al.) 26 June 2003 (26.06.2003), paragraphs 01-04, 01-0042, 0047.	1-37
☐ Further documents are listed in the continuation of Box C. ☐ See parent family member.		
D. SPECIAL FEATURES OF THE INVENTION "A" document disclosing the general state of the art, which is not concerned to be of particular relevance "B" further information in patent publications or other documents not being data "C" document disclosing the details of the invention, which is not concerned to be of particular relevance "D" document published prior to the invention, which is not concerned to be of particular relevance "E" document published prior to the invention, which is not concerned to be of particular relevance "F" document published prior to the invention, which is not concerned to be of particular relevance "G" document published prior to the invention, which is not concerned to be of particular relevance "H" document published prior to the invention, which is not concerned to be of particular relevance "I" document published prior to the invention, which is not concerned to be of particular relevance "J" document published prior to the invention, which is not concerned to be of particular relevance "K" document published prior to the invention, which is not concerned to be of particular relevance "L" document published prior to the invention, which is not concerned to be of particular relevance "M" document published prior to the invention, which is not concerned to be of particular relevance "N" document published prior to the invention, which is not concerned to be of particular relevance "O" document published prior to the invention, which is not concerned to be of particular relevance "P" document published prior to the invention, which is not concerned to be of particular relevance "Q" document published prior to the invention, which is not concerned to be of particular relevance "R" document published prior to the invention, which is not concerned to be of particular relevance "S" document published prior to the invention, which is not concerned to be of particular relevance "T" document published prior to the invention, which is not concerned to be of particular relevance "U" document published prior to the invention, which is not concerned to be of particular relevance "V" document published prior to the invention, which is not concerned to be of particular relevance "W" document published prior to the invention, which is not concerned to be of particular relevance "X" document published prior to the invention, which is not concerned to be of particular relevance "Y" document published prior to the invention, which is not concerned to be of particular relevance "Z" document published prior to the invention, which is not concerned to be of particular relevance		
Date of international application of the international patent 02 September 2003 (02.09.2003)		Date of filing of the international search report 04 JAN 2004
Name and mailing address of the applicant Mail Stop PCT, Attn: EA/US Commercial Relations P.O. Box 1450 Alexandria, Virginia 22314-0450 Facsimile No. (703) 305-3294		Authorized officer John R. R. PAX PATENT EXAMINER Telephone No. (571) 272-1800 (7:30-11:30)

Form PCT/ISA/210 (revised sheet) (April 2003)

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/BR02/1481

Continuation of B PITEUS SEARCHED Item 3:
FAIT
rapidly active hydrogen peroxide, lesions, wounds, ulcers

MÉTODOS Y COMPOSICIONES PARA LA CICATRIZACIÓN DE HERIDAS
DESCRIPCIÓN DE LA INVENCION

Campo de la invención

[0001] Esta solicitud reclama el beneficio de prioridad de la Solicitud de Utilidad de los Estados Unidos No. 10/871.158, presentada el 18 de junio de 2004, cuya descripción se incorpora en su totalidad a la presente mediante referencia.

[0002] La presente invención concierne de modo general a métodos y composiciones para tratar heridas.

Antecedentes de la invención

[0003] Durante años se ha considerado que las especies reactivas de oxígeno (ROS) son primordialmente dañinas para las células vivas. De hecho, ROS tales como el peróxido de hidrógeno y el ozono se han usado como desinfectantes por su potente efecto oxidante. Estos efectos oxidantes son inespecíficos; además de la destrucción de microorganismos no deseados, se produce un considerable daño colateral. De este modo, en las dosis tradicionalmente usadas para desinfección, el peróxido de hidrógeno es destructivo para el tejido vivo.

[0004] Sin embargo, se ha descubierto que en dosis más bajas, el peróxido de hidrógeno tiene sorprendentes efectos en el proceso de cicatrización. La presente invención concierne al uso de dosis bajas de peróxido de hidrógeno por sus efectos sobre la cicatrización de heridas.

RESUMEN DE LA INVENCION

[0005] La presente invención proporciona métodos para aumentar la velocidad de cicatrización de lesiones en mamíferos, que comprenden aplicar a

la lesión entre aproximadamente 500 nanomoles y aproximadamente 50 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión. En ciertas realizaciones, los métodos comprenden aplicar entre aproximadamente 1 y 50, o entre aproximadamente 1 y 10, o entre aproximadamente 1 y 2 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión. El peróxido de hidrógeno se puede aplicar a la lesión en una fuente elegida, por ejemplo, entre fuentes enzimáticas y fuentes químicas. En algunas realizaciones, la fuente de peróxido de hidrógeno es química y la fuente es peróxido de hidrógeno.

[0006] La invención también proporciona métodos para aumentar la velocidad de cicatrización de la lesión en un mamífero, que comprenden aplicar a la lesión peróxido de hidrógeno en una concentración entre aproximadamente 500 nanomoles y aproximadamente 50 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión durante un período que dura entre aproximadamente 12 horas y aproximadamente 24 horas. En algunas realizaciones, el peróxido de hidrógeno se aplica en una concentración entre aproximadamente 1 y aproximadamente 10 micromoles. El peróxido de hidrógeno se puede aplicar en una composición adecuada para uso farmacéutico, que puede estar en una forma seleccionada, por ejemplo, entre geles, lociones, ungüentos, cremas, pastas y líquidos. El peróxido de hidrógeno se puede aplicar en un dispositivo adecuado para uso farmacéutico, incluyendo, sin limitación, vendajes, apósitos quirúrgicos, gasas, tiras adhesivas, grapas quirúrgicas, clips, hemostáticos, dispositivos intrauterinos, suturas, trócares, catéteres, tubos e implantes. Los implantes incluyen, sin limitación, píldoras, pélets, cilindros, obleas, discos y comprimidos.

[0007] El dispositivo puede comprender un material polimérico, que puede comprender un material absorbible. En algunas realizaciones el material absorbible comprende un material sintético. Los materiales sintéticos se pueden seleccionar entre polímeros celulósicos, polímeros de ácido glicólico, polímeros de metacrilato, polímeros de etilen vinil acetato, copolímeros de etilen vinil alcohol, policaprolactam, poliacetato, copolímeros de lacturo y glicoluro, polidioxanona, poliglactina, poliglecaprona, poliglucanato, poliglucanato y combinaciones de estos polímeros. En algunas realizaciones, el material absorbible comprende un material no sintético. El material no sintético se puede seleccionar entre catgut, membrana de cargile, fascia lata, gelatina, colágeno y combinaciones de estos materiales.

[0008] El dispositivo puede comprender un material polimérico, que puede comprender un material no absorbible. En algunas realizaciones, el material no absorbible comprende un material sintético. Los materiales sintéticos se pueden seleccionar entre nailon, rayones, poliésteres, poliolefinas y combinaciones de estos materiales. En algunas realizaciones, el material no absorbible comprende un material no sintético. Los materiales no sintéticos se pueden elegir entre seda, seda dérmica, algodón, lino y combinaciones de estos materiales.

[0009] El método se puede usar para tratar lesiones elegidas entre heridas, úlceras y quemaduras. Las heridas se pueden seleccionar entre heridas agudas y heridas crónicas. Las heridas se pueden seleccionar entre heridas de espesor total y heridas de espesor parcial. Las heridas agudas se pueden seleccionar, por ejemplo, entre heridas quirúrgicas, heridas penetrantes, lesiones por desgarró, lesiones por aplastamiento, lesiones por

cortes, lesiones por quemaduras, laceraciones y heridas por mordeduras. Las heridas crónicas se pueden seleccionar, por ejemplo, entre úlceras arteriales, úlceras venosas, úlceras por compresión y úlceras diabéticas.

[0010] La presente invención también proporciona un dispositivo para administrar peróxido de hidrógeno en una lesión, que comprende peróxido de hidrógeno y un material de vehículo, donde el dispositivo libera dicho peróxido de hidrógeno durante un período de por lo menos aproximadamente 12 horas, donde el peróxido de hidrógeno liberado por el dispositivo está en una concentración insuficiente para producir necrosis de la lesión. En algunas realizaciones, el dispositivo libera entre aproximadamente 0,5 y 50 μmol de peróxido de hidrógeno/ cm^2 de herida/12 horas y aproximadamente de 0,5 a 50 μmol de peróxido de hidrógeno/ cm^2 de herida/24 horas. El material de vehículo puede comprender un material polimérico. En algunas realizaciones, el material polimérico comprende un material absorbible. En algunas realizaciones, el material polimérico comprende un material sintético.

[0011] La invención también proporciona una composición para tratar lesiones en mamíferos que comprende: peróxido de hidrógeno y un vehículo adecuado para uso farmacéutico, donde la dosis unitaria de la composición comprende entre aproximadamente 0,5 y 50 μmol de peróxido de hidrógeno/ cm^2 de herida. En algunas realizaciones, el vehículo comprende un material en forma de gel y en algunas realizaciones el vehículo comprende un material líquido.

[0012] Otros objetos y ventajas de la invención se expondrán en parte en la descripción siguiente y en parte resultarán evidentes a partir de la descripción o podrán conocerse con la práctica de la invención. Los objetos y

ventajas de la invención serán realizados y obtenidos por medio de los elementos y combinaciones indicados particularmente en las reivindicaciones adjuntas.

[0013] Debe entenderse que tanto la descripción general precedente como la siguiente descripción detallada son únicamente de tipo ejemplificativo y explicativo, y no restringen la invención de acuerdo con las reivindicaciones.

BREVE DESCRIPCIÓN DE LAS FIGURAS

[0014] **Figura 1. Presencia de ROS en el sitio de la herida. A. Concentración de H_2O_2 en el fluido de la herida.** Se implantaron cilindros de malla de alambre de Hunt/Schilling en el tejido subcutáneo del dorso de ratones C57BL/6 de 5 semanas de edad por medio de una incisión. Al cabo de 5 días, se recolectó el fluido de la herida y se midió la concentración de H_2O_2 en estado estacionario en el líquido usando una técnica electroquímica en tiempo real, como se describe más adelante en la presente. La muestra de línea de base se recolectó en PBS. Se agregó fluido de la herida (0,15 ml) a DPBS (1 ml) en el momento indicado con una flecha. Usando una curva de estándar, la concentración de H_2O_2 determinada en el fluido de la herida fue de 1,1 μM . **B. Espectros EPR del aducto DMPO medido en el líquido de enjuague de la herida.** Los espectros se obtuvieron de los efluentes del DPMO (100 mM, 0,1 ml) recolectado de la cavidad de la herida a las 0 h (control simulado, panel superior) y 12 h después de la herida (panel inferior). Los espectros en el panel inferior se identificaron como los del DMPO-OH con las siguientes constantes de acoplamiento: $a_N = 14,90$ G; $a_H = 14,90$. Los parámetros de adquisición de datos fueron: frecuencia de microondas 9,8682 GHz; ancho de barrido 100 G; potencia de microondas 20 mW; amplitud de modulación 0,5 G; frecuencia de

modulación 100 kHz; constante de tiempo 80 mseg. C. *Producción de superóxido en piel normal y en tejido del borde de la herida.* Las muestras del borde de la herida se cosecharon a las 12 h de practicada la herida y se las congeló inmediatamente en OCT. Se incubaron cortes frescos de 30 micrones con DH (0,01 mM, 20 min., 200x) para detectar O_2 y se los observó con microscopio confocal.

[0015] **Figura 2.** La sobreexpresión de catalasa reduce la cicatrización. La piel donde se iba a practicar la herida se inyectó una vez con catalasa y adenovirus LacZ (control) (10^{11} pfu) 5 días antes de practicar la herida para permitir la máxima sobreexpresión de catalasa en el sitio de la herida. Se colocaron dos heridas de espesor completo de 8 x 16 mm con cicatrización por segunda intención en la piel del dorso de ratones C57BL/6 de 8 semanas de edad (Figura 2). A. Western blot de la piel infectada mostrando la sobreexpresión de catalasa en el lado tratado con virus AdCatalasa (AdCat) en comparación con el lado tratado con virus Ad LacZ de Control. Las transferencias se investigaron nuevamente con β actina para demostrar la carga igual de las muestras. B. Los cierres de la herida se muestran como porcentaje del área inicial de la herida, determinada el día indicado después de practicar la herida. La línea punteada representa la curva de cicatrización estándar de los ratones C57BL/6 tratados con solución fisiológica (círculos vacíos o) sin infección viral. Tratamiento AdCat (triángulos llenos ▼); tratamiento AdLacZ (círculos llenos ●); * $p < 0,05$ en comparación con el lado tratado con LacZ. C. Se tiñeron con tinción tricrómica de Masson cortes de piel regenerada en el sitio de la herida fijadas con formalina y obtenidas el día de cierre de ambas heridas. El lado AdCat muestra una región HE más amplia,

indicativa de regeneración incompleta (vs. control) de la piel, consistente con un cierre más lento. Es: escara; G: tejido de granulación; HE.

[0016] **Figura 3. H_2O_2 tópico en el cierre de la herida: la dosis es un aspecto clave.** Se colocaron dos heridas de espesor completo de 8 x 16 mm obtenidas por disección (recuadro) en la piel dorsal de ratones macho C57BL/6 (8 semanas de edad). Cada una de las dos heridas fue tratada con H_2O_2 o solución fisiológica. **A. Dosis baja de H_2O_2 (1,25 micromoles/herida; o 0,025 ml de solución al 0,15%/herida; el tratamiento una vez diaria, días 0 – 4, círculos vacíos o) facilitó moderadamente el cierre en comparación con el lado tratado con placebo (círculos llenos ●); $p < 0,05$.** **B. El tratamiento con dosis bajas de H_2O_2 no es tóxico para la microflora de la herida.** Para determinar la microflora superficial, se pasó un hisopo a las heridas (tratadas con 1,25 micromoles de H_2O_2 /herida, barra vacía, o solución fisiológica, barra llena) con un aplicador con punta de alginato. Se hizo la evaluación cuantitativa de la carga bacteriana de la superficie. Para la microflora del tejido profundo de la herida, 48 h después de practicar la herida se removió el tejido de escara, se obtuvieron muestras del tejido del lecho de la herida por debajo de la escara y se realizó la evaluación de la carga bacteriana. Los valores mostrados representan las medias $\pm$ SD de CFU de cuatro observaciones. **C. El tratamiento una vez diaria en los días 0 – 4 con dosis alta de H_2O_2 (alta, 25 micromoles/herida; círculos llenos ●; 0,025 ml de solución al 3%) versus baja (1,25 micromoles/herida o 0,025 ml de solución al 0,15%, círculos vacíos o) afectó adversamente el cierre; $p < 0,05$; comparado con el tratamiento con dosis baja de H_2O_2 . Un tratamiento con concentración mayor de H_2O_2 (recuadro) (62,5 micromoles/herida, lado izquierdo tratado; 0,025 ml de solución al 7,5%/herida**

una vez el día 0) causa daño tisular necrótico y lesión severa que lleva a la muerte de los ratones.

[0017] Figura 4. Cambios inducidos por la herida y por H_2O_2 en los genes relacionados con la angiogénesis, la vascularización y el riego sanguíneo en el borde de la herida. Se trataron heridas apareadas disecadas (Figura 2) con solución fisiológica placebo o H_2O_2 (1,25 micromoles/herida; días 0 – 4, una vez diaria). Se recolectó tejido del borde de la herida en los tiempos indicados después de practicar la herida. A. Ensayo de protección a la ribonucleasa (RPA) que muestra la cinética de la expresión de mRNA relacionado con angiogénesis en una herida tratada con placebo. B. El tratamiento de la herida con dosis baja de H_2O_2 (1,25 micromoles/herida, una vez diaria, días 0 – 4) aumentó más las expresiones de mRNA de Fit1 y VEGF inducidas por la herida, según determinación hecha con RPA. C. Las imágenes del riego sanguíneo de las heridas se obtuvieron en forma no invasiva usando un flujómetro de láser Doppler. Se muestran imágenes que reflejan el riego sanguíneo (panel derecho) y una foto digital (región de interés; panel izquierdo) del tejido posterior a la cicatrización. Se presentan los datos, medias $\pm$ SD, de riego sanguíneo (gráfico de barras). La media representa la media aritmética de todos los valores válidos de flujo sanguíneo para píxeles dentro de la región de interés. Los resultados muestran que el tratamiento resultó en un aumento del flujo sanguíneo, indicador funcional de angiogénesis aumentada. D. El día 8 después de practicar la herida, se hicieron cortes con criosección del borde de la herida y se estimó la vascularización mediante tinción para CD31 (rojo, rodamina) y DAPI (azul, núcleos); la mayor abundancia de tinción roja CD31 en

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el corte obtenido del lado tratado con H_2O_2 (abajo) refleja mejor vascularización vs. el control (arriba).

[0018] Figura 5. Fosforilación inducida por H_2O_2 de la quinasas de adhesión focal (FAK) en células del endotelio microvascular y el tejido del borde de la herida. Se trataron células de endotelio microvascular humano (HMEC-1) con H_2O_2 en las dosis y duración indicadas. La fosforilación de la FAK se detectó con Western blot y anticuerpos anti-FAK específicos del sitio de fosforilación. Se hizo la transferencia de FAK natural o β actina para mostrar igual carga. A. Efecto de diversas dosis de tratamiento con H_2O_2 sobre el estado de fosforilación (Ty 925) de FAK. B. Cinética de la activación específica del sitio de la fosforilación de FAK en células HMEC después del tratamiento con H_2O_2 (0,1 mM). C. Se trataron heridas apareadas disecadas (Figura 2) con solución fisiológica placebo o H_2O_2 (1,25 micromoles/herida). Se recolectó tejido del borde de la herida 30 minutos después de practicar la herida. Se determinó la fosforilación de FAK en el tejido del borde de la herida con Western Blot. Se muestran los datos de tres animales (N° 1 - N° 3).

[0019] Figura 6. El déficit de MCP-1 y p47phox disminuye la cicatrización dérmica. Se colocaron heridas disecadas (Figura 2) en la piel dorsal de ratones de 8 semanas C57BL/6, atontados para MCP-1 o p47phox. Cada una de las dos heridas se trató con solución fisiológica o con H_2O_2 (1,25 micromoles/herida; días 0 - 4). A. RPA que muestra la cinética de la expresión de mRNA relacionado con la proteína de quimiotaxis de monocitos/macrófagos en heridas tratadas con placebo de ratones silvestres (C57 BL/6). B. Los cierres de la herida en heridas tratadas con solución fisiológica (círculos llenos •) de C57BL/6 y ratones atontados para MCP-1 tratados con H_2O_2 (triángulos

llenos ▼) o solución fisiológica (círculos vacíos ○) se muestran como porcentaje del área de la herida inicial. *p < 0,05; comparado con tratamiento con solución fisiológica en ratones C57BL/6. Nº, p < 0,05; comparado con tratamiento con solución fisiológica en ratones atontados. C. Los cierres de la herida en heridas tratadas con solución fisiológica (círculos llenos ●) de C57BL/6 y ratones atontados para p47 Phox tratados con H₂O₂ (triángulos llenos ▼) o solución fisiológica (círculos vacíos ○) se muestran como porcentaje del área de la herida inicial. *p < 0,05; comparado con tratamiento con solución fisiológica en ratones C57BL/6. Nº, p < 0,05; comparado con tratamiento con solución fisiológica en ratones atontados. D. Expresión de queratina 14 (fluorescencia verde) en la piel de ratones atontados para p47 phox cosechada de sitios de herida después del cierre el día 18 después de practicar la herida. Obsérvese la mayor expresión de queratina 14 en el lado control en comparación con el lado tratado con H₂O₂ indicando que la cicatrización está en proceso en el lado control, mientras que el lado tratado con H₂O₂ muestra una expresión de queratina 14 similar a la de la piel normal.

DESCRIPCIÓN DE LAS REALIZACIONES

[0020] La presente invención se describirá ahora con referencia a realizaciones más detalladas. No obstante, esta invención se puede realizar en diferentes formas y no debe interpretarse como limitada a las realizaciones que figuran en la presente. Por el contrario, estas realizaciones se proporcionan para explicar mejor y completar la memoria descriptiva y transmitir el alcance de la invención para el experto en la técnica.

[0021] A menos que se defina de otro modo, todos los términos técnicos y científicos usados en la presente tienen el mismo significado que se les

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asigna comúnmente en la técnica a la que pertenece esta invención. La terminología usada en la descripción de la presente invención está destinada a describir solamente realizaciones particulares y no limita la invención. Tal como se usa en la memoria descriptiva de la invención y en las reivindicaciones adjuntas, las formas singulares "uno", "un" y "el" incluyen también los plurales, a menos que el contexto indique claramente lo contrario. Todas las publicaciones, solicitudes de patente, patentes y demás referencias mencionadas en la presente quedan incorporadas en su totalidad mediante referencia.

[0022] A menos que se indique lo contrario, todos los números que expresan cantidades de ingredientes, condiciones de recedones, etcétera, usados en la memoria descriptiva y en las reivindicaciones deben entenderse modificados por el término "aproximadamente". Del mismo modo, a menos que se indique lo contrario, los parámetros numéricos expresados en la siguiente memoria descriptiva y en las reivindicaciones adjuntas son aproximaciones que pueden variar de acuerdo con las propiedades que se deseen obtener con la presente invención. Como mínimo, y al intentar limitar la aplicación de la doctrina de los equivalentes al alcance de las reivindicaciones, cada parámetro numérico debe interpretarse a la luz del número de dígitos significativos con los redondeos ordinarios.

[0023] A pesar de que los límites y parámetros numéricos que expresan el amplio alcance de la invención son aproximaciones, los valores numéricos consignados en ejemplos específicos se reportan con la mayor precisión posible. No obstante, todo valor numérico contiene cierto error inherente que resulta de la desviación estándar encontrada en las respectivas mediciones de

las pruebas. Todo rango numérico consignado en la presente memoria descriptiva incluirá todos los rangos más estrechos que se encuentran dentro de dicho rango numérico más amplio del mismo modo que si dichos rangos numéricos más estrechos figuraran en su totalidad escritos en la presente.

[0024] La presente invención concierne de modo general al uso del peróxido de hidrógeno de sus fuentes en la cicatrización de lesiones. En algunas realizaciones, aumenta la velocidad de la cicatrización y en algunas realizaciones se reduce la formación de tejido cicatrizal. La invención se puede usar de modo general para tratar cualquier daño en un organismo vivo en el que haya procesos naturales de reparación del organismo. La invención se puede usar para tratar lesiones en animales, tales como mamíferos, e incluye específicamente humanos.

[0025] El término "lesión" se usa en la presente en su sentido genérico, lo que significa que abarca todo tipo de heridas y lesiones. "Herida" también se puede usar en su sentido genérico, lo que significa que abarca heridas, quemaduras, úlceras, etc. En la presente, "herida" y "lesión" se pueden usar indistintamente y, a menos que el contexto específicamente indique lo contrario, no corresponde distinción alguna. Las lesiones pueden ser heridas, quemaduras, úlceras, etc. Las lesiones/heridas pueden ser agudas o crónicas. Las heridas pueden ser de espesor completo, es decir, que penetran todas las capas de la piel, o de espesor parcial, es decir, que no penetran todas las capas de la piel. Ejemplos de heridas agudas incluyen, sin limitación, heridas quirúrgicas, heridas penetrantes, lesiones por desgarró, lesiones por aplastamiento, lesiones por cortes, lesiones por quemaduras, laceraciones y heridas por mordeduras. Ejemplos de heridas crónicas incluyen, sin limitación,

úlceras, tales como úlceras arteriales, úlceras venosas, úlceras por compresión y úlceras diabéticas. Desde ya, las heridas agudas se pueden convertir en heridas crónicas.

[0026] La composición que se aplica a la lesión a ser tratada contiene peróxido de hidrógeno o una fuente de peróxido de hidrógeno. La concentración de peróxido de hidrógeno aplicada a la lesión es menor que la cantidad que se usa convencionalmente y, en algunas realizaciones, es menor que la cantidad que produce un efecto de oxidación sobre microbios u otras células vivas y, en algunas realizaciones, es menor que la cantidad que produce un efecto necrótico en el tejido contactado. En algunas realizaciones, la cantidad de peróxido de hidrógeno aplicada a una lesión está entre aproximadamente 500 nanomoles (nmol) y aproximadamente 50 micromoles (μmol) por centímetro cuadrado (cm^2) de lesión. En algunas realizaciones, la cantidad de peróxido de hidrógeno aplicada a una lesión está entre aproximadamente 5 μmol y aproximadamente 500 μmol por centímetro cúbico (cm^3) de lesión.

[0027] La cantidad de peróxido de hidrógeno que se aplica a una lesión puede estar entre aproximadamente 500, 600, 700, 800 o 900 nmol, o 1, 2, 3, 4, 5, 6, 7, 8, 9, o aproximadamente 10 μmol o más y aproximadamente 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40 o aproximadamente 50 μmol por centímetro cuadrado (cm^2) de lesión. La cantidad puede estar, por ejemplo, entre 1-50, 1-25, 1-10, o 1-2 μmol por centímetro cuadrado (cm^2) de lesión. La cantidad de peróxido de hidrógeno aplicada a una lesión puede estar entre aproximadamente 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90, o aproximadamente 100 μmol o más, y aproximadamente 20, 30, 40, 50, 60, 70,

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80, 90, 100, 150, 200, 250, 300, 400, o aproximadamente 500 μmol por centímetro cúbico (cm^3) de lesión. La cantidad puede estar, por ejemplo, entre 10-500, 10-250, 10-100 o 10-20 μmol por centímetro cúbico (cm^3) de lesión.

[0028] La concentración de peróxido de hidrógeno aplicada a una lesión puede estar entre aproximadamente 10, 15, 20, 25, 30, 35, 40, 45, 50, o aproximadamente 75 mM y aproximadamente 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 o 100 mM o más. Por lo tanto, la concentración de peróxido de hidrógeno puede estar entre aproximadamente 10 y aproximadamente 100 mM, o entre aproximadamente 25 y aproximadamente 75 mM, o entre aproximadamente 40 y aproximadamente 60 mM.

[0029] El peróxido de hidrógeno se puede aplicar en cualquier forma o vehículo incluyendo, sin limitación, líquidos, geles, lociones, cremas, pastas y ungüentos. El medio de aplicación dependerá de la forma que adopte el peróxido de hidrógeno: los líquidos, se pueden rociar o verter, por ejemplo; los geles, lociones, cremas, pastas y ungüentos se pueden frotar o masajear, por ejemplo. Estas y otras formas y/o vehículos para la administración de peróxido de hidrógeno se describen en publicaciones tales Remington's Pharmaceutical Science, y en otras publicaciones similares.

[0030] Las formas de administración pueden ser homogéneas, p. ej., formas en las que el peróxido de hidrógeno está en solución, o heterogéneas, p. ej., formas en las que el peróxido de hidrógeno está contenido dentro de liposomas o microesferas. Las formas pueden producir un efecto inmediato y pueden, en forma alternativa o adicional, producir un efecto extendido. Por ejemplo, se pueden usar liposomas o microesferas, u otros medios similares para proporcionar liberación extendida del peróxido de hidrógeno, para

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extender el período durante el cual el peróxido de hidrógeno está expuesto a la lesión; también se puede proporcionar peróxido de hidrógeno no encapsulado para obtener un efecto inmediato.

[0031] Las formas de administración también pueden adoptar la forma de dispositivos que pueden suministrar peróxido de hidrógeno a una lesión durante un período deseado de tiempo. Los dispositivos incluyen, sin limitación, vendajes, apósitos quirúrgicos, gasas, tiras adhesivas, grapas quirúrgicas, clips, hemostáticos, dispositivos intrauterinos, suturas, trocates, catéteres, tubos e implantes. Los implantes incluyen, sin limitación, píldoras, pellets, cilindros, obleas, discos y comprimidos.

[0032] Dispositivos de acuerdo con la invención se puedan preparar de acuerdo con métodos conocidos y pueden incluir material polimérico, o estar fabricados con este material. En algunos casos, el material polimérico será un material absorbible y en otros casos será un material no absorbible. Desde ya, los dispositivos puedan incluir tanto materiales absorbibles como no absorbibles.

[0033] Los materiales absorbibles pueden ser materiales sintéticos y materiales no sintéticos. Los materiales sintéticos absorbibles incluyen, sin limitación, polímeros celulósicos, polímeros de ácido glicólico, polímeros de metacrilato, polímeros de etilen vinil acetato, copolímeros de etilen vinil alcohol, policaprolactam, poliacetato, copolímeros de lacturo y glicoluro, polidioxanona, poliglactina, poliglecapróna, poligliconato, poligluconato y combinaciones de estos materiales. Los materiales no sintéticos absorbibles incluyen, sin limitación, catgut, membrana de cargila, fascia lata, gelatina, colágeno y combinaciones de estos materiales.

[0034] Los materiales sintéticos no absorbibles incluyen, sin limitación, nailon, rayones, poliésteres, poliolefinas y combinaciones de estos materiales. Los materiales no sintéticos no absorbibles incluyen, sin limitación, seda, seda dérmica, algodón, lino y combinaciones de estos materiales.

[0035] También están previstas combinaciones de los dispositivos y vehículos/portadores precedentes. Por ejemplo, un gel o unguento de peróxido de hidrógeno puede estar impregnado en un vendaje o apósito de heridas para administrar el peróxido de hidrógeno al lugar deseado. En otro ejemplo, un dispositivo absorbible implantable puede cargarse con una solución de peróxido de hidrógeno y liberar la solución del dispositivo durante un período que se desee. La forma física usada para administrar el peróxido de hidrógeno no es crítica y la elección o el diseño de dichos dispositivos están dentro de las habilidades del experto en la técnica.

[0036] El peróxido de hidrógeno se puede hacer llegar al sitio deseado como peróxido de hidrógeno en sí o se puede hacer llegar como precursor. Por ejemplo, el superóxido se transforma en peróxido de hidrógeno por acción de la superóxido dismutasa, que está presente en forma natural en los animales. De este modo, el peróxido de hidrógeno se puede hacer llegar a un sitio deseado administrando superóxido, que se transforma en peróxido de hidrógeno. Se pueden administrar peróxidos del tipo peróxido de hidrógeno administrando, por ejemplo, terbutil hidroperóxido. Todos estos tipos de fuentes se pueden considerar fuentes químicas de peróxido de hidrógeno.

[0037] El peróxido de hidrógeno también se forma naturalmente en el organismo por una reacción entre la hemoglobina y el oxígeno que produce superóxido, que luego se convierte en peróxido de hidrógeno por acción de la

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superóxido dismutasa. El peróxido de hidrógeno se degrada naturalmente en el organismo por acción de una enzima llamada catalasa. Se puede hacer que se acumule peróxido de hidrógeno en el sitio de una lesión administrando un inhibidor de la catalasa en el sitio deseado. También se puede hacer que se acumule peróxido de hidrógeno administrando superóxido dismutasa adicional. La administración de peróxido de hidrógeno al sitio de una lesión de esta forma se considera realizada a partir de una fuente enzimática. También se la puede considerar una fuente natural de peróxido de hidrógeno, por oposición a una fuente externa.

[0038] El peróxido de hidrógeno también se puede generar como subproducto de una serie de reacciones, incluyendo, por ejemplo: 1) glucosa + glucosa oxidasa; 2) xantina + xantina oxidasa; 3) hipoxantina + xantina oxidasa; y 4) ascorbato + ascorbato oxidasa. La concentración de peróxido de hidrógeno también se puede aumentar en un organismo mediante la sobreexpresión de rac1 y rac2, NADPH oxidasa y superóxido dismutasa. Se considera que todas son fuentes enzimáticas y están dentro del alcance de la invención.

[0039] El peróxido de hidrógeno se administra al sitio deseado por lo menos una vez. En algunas realizaciones, el peróxido de hidrógeno se administra al sitio deseado dos, tres, cuatro, cinco, seis, siete, ocho, nueve, diez o más veces. La administración se puede hacer con una frecuencia de una vez cada dos, cuatro, seis, ocho, diez, doce, catorce, dieciséis, dieciocho, veinte, veintidós o veinticuatro horas, o más. Cuando se desean dosis repetidas, se pueden "programar" dispositivos u otros vehículos para que liberen dosis de peróxido de hidrógeno en los tiempos deseados. Por ejemplo, una formulación en microesferas puede incluir peróxido de hidrógeno no

encapsulado para obtener un efecto inmediato a la administración; un componente encapsulado que suministra una segunda dosis a las veinticuatro horas; y un componente encapsulado que suministra una tercera dosis a las cuarenta y ocho horas. La estrategia del tratamiento queda a cargo del profesional; el diseño de los dispositivos o vehículos está dentro del nivel del experto en el arte.

[0040] Puede resultar deseable proporcionar otras condiciones en la práctica de la presente invención. Por ejemplo, puede resultar deseable asegurar que la región seleccionada de la lesión está suficientemente oxigenada; por lo general, es suficiente que esté presente el oxígeno atmosférico. También puede resultar deseable mantener un nivel deseado de humedad y una temperatura en particular; en algunas realizaciones resulta deseable un medio caliente y húmedo. Aunque no es un requisito, también puede resultar deseable crear o mantener un medio estéril.

[0041] Adicionalmente, puede resultar deseable incluir en la formulación otros agentes terapéuticamente beneficiosos. Por ejemplo, los vehículos o portadores pueden incluir también humectantes o humidificantes para mantener un nivel deseado de humedad en el área tratada. Otras posibilidades incluyen drogas tales como anestésicos o antibióticos, que proporcionan otros efectos deseados. Nuevamente, las posibilidades no están limitadas y quedan a criterio del profesional.

[0042] Los siguientes Ejemplos se incluyen para describir y explicar la invención con mayor claridad.

[0043] EJEMPLOS

[0044] Ejemplo 1: Presencia de ROS en el sitio de la herida.

[0045] *Concentración de H_2O_2 en el fluido de la herida.* Se implantaron cilindros de malla de alambre de Hunt/Schilling en el tejido subcutáneo del dorso de ratones C57BL/6 de 5 semanas de edad por medio de una incisión. Al cabo de 5 días, se recolectó el fluido de la herida y se midió la concentración de H_2O_2 en estado estacionario en el líquido usando una técnica electroquímica en tiempo real, como la que se describe en Lui y Zweier (Free Radic Biol Med. 2001 Oct 1;31(7): 894-901). La muestra de línea de base se recolectó en PBS. Los resultados se muestran en la Figura 1A.

[0046] Se agregó fluido de la herida (0,15 ml) a DPBS (1 ml) en el momento indicado con una flecha. Usando una curva de estándar, la concentración de H_2O_2 determinada en el fluido de la herida fue de 1,1 μM . Estos resultados contrastan con las mediciones en plasma sanguíneo, que no muestran peróxido de hidrógeno medible.

[0047] *Detección de H_2O_2 en el borde de la herida.*

[0048] Se hizo el ensayo para peróxido de hidrógeno en el borde de la herida mediante detección de huellas de radical de peróxido de hidrógeno en la cavidad de la herida usando una solución de spin trap para enjuagar la herida expuesta. Doce horas después de practicar la herida, el sitio se trató con el spin trap, DMPO (5,5-dimetil-pirrolin-1-oxil). Para obtener datos de control, se sometió una herida recién practicada al mismo tratamiento con spin trap. Al cabo de 15 minutos se extrajo la solución de spin trap de la cavidad de la herida y se la sometió al ensayo con resonancia paramagnética electrónica (EPR).

[0049] Las mediciones con EPR se realizaron con un espectrómetro EPR Bruker ER 300 operando en banda X con una cavidad TM 110. La Figura 1B

muestra los espectros EPR del aducto DMPO medido en el líquido de enjuague de la herida. Los espectros se obtuvieron de los efluentes del DPMO (100 mM, 0,1 ml) recolectado de la cavidad de la herida a las 0 h (control simulado, panel superior) y 12 h después de practicar la herida (panel inferior). Los espectros en el panel inferior se identificaron como los del DMPO-OH con las siguientes constantes de acoplamiento: $a_N = 14,90$ G; $a_H = 14,90$. Los parámetros de adquisición de datos fueron: frecuencia de microondas 9,8882 GHz; ancho de barrido 100 G; potencia de microondas 20 mW; amplitud de modulación 0,5 G; frecuencia de modulación 100 kHz; constante de tiempo 80 mseg.

[0050] Mientras que el espectro de la simulación de solución tratada con spin trap no mostró ningún aducto prominente del spin, el espectro obtenido del líquido de enjuague de la herida a las 12 horas mostró un claro patrón de cuarteto 1:2:2:1. Los componentes individuales se identificaron como DMPO-OH (aducto con radical hidroxilo) por simulación.

[0051] *Producción de superóxido en piel normal y en tejido del borde de la herida.* Como resultado funcional de la actividad de la NADPH oxidasa, la generación de O_2^- se mide a menudo en cortes por criosección usando dihidroetidio (DHE) como tinción fluorescente sensible a ROS. En resumen, las muestras del borde de la herida se cosecharon a las 12 horas de practicada la herida y se las congeló inmediatamente en OCT. Se incubaron cortes frescos de 30 micrones con DHE (0,01 mM, 20 min., 200x) para detectar O_2^- y se los observó con microscopio confocal. Los resultados se muestran en la Figura 1C.

[0052] Empleando este método, se observó claramente que el borde del tejido de la herida se tiñó en forma mucho más prominente que la piel normal

ración cortada. Este hallazgo constituye una nueva demostración de que el sitio de la herida está enriquecido en ROS.

[0053] En conjunto, estos experimentos demuestran claramente que los ROS, incluyendo el peróxido de hidrógeno, están presentes en el tejido de cicatrización de la herida.

[0054] Ejemplo 2: Efecto del exceso de catalasa sobre la cicatrización de heridas.

[0055] Como se demostró la presencia de peróxido de hidrógeno en el tejido de cicatrización de heridas, se realizaron experimentos para determinar si la disminución de su concentración perturba la cicatrización. La catalasa es la enzima natural que hidroliza el peróxido de hidrógeno, por lo que se la introdujo en las heridas a ensayar.

[0056] En resumen, se introdujo la catalasa en las heridas a través de su sobreexpresión, usando un vector adenoviral. Este vector permitió obtener una alta eficiencia de sobreexpresión en la piel murina. La piel donde se iba a practicar la herida se inyectó una vez con catalasa o adenovirus LacZ (control) (10^{11} pfu) cinco días antes de practicar la herida para permitir la máxima sobreexpresión de catalasa en el sitio de la herida. Se colocaron dos heridas de espesor completo de 8 x 16 mm con cicatrización por segunda intención en la piel del dorso de ratones C57BL/6 de 8 semanas de edad.

[0057] La Figura 2A muestra un Western blot de la piel infectada mostrando la sobreexpresión de catalasa en el lado tratado con virus Ad-catalasa (AdCat) en comparación con el lado tratado con virus Ad-LacZ de Control. Las transferencias se investigaron nuevamente con β actina para demostrar la carga igual de las muestras.

[0058] La Figura 2B muestra cierres de la herida como porcentaje del área inicial de la herida, determinada el día indicado después de practicar la herida. La línea punteada representa la curva de cicatrización estándar de los ratones C57BL/6 tratados con solución fisiológica (círculos vacíos ○) sin infección viral. Tratamiento AdCat (triángulos llenos ▼); tratamiento AdLacZ (círculos llenos ●); $p < 0,05$ en comparación con el lado tratado con LacZ.

[0059] La Figura 2C muestra la tinción tricrómica de Masson realizada en cortes de piel regenerada en el sitio de la herida fijadas con formalina y obtenidas el día de cierre de ambas heridas. El lado AdCat muestra una región HE más amplia, indicativa de regeneración incompleta (vs. control) de la piel, consistente con un cierre más lento. Es: escara; G: tejido de granulación; HE: epitelio hiperproliferativo.

[0060] Ejemplo 3: Efecto del peróxido de hidrógeno sobre el cierre de heridas.

[0061] Como se demostró claramente que el peróxido de hidrógeno está presente en las heridas que cicatrizan (Ejemplo 1), y que su ausencia de las heridas que cicatrizan atenúa el proceso de cicatrización (Ejemplo 2), se realizaron experimentos para examinar el efecto del agregado de peróxido de hidrógeno.

[0062] En resumen, Se colocaron dos heridas de espesor completo de 8 x 16 mm obtenidas por disección (Figura 3, recuadros) en la piel dorsal de ratones macho C57BL/6 (8 semanas de edad). Cada una de las dos heridas fue tratada con H_2O_2 o solución fisiológica.

[0063] La Figura 3A muestra que el tratamiento con dosis baja de H_2O_2 (1,25 micromoles/herida; o 0,025 ml de solución al 0,15%/herida, una vez

diaria, días 0 – 4, círculos vacíos ○) facilitó moderadamente el cierre en comparación con el lado tratado con placebo (círculos llenos ●); $p < 0,05$.

[0064] La Figura 3B muestra que el tratamiento con dosis bajas de H_2O_2 no influye sobre la microflora de la herida. Para determinar la microflora superficial, se pasó un hisopo (24 – 48 horas después de practicar las heridas) a las heridas (tratadas con 1,25 micromoles de H_2O_2 /herida, barra vacía, o solución fisiológica, barra llena) con un aplicador con punta de alginato durante 20 segundos. Se hizo la evaluación cuantitativa de la carga bacteriana de la superficie. Para la microflora del tejido profundo de la herida, 48 h después de practicar la herida se removió el tejido de escara, se obtuvieron muestras del tejido del lecho de la herida por debajo de la escara y se realizó la evaluación de la carga bacteriana. Los valores mostrados representan las medias $\pm$ SD de CFU de cuatro observaciones.

[0065] La Figura 3C muestra que la dosis alta de H_2O_2 (alta, 25 micromoles/herida; círculos llenos ●; 0,025 ml de solución al 3% versus baja 1,25 micromoles/herida ○ 0,025 ml de solución al 0,15%, círculos vacíos ○ una vez diaria en los días 0 – 4) afectó adversamente el cierre. ($p < 0,05$; comparado con el tratamiento con dosis baja de H_2O_2). Un tratamiento con concentración mayor de H_2O_2 (recuadro) (62,5 micromoles/herida, lado izquierdo tratado; 0,025 ml de solución al 7,5%/herida una vez el día 0) causó daño tisular necrótico y lesión severa, llevando a la muerte de los ratones.

[0066] Ejemplo 4: Cambios inducidos por la herida y por H_2O_2 en los genes relacionados con la angiogénesis, la vascularización y el riego sanguíneo en el borde de la herida.

[0067] Se realizaron experimentos adicionales para estudiar el mecanismo mediante el cual dosis bajas de peróxido de hidrógeno aumentaban la velocidad de cicatrización de la herida.

[0068] Se trataron heridas apareadas disecadas con solución fisiológica placebo o H_2O_2 (1,25 micromoles/herida; días 0 – 4, una vez diaria). Se recolectó tejido del borde de la herida en los tiempos indicados después de practicar la herida. La Figura 4A muestra un ensayo de protección a la ribonucleasa (RPA) que muestra la cinética de la expresión de mRNA relacionado con angiogénesis en una herida tratada con placebo. La Figura 4B muestra cómo el tratamiento de la herida con dosis baja de H_2O_2 (1,25 micromoles/herida, una vez diaria, días 0 – 4) aumentó más las expresiones de mRNA de Fit1 y VEGF inducidas por la herida, según determinación hecha con RPA.

[0069] La Figura 4C muestra las imágenes del riego sanguíneo de las heridas que se obtuvieron en forma no invasiva usando un flujómetro de láser Doppler. Se muestran imágenes que reflejan el riego sanguíneo (panel derecho) y una foto digital (región de interés; panel izquierdo) del tejido posterior a la cicatrización. Los datos de riego sanguíneo se presentan como medias $\pm$ SD, (gráfico de barras). La media representa la media aritmética de todos los valores válidos de flujo sanguíneo para píxeles dentro de la región de interés. Los resultados muestran que el tratamiento resultó en un aumento del flujo sanguíneo, indicador funcional de angiogénesis aumentada.

[0070] La Figura 4D muestra los resultados del día 8 después de practicar la herida. Se hicieron cortes con criosección del borde de la herida y se estimó la vascularización mediante tinción para CD31 (rojo, rodamina) y

DAPI (azul, núcleos); la mayor abundancia de tinción roja CD31 en el corte obtenido del lado tratado con H_2O_2 (abajo) refleja mejor vascularización vs. el control (arriba).

[0071]Ejemplo 5: Fosforilación Inducida por H_2O_2 de la quinasa de adhesión focal (FAK) en células del endotelio microvascular y el tejido del borde de la herida.

[0072]Se trataron células de endotelio microvascular humano (HMEC-1) con H_2O_2 en las dosis y duración indicadas. La fosforilación de la FAK se detectó con Western blot y anticuerpos anti-FAK específicos del sitio de fosforilación. Se hizo la transferencia de FAK natural o β actina para mostrar igual carga.

[0073]La Figura 5A muestra el efecto de diversas dosis de tratamiento con H_2O_2 sobre el estado de fosforilación (Ty 925) de FAK. La Figura 5B muestra la cinética de la activación específica del sitio de la fosforilación de FAK en células HMEC después del tratamiento con H_2O_2 (0,1 mM).

[0074]En la Figura 5C, se trataron heridas apareadas disecadas con solución fisiológica placebo o H_2O_2 (1,25 micromoles/herida). Se recolectó tejido del borde de la herida 30 minutos después de practicar la herida. Se determinó la fosforilación de FAK en el tejido del borde de la herida con Western Blot. Se muestran los datos de tres animales (Nº 1 – Nº 3).

[0075]Ejemplo 6: El déficit de MCP-1 y p47phox disminuye la cicatrización dérmica.

[0076]Al atraer macrófagos productores de peróxido de hidrógeno, la proteína 1 de quimiotaxis/quimioatracción de monocitos/macrófagos (MCP-1) juega un papel clave en el desarrollo y la resolución de la respuesta

Inflamatoria aguda a las heridas. La $P47^{phox}$ es una subunidad reguladora de la NADPH oxidasa, que participa en la producción de ROS. Debido a la importancia de estos factores en la producción de ROS y en la cicatrización de heridas, se realizaron pruebas para examinar cómo el peróxido de hidrógeno afecta las heridas en animales que carecen de estos factores.

[0077] En resumen, se colocaron heridas disecadas en la piel dorsal de ratones de 8 semanas C57BL/6, atontados para MCP-1 o $p47^{phox}$. Cada una de las dos heridas se trató con solución fisiológica o con H_2O_2 (1,25 micromoles/herida; días 0 – 4).

[0078] La Figura 6A muestra un ensayo de protección a la RNasa que muestra la cinética de la expresión de mRNA relacionado con la proteína de quimiotaxis de monocitos/macrófagos en heridas tratadas con placebo de ratones silvestres (C57 BL/6). La Figura 6B muestra los cierres de la herida en heridas tratadas con solución fisiológica (círculos llenos ●) de C57BL/6 y de ratones atontados para MCP-1 tratados con H_2O_2 (triángulos llenos ▼) o solución fisiológica (círculos vacíos ○) como porcentaje del área de la herida inicial. (* $p < 0,05$; comparado con tratamiento con solución fisiológica en ratones C57BL/6. N*, $p < 0,05$; comparado con tratamiento con solución fisiológica en ratones atontados). La Figura 6C muestra los cierres de la herida en heridas tratadas con solución fisiológica (círculos llenos ●) de C57BL/6 y en ratones atontados para $p47^{phox}$ tratados con H_2O_2 (triángulos llenos ▼) o solución fisiológica (círculos vacíos ○) se muestran como porcentaje del área de la herida inicial. * $p < 0,05$; comparado con tratamiento con solución fisiológica en ratones C57BL/6. N*, $p < 0,05$; comparado con tratamiento con solución fisiológica en ratones atontados.

[0079] La queratina 14 apoya la diferenciación y regeneración epidérmica; su expresión es desencadenada por heridas dérmicas. La Figura 6D muestra la expresión de queratina 14 (fluorescencia verde) en la piel de ratones atontados para p47^{phox} cosechada de sitios de herida después del cierre el día 16 después de practicar la herida. Obsérvese la mayor expresión de queratina 14 en el lado control en comparación con el lado tratado con H₂O₂ indicando que la cicatrización está en proceso en el lado control, mientras que el lado tratado con H₂O₂ muestra una expresión de queratina 14 similar a la de la piel normal, indicando cicatrización completa.

[0080] Otras realizaciones de la invención resultarán evidentes para el experto en la técnica a partir de la consideración de la memoria descriptiva y de la práctica de la invención revelada en la presente. La memoria descriptiva y los ejemplos deben considerarse únicamente como ejemplificativos, quedando indicado el verdadero alcance y espíritu de la invención en las reivindicaciones adjuntas.

REIVINDICACIONES

Se reivindica lo siguiente:

- 1. Un método para aumentar la velocidad de cicatrización de una lesión en mamíferos, que comprende aplicar a la lesión entre aproximadamente 500 nanomoles y aproximadamente 50 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión.**
- 2. El método de acuerdo con la reivindicación 1, que comprende aplicar entre aproximadamente 1 y aproximadamente 50 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión.**
- 3. El método de acuerdo con la reivindicación 2, que comprende aplicar entre aproximadamente 1 y aproximadamente 10 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión.**
- 4. El método de acuerdo con la reivindicación 3, que comprende aplicar entre aproximadamente 1 y aproximadamente 2 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión.**
- 5. El método de acuerdo con la reivindicación 1, donde el peróxido de hidrógeno se aplica a la lesión en una fuente seleccionada entre fuentes enzimáticas y químicas.**
- 6. El método de acuerdo con la reivindicación 5, donde la fuente de peróxido de hidrógeno es química y la fuente es peróxido de hidrógeno.**
- 7. Un método para aumentar la velocidad de cicatrización de una lesión en mamíferos, que comprende aplicar a la lesión una concentración entre aproximadamente 500 nanomoles y aproximadamente 50 micromoles de peróxido de hidrógeno por centímetro cuadrado de lesión durante un período entre aproximadamente 12 horas y aproximadamente 24 horas.**

8. El método de acuerdo con la reivindicación 7, donde el peróxido de hidrógeno se aplica en una concentración entre aproximadamente 1 y aproximadamente 10 micromoles.

9. El método de acuerdo con la reivindicación 7, donde el peróxido de hidrógeno se aplica en una composición adecuada para uso farmacéutico.

10. El método de acuerdo con la reivindicación 9, donde la composición adecuada para uso farmacéutico está en una forma seleccionada entre geles, lociones, ungüentos, cremas, pastas y líquidos.

11. El método de acuerdo con la reivindicación 7, donde el peróxido de hidrógeno se aplica en un dispositivo adecuado para uso farmacéutico.

12. El método de acuerdo con la reivindicación 11, donde el dispositivo adecuado para uso farmacéutico se selecciona entre vendajes, apósitos quirúrgicos, gasas, tiras adhesivas, grapas quirúrgicas, clips, hemostáticos, dispositivos intrauterinos, suturas, trócares, catéteres, tubos e implantes.

13. El método de acuerdo con la reivindicación 12, donde el implante se selecciona entre píldoras, pélets, cilindros, obleas, discos y comprimidos.

14. El método de acuerdo con la reivindicación 11, donde el dispositivo comprende un material polimérico.

15. El método de acuerdo con la reivindicación 14, donde el material polimérico comprende un material absorbible.

16. El método de acuerdo con la reivindicación 15, donde el material absorbible comprende un material sintético.

17. El método de acuerdo con la reivindicación 16, donde el material sintético se selecciona entre polímeros de celulosa, polímeros de ácido glicólico, polímeros de metacrilato, polímeros de etilen vinil acetato,

copolímeros de etilen vinil alcohol, policaprolactam, poliácetato, copolímeros de lacturo y glicoluro, polidioxanona, poliglactina, poliglicaprona, poliglucanato, poliglucanato y combinaciones de estos polímeros.

18. El método de acuerdo con la reivindicación 15, donde dicho material absorbible comprende un material no sintético.

19. El método de acuerdo con la reivindicación 18, donde dicho material no sintético se selecciona entre catgut, membrana de cergile, fascia lata, gelatina, colágeno y combinaciones de estos materiales.

20. El método de acuerdo con la reivindicación 14, donde el material polimérico comprende un material no absorbible.

21. El método de acuerdo con la reivindicación 20, donde el material no absorbible comprende un material sintético.

22. El método de acuerdo con la reivindicación 21, donde el material sintético se selecciona entre nailon, rayones, poliésteres, poliolefinas y combinaciones de estos materiales.

23. El método de acuerdo con la reivindicación 20, donde el material no absorbible comprende un material no sintético.

24. El método de acuerdo con la reivindicación 23, donde dicho material no sintético se selecciona entre seda, seda dérmica, algodón, lino y combinaciones de estos materiales.

25. El método de acuerdo con la reivindicación 7, donde la lesión se selecciona entre heridas, úlceras y quemaduras.

26. El método de acuerdo con la reivindicación 25, donde las heridas se seleccionan entre heridas agudas y heridas crónicas.

27. El método de acuerdo con la reivindicación 25, donde las heridas se seleccionan entre heridas de espesor total y heridas de espesor parcial.

28. El método de acuerdo con la reivindicación 26, donde las heridas agudas se seleccionan entre heridas quirúrgicas, heridas penetrantes, lesiones por desgarró, lesiones por aplastamiento, lesiones por cortes, lesiones por quemaduras, laceraciones y heridas por mordeduras.

29. El método de acuerdo con la reivindicación 26, donde las heridas crónicas se seleccionan entre úlceras arteriales, úlceras venosas, úlceras por compresión y úlceras diabéticas.

30. Un dispositivo de liberación de peróxido de hidrógeno para la administración en una lesión, que comprende peróxido de hidrógeno y un material de vehículo, donde dicho dispositivo libera dicho peróxido de hidrógeno durante un período de tiempo de por lo menos aproximadamente 12 horas, donde el peróxido de hidrógeno liberado del dispositivo está en concentración insuficiente para producir necrosis de la lesión.

31. El dispositivo de liberación de peróxido de hidrógeno de acuerdo con la reivindicación 30, donde el dispositivo libera entre aproximadamente 0,5 y 50 μmol de peróxido de hidrógeno/ cm^2 de herida/12 horas y aproximadamente 0,5 y 50 μmol de peróxido de hidrógeno/ cm^2 de herida/24 horas.

32. El dispositivo de liberación de peróxido de hidrógeno de acuerdo con la reivindicación 31, donde el material de vehículo comprende un material polimérico.

33. El dispositivo de liberación de peróxido de hidrógeno de acuerdo con la reivindicación 32, donde el material polimérico comprende un material absorbible.

34. El dispositivo de liberación de peróxido de hidrógeno de acuerdo con la reivindicación 32, donde el material polimérico comprende un material sintético.

35. Una composición para tratar lesiones en mamíferos, que comprende: peróxido de hidrógeno, un vehículo adecuado para uso farmacéutico, donde una dosis unitaria de la composición comprende entre aproximadamente 0,5 y aproximadamente 50 μmol de peróxido de hidrógeno/ cm^2 de herida.

36. La composición de acuerdo con la reivindicación 35, donde el vehículo comprende un material en forma de gel.

37. La composición de acuerdo con la reivindicación 35, donde el vehículo comprende un material líquido.

RESUMEN

Métodos, dispositivos y composiciones para tratar lesiones en animales, que comprenden peróxido de hidrógeno en concentraciones que son menores a las usadas convencionalmente. Los métodos, dispositivos y composiciones aceleran la cicatrización de heridas.

Figura 1	A	H ₂ O ₂ concentration current (A) time (sec)	Concentración de H ₂ O ₂ corriente (A) tiempo (seg.)
	B	magnetic field (Gauss)	Campo magnético (Gauss)
	C	skin wound edge	piel borde de la herida
Figura 2	A	catalase β-actin	catalasa β actina
	B	% original wound post-wounding day	% de la herida original día posterior a la herida
Figura 3	A	% original wound post-wounding day	% de la herida original día posterior a la herida
	B	Cfu/wound surface deep tissue	Cfu/herida superficie tejido profundo
	C	% original wound post-wounding day	% de la herida original día posterior a la herida
Figura 4	A	Thrombin R endoglin Angiopoietin-1	Trombina R endoglina Angiopoyetina-1
	C	hind limb tail increased blood flow blood flow (arbitrary units) treated	pata trasera cola flujo sanguíneo aumentado flujo sanguíneo (unidades arbitrarias) tratada
Figura 5	B	min after H ₂ O ₂ treatment	min. después de tratamiento con H ₂ O ₂
	C	replicate β-actin	replicado β actina
Figura 6	B	% original wound	% de la herida original

75

C	% original wound post-wounding day	% de la herida original día posterior a la herida
---	---------------------------------------	--

76

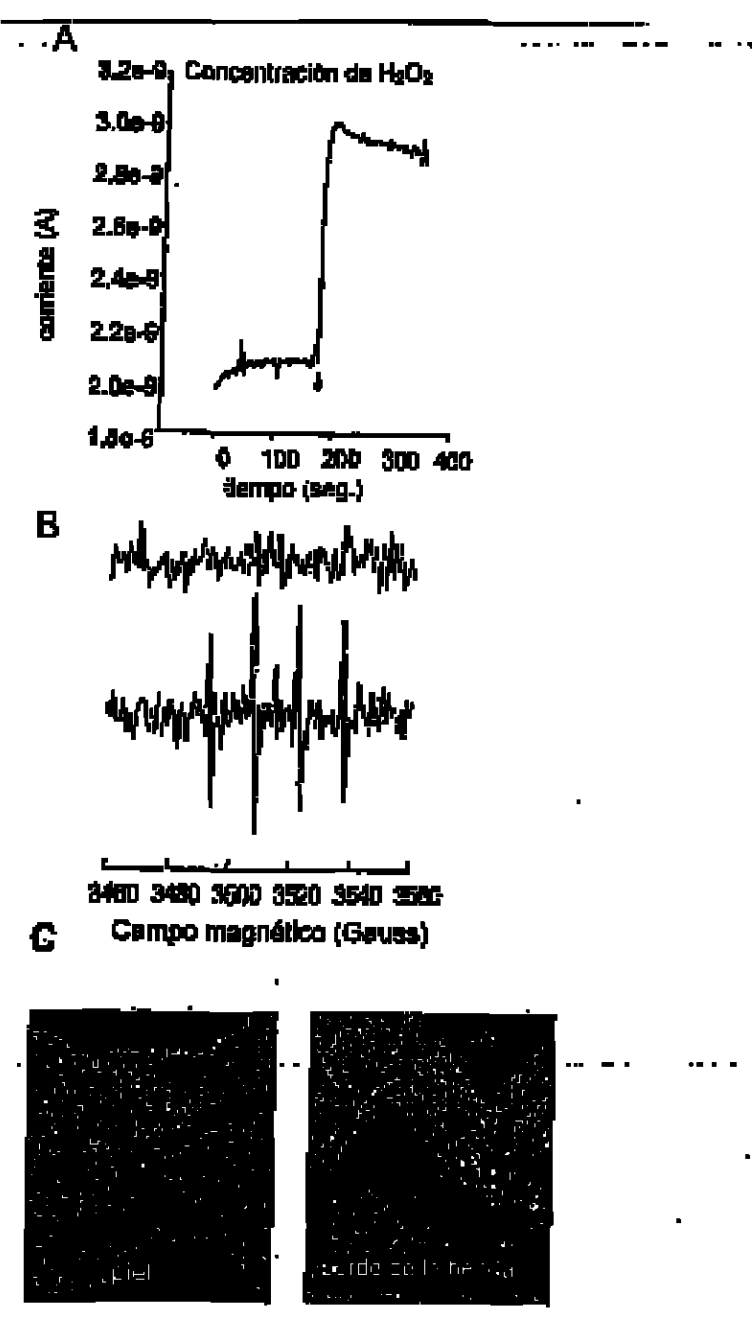


Figura 1

97
77

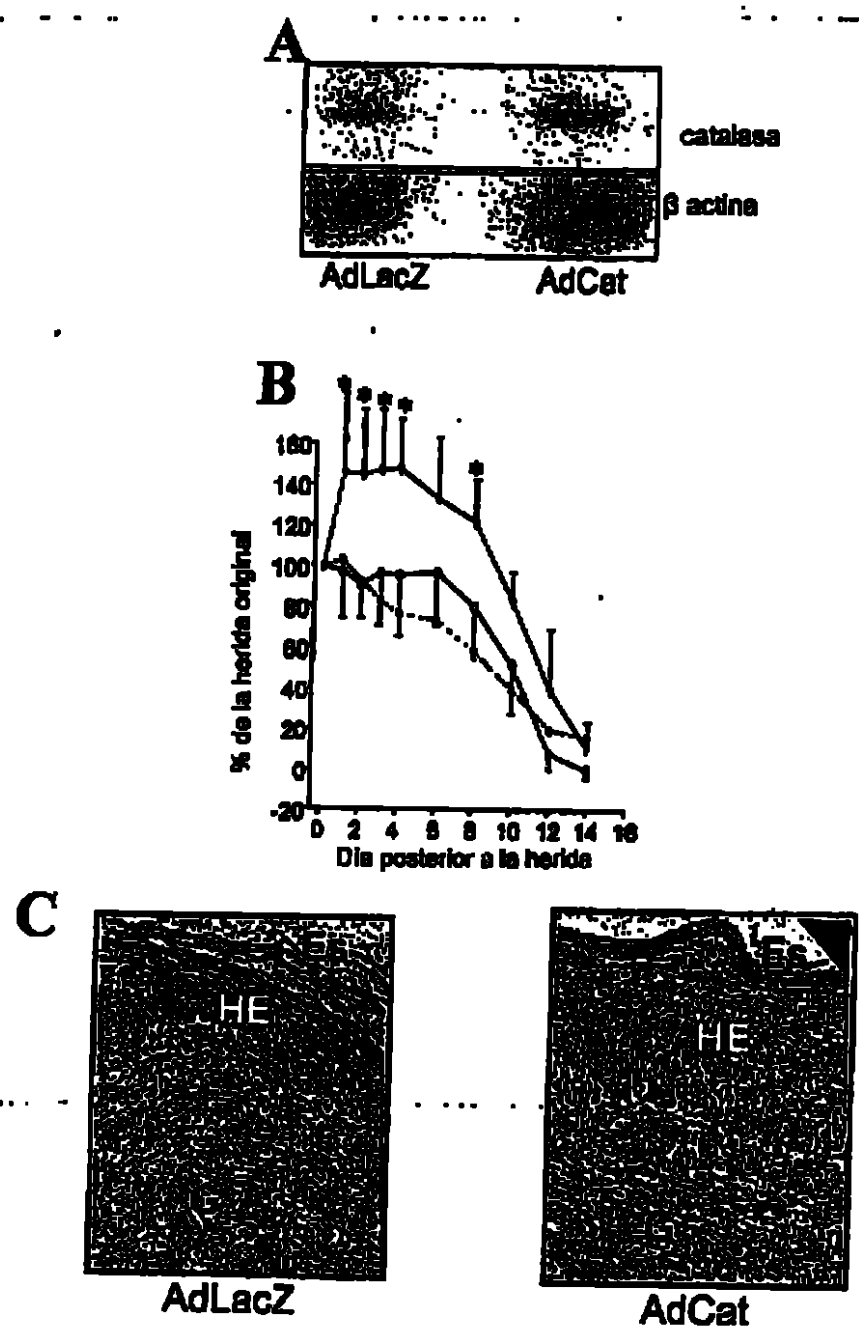


Figura 2

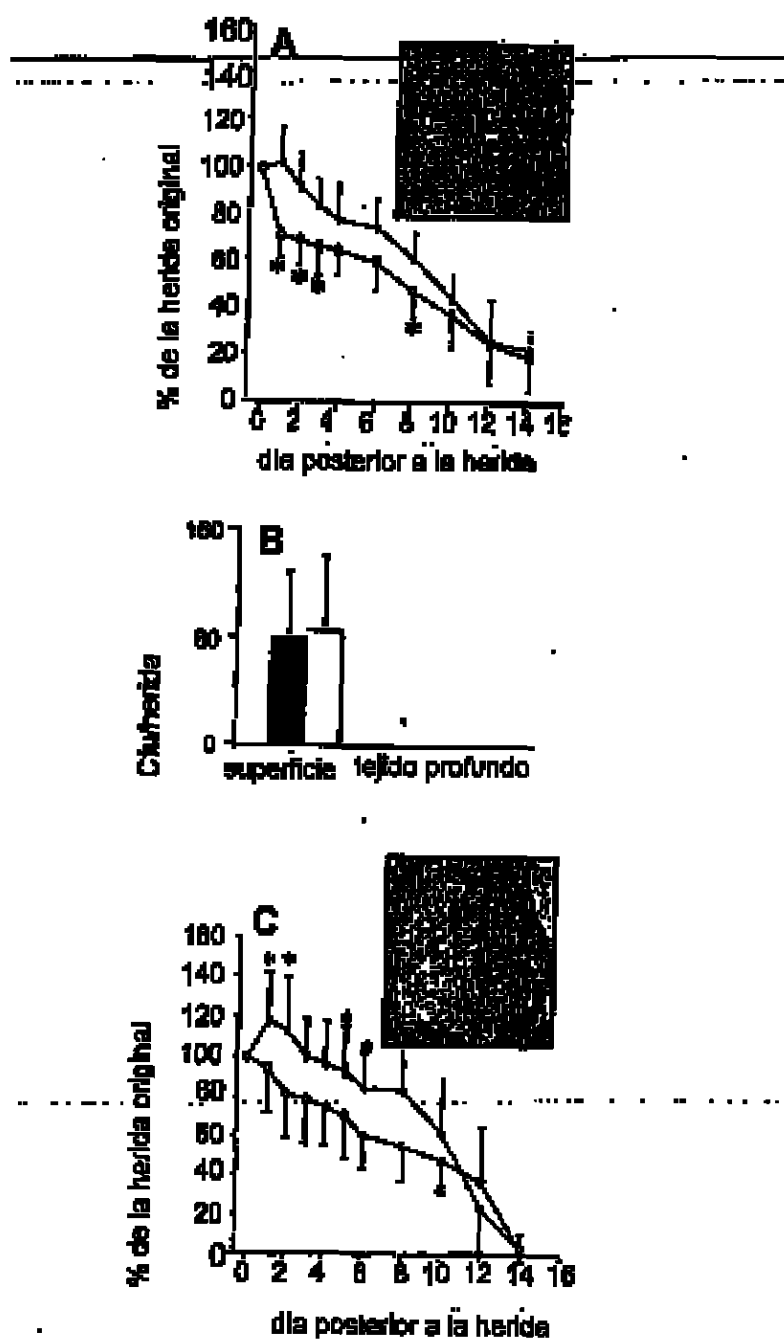


Figura 3

79

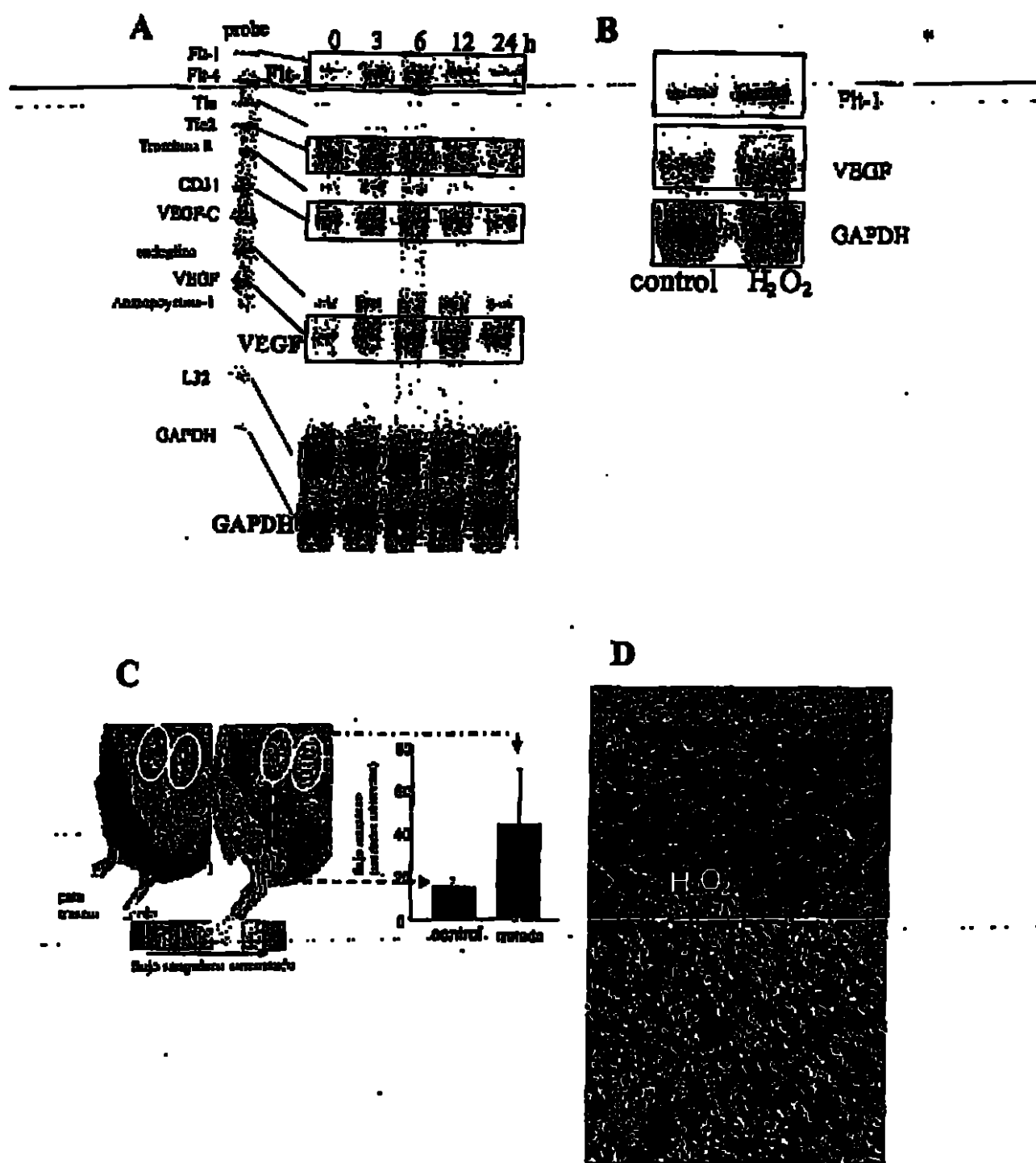


Figura 4

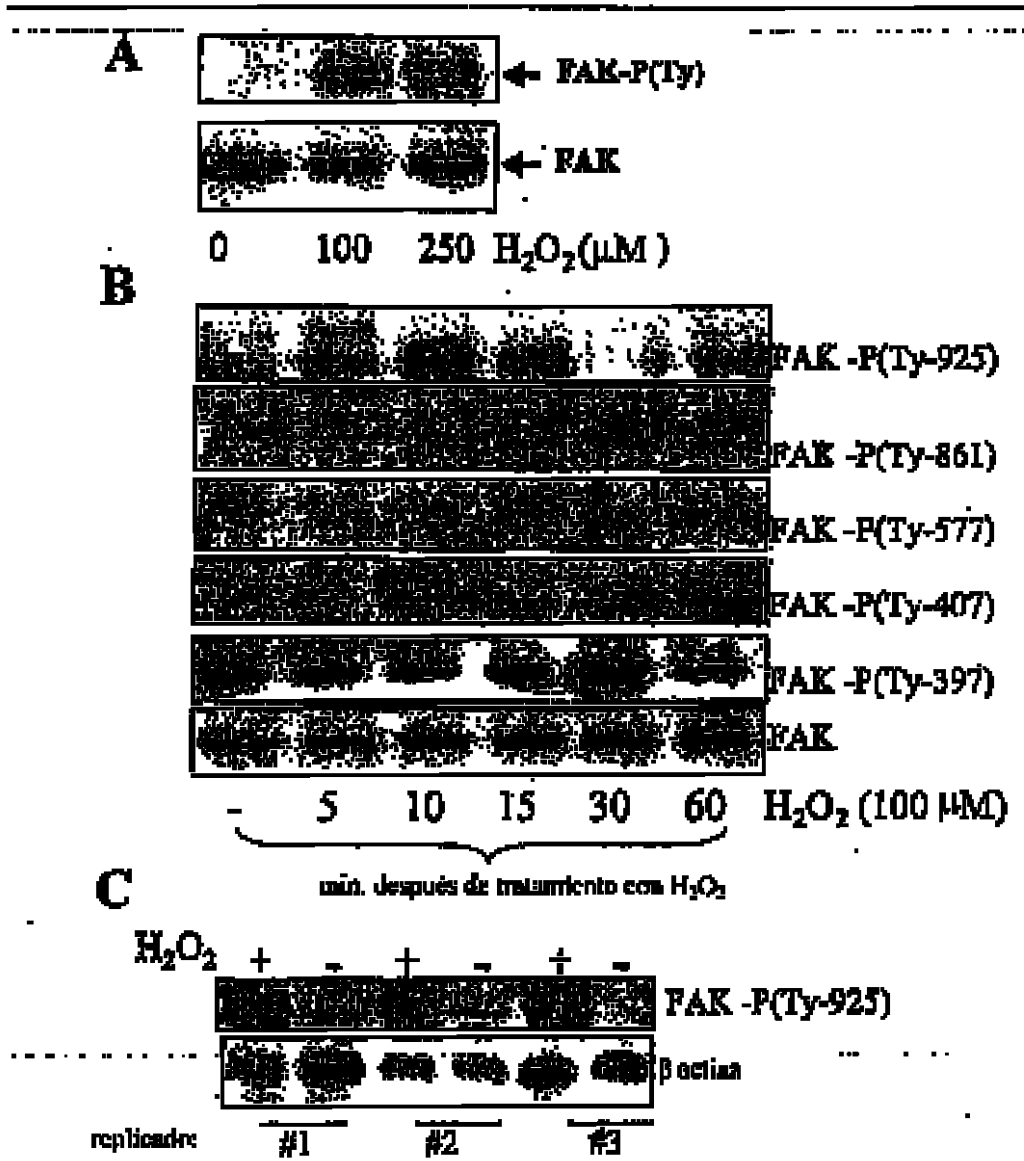


Figura 5

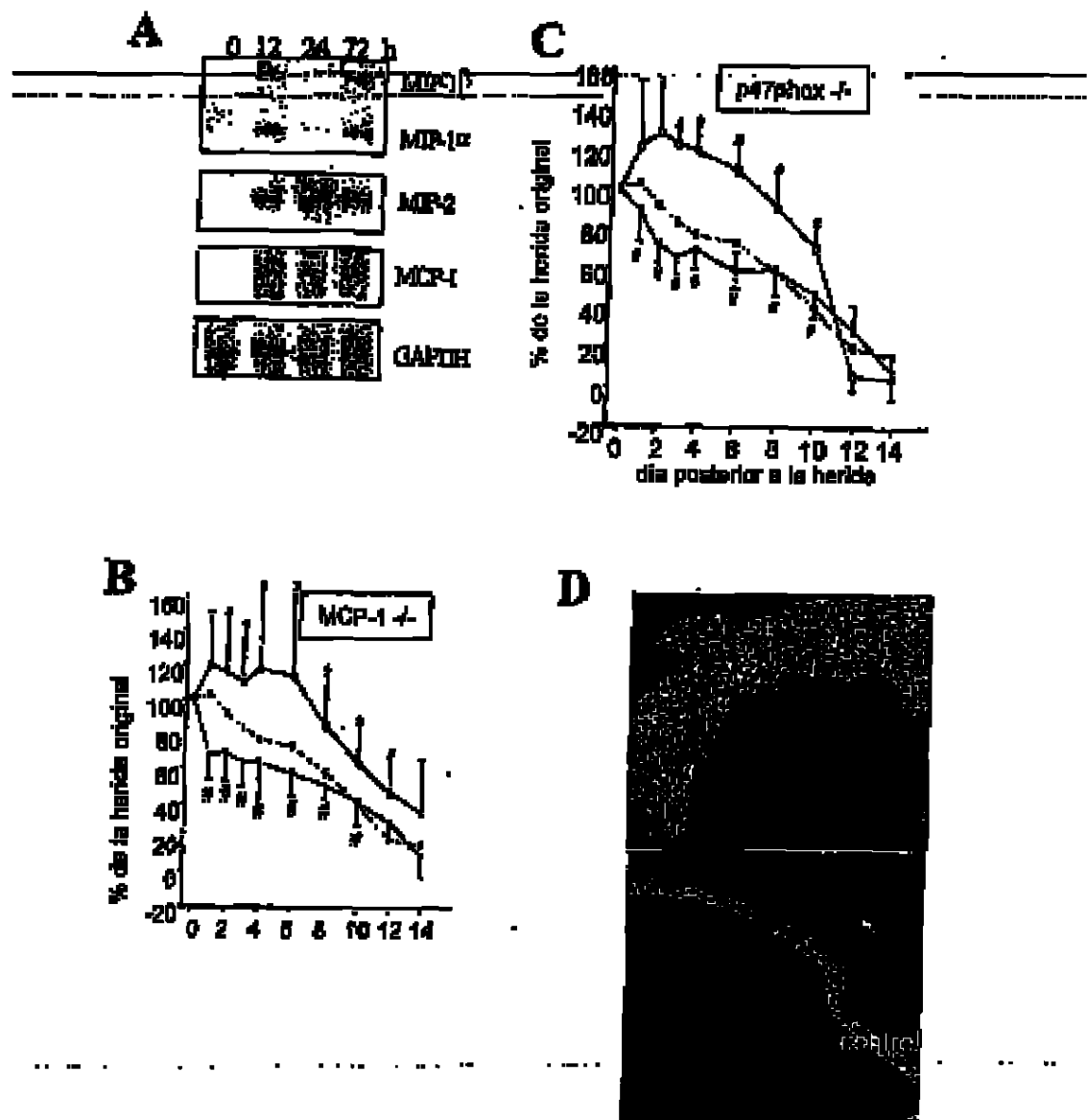


Figura 6

RECEIVED

PATENT COOPERATION TREATY

JAN 0 2006

From the INTERNATIONAL SEARCHING AUTHORITY

STENT TO
ONE COLUMBIA UNIVERSITY
PCT JAN 0 2006

To:
SEAN C. MYERS-PAYNE
CALFEE, HALTER & GRIFFIN LLP
1100 FIFTH THIRD CENTER
21 EAST STATE STREET
COLUMBUS, OH 43215

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

Applicant's name, file reference 2173704-129	Date of mailing (day/month/year)
International application No. PCT/US03/1477	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION	Issued filing date (day/month/year) 17 June 2005 (17.06.2005)

1. ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statements under Article 19:
The applicant is notified that, if he or she wishes, to amend the claims of the international application (see Rule 46):

When? The time limit for filing amendments is seven days from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 24, Switzerland, and, Facsimile No: (41-22) 318.52.70

For more detailed instructions see the notes on the accompanying sheet.

2. ☐ The applicant is hereby notified that the international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.

3. ☐ With regard to the protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

☐ the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

☐ no decision has been made yet on the protest, the applicant will be notified as soon as a decision is made.

4. **Remarks:**

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis 1 and 90bis 3, respectively, before the completion of the technical preparations for international publication.

The applicant may submit comments on the international search report or the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments would also be made available to the public but not before the expiration of 20 months from the priority date.

Within 18 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 18 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 18 months.

See the Annex to Form PCT/IB/301 and, for details about the applicable time limits, Office by Office, see the PCT Applicant's Guide, Volume II, National Chapters and the WIPO Internet site.

Name and mailing address of the ISA/ ILS Mail Stop PCT, Attn: ISA/US Communication for Patents P.O. Box 1430 Alexandria, Virginia 22313-1430 Facsimile No. (703) 245-1113	Authorized officer John Pak Telephone No. (571) 772-1400
--	--

Form PCT/IB/4220 (January 2004)

(See notes on accompanying sheet)

[Handwritten signature]

JAN 6 2006

PATENT COOPERATION TREATY

RECEIVED

JAN 6 2006

From the INTERNATIONAL SEARCHING AUTHORITY

To:
SEAN C. MYERS-PAYNE
CALVEE, HALLER & GRIFFOLD LLP
100 FIFTH THIRD CENTER
21 EAST STATE STREET
COLUMBUS, OH 43215

NO. COLUMBUS IP
PCT

NOTIFICATION OF TRANSMITTAL OF
THE INTERNATIONAL SEARCH REPORT AND
THE WRITTEN OPINION OF THE INTERNATIONAL
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 40.1)

Applicant's or agent's file number 213704328	Date of mailing (day/month/year) 04 JAN 2006
International Application No. PCT/US05/01477	International filing date (day/month/year) 17 June 2005 (17.06.2005)
Applicant THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION	

- 1 ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.

Filing of amendments and statements under Article 17:
The applicant is notified, if he so wishes, to amend the claims of the international application (see Rule 40.2).

When? The time limit for filing such amendments is normally two months from the date of transmittal of the international search report.

Where? Directly to the International Bureau of WIPO, 34 chemin des Colombettes
1211 Geneva 20, Switzerland, Facsimile No: (41-22) 368.82 70.

For more detailed instructions, see the notes on the accompanying sheet.
- 2 ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) in that effect and the written opinion of the International Searching Authority are transmitted herewith.
- 3 ☐ With regard to the previous national payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
 - ☐ the payment together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the same to the fee paying and the decision thereon to the designated Office;
 - ☐ no decision has been made yet on the payment; the applicant will be notified as soon as a decision is made.
- 4 **Resolutions**

Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90bis.1 and 90bis.3, respectively, before the expiration of the relevant corresponding time limit for international publication.

The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established. These comments could also be made available to the public but not before the expiration of 18 months from the priority date.

Within 18 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 34 months from the priority date (in some Offices even later); otherwise, the applicant must, within 30 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices.

In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 18 months.

See the Annex to Form PCT/ISAR/01 and the details about the applicable time limits, Office by Office, on the PCT Applicant's Guide, Volume II, National Chapters and the WIPO Internet site.

Nearest mailing address of the ISA/US Mail Stop PCT, Attn: ISAR/US Communications for Patents P.O. Box 1450 Alexandria, Virginia 22313-2450 Facsimile No. (703) 245-7338	Authorized officer John Felt Telephone No. (771) 271-1000
--	--

Form PCT/ISAR/01 (January 2004)

(See notes on accompanying sheet)

[Handwritten signature]

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 22723/04/79	FOR FURTHER ACTION see Form PCT/ISA/220 as well as, when applicable, item 7 below	
International application No. PCT/US03/21477	International filing date (day/month/year) 17 June 2003 (17.06.2003)	(Earliest) Priority Date (day/month/year) 18 June 2002 (18.06.2002)
Applicant THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION		

This International Search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International search report consists of a total of _____ sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the Report

a. With regard to the language, the international search was carried out on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purpose of international search (Rule 12.2(a) and 21.1(b))

b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Rule No. 1.

2. ☐ Certain claims were found unsearchable (See Rule No. 3)

3. ☐ Unity of invention is lacking (See Rule No. 5)

4. With regard to the title,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established by this Authority to read as follows:

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant.
☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Item No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. _____
☐ as suggested by the applicant.
☐ as selected by this Authority, because the applicant failed to suggest a figure.
☐ as selected by this Authority, because this figure best characterizes the invention.
- b. ☒ none of the figures is to be published with the abstract.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 42 and 44)

Applicant's or applicant's representative's reference number 1277704329	FOR FURTHER ACTION as well as, where applicable, Item 2 below	
International application No. P/TAS/2014/017	International filing date (day/month/year) 17 June 2015 (17.06.2015)	(Earliest) Priority Date (day/month/year) 18 June 2014 (18.06.2014)
Applicant THE GEO STATE UNIVERSITY RESEARCH FOUNDATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of _____ sheets.

☒ It is also accompanied by a copy of each printing document cited in this report.

1. Basis of the Report

- a. With regard to the language, the international search was carried out on the basis of
- ☒ the international application in the language in which it was filed
- ☐ a translation of the international application into _____, which is the language of a translation furnished for the purpose of examination under (Rule 22.3(a) and 22.1(b))
- b. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. 1.
2. ☐ Certain claims were found unsearchable (See Box No. 8)
3. ☐ Unity of invention is lacking (See Box No. 10)
4. With regard to the title,
- ☒ the text is approved as submitted by the applicant.
- ☐ the text has been established by this Authority in particular as follows:

5. With regard to the abstract,

- ☒ the text is approved as submitted by the applicant.
- ☐ the text has been established, according to Rule 36.2(b), by this Authority as it appears in Doc. No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. With regard to the drawings,

- a. the figure of the drawings to be published with the abstract is Figure No. _____
- ☐ as suggested by the applicant.
- ☐ as selected by this Authority, because the applicant failed to request a figure.
- ☐ as selected by this Authority, because this figure better demonstrates the invention.
- b. ☒ none of the figures is to be published with the abstract.

INTERNATIONAL SEARCH REPORT

International Classification No. 44

PC 7/1805/2 1477

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : 66K0040

US Cl. : 424612,516

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbol)
 US : 424612,516

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 Please See Classification Head

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5002/021539 A (KACZEL et al.) 27 November 2000 (27.11.2000), claims 1, 3, 6, 9, 14, 15	33-37
X	US 4,163,800 A (WICKETT et al.) 07 August 1979 (07.08.1979), column 3, lines 28-33, column 4, lines 17-21, column 5, lines 4-13, column 6, lines 6-10, column 7, lines 1-5, column 8, lines 15-24	1, 2, 3-7, 9-11, 30, 37 3, 4, 8, 12-29, 31-24
X	US 2004/0005277 A (WILLISON et al.) 28 January 2004 (01.01.2004), paragraphs 0147, 0148-0149, 0175-0179	30-37
X	US 2004/0001878 A (DELUKKE et al.) 01 January 2004 (01.01.2004), paragraphs 0031, 2071, 0044-0050, 0091-0793, 0804	1-37
X	US 2003/0019985 A (SEHL et al.) 26 June 2003 (26.06.2003), paragraphs 0050-0062, 0067	1-37

☐ Foreign documents are listed in the continuation of Doc. C.	☐ Non patenting foreign sources.
* documents defining the prior art in the art which must be taken into account in the present invention	* Low priority published after the international filing date or priority date and not in compliance with the application for a patent in the country of origin
* single application reports published on or after the international filing date	* documents of prior art references, the claimed invention is not for non-patentable subject matter or cannot be considered to have an inventive step when the prior art is taken into account
* documents which may be considered as primary documents or which needed to establish the publication of prior art references, or other relevant reasons for specific	* documents of prior art references, the claimed invention cannot be considered to have an inventive step when the prior art is taken into account and other relevant documents, and considerations being taken into a patent office in the art
* foreign patents given in the countries, etc., additional or other reasons	* documents which are of the prior art in the art
* documents which are of the prior art in the countries, etc., additional or other reasons	

Date of the actual completion of the international search (22 October 2003 (02.10.2003))	Date of mailing of the international search report 04 JAN 2004
Name and mailing address of the ISA/US 3600 Reg. PC2, Ave. 28/28 Carmichael, St. Paul P.O. Box 1430 Alexandria, Virginia 22113-1430 Telephone No. (703) 365-3230	Authorized officer John Fox Telephone No. (703) 365-1600

Form PC 7/1805/210 (second sheet) (April 2003)

DMCh

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/0107

87

Continuation of B. FIELD'S SEARCHED Item J.
EAST
search terms: hydrogen peroxide, leucine, wounds, clots

PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:
MR. C. MYERS-PAYNE
CALIFFE, HALLIK & CRISWOLD LLP
1109 FIFTH THIRD CENTER
21 EAST STATE STREET
COLUMBUS, OH 43215

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43bis.1)

Date of mailing
(day/month/year) 04 JAN 2006

Applicant's address as indicated in the international

2272704339

FOR FURTHER ACTION

See paragraph 2 below

International application No.

PCT/US2005/01477

Date of filing (day/month/year)

17 June 2005 (17.06.2005)

Priority date (day/month/year)

18 June 2004 (18.06.2004)

International Patent Classification (IPC) or both national classification and IPC:

IPC(7): A61K31/00 and US CL: 424/612.616

Applicant

THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION

1. This opinion comprises indications relating to the following items:

- ☒ Box No. I Date of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain other matters on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant declares an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66 bis(2) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 2 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/230.

3. For further details, see notes to Form PCT/ISA/220

Name and mailing address of the ISA/US
Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1460
Alexandria, Virginia 22303-1460
Facsimile No. (703) 305-3230

Date of completion of this opinion

17 November 2005 (17.11.2005)

Authorized Officer

John Pate

Telephone No. (571) 272-1100

Form PCT/ISA/217 (former draft) (April 2005)

[Handwritten signature]

88

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.

PC/TLS033147

Box No. 1 Marks of I/O's opinion

1 With regard to the language, the opinion has been established on the basis of:

- ☒ the international application in the language in which it was filed
☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2 With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the opinion has been established on the basis of:

a. type of material

- ☐ a sequence listing
☐ table(s) related to the sequence listing

b. format of material

- ☐ on paper
☐ in electronic form

c. time of filing/submitting

- ☐ contained in the international application as filed.
☐ Filed together with the international application in electronic form.
☐ Furnished subsequently to this Authority for the purposes of search.

3 ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies (or editions) is that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/JP03/21471

90

Box No. V Reasoned statement under Rule 43 bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims 1, 4, 12, 29, 31-34	YES
	Claims 1, 2, 3, 9, 11, 10, 37	NO
Inventive step (IS)	Claims NONE	YES
	Claims 1-37	NO
Industrial applicability (IA)	Claims 1-37	YES
	Claims NONE	NO

2. Citations and explanations

Please See Continuation Sheet

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International Application
PCT/US95/1184

Supplemental Box
to use the space in any of the preceding boxes is not sufficient.

V. 2. Citations and Explanations:

Claims 3,4,8,12-29,31-34 meet the criteria set out in PCT Article 33(2), because no single prior art reference explicitly discloses the claimed amount of hydrogen peroxide, the specified device structure or devices containing polyvinyl alcohol, or the specified amounts of water.

Claims 1-37 are not set out in PCT Article 33(4), and thus meet formal applicability because the subject matter claimed can be made or used in industry.

Claims 15-37 lack novelty under PCT Article 33(1) as being anticipated by Segal et al. (US 2003/021938W).

Segal et al. expressly discloses a composition containing a peroxide density of less than about 1.3 mg/cm², which further comprises water or geling agent or is present in ranges of between about 0.01 mg/cm² and about 1.3 mg/cm² or about 0.5 mg/cm² and about 1.3 mg/cm². (Claims 1,3,6,9,14,19) falling within the scope of applicant's claims.

Claims 1,2,5-7,9-11,30,37 lack novelty under PCT Article 33(1) as being anticipated by Wikstrom et al. (US 4,162,800).

Wikstrom et al. expressly discloses a method of treating teeth by topically applying a composition containing 3% hydrogen peroxide, water, and water to a tooth at 0.05 g/cm² min falling within the scope of applicant's claims (Claims 8, Items 15-24).

Claims 30-37 lack an inventive step under PCT Article 33(3) as being obvious over Williams et al. (US 2004/0005277).

Williams et al. discloses a product in which discloses absorbing an aqueous hydrogen peroxide solution on or in a layer formed from PVP, Polyalkylene glycol and acrylic acid esters (paragraphs 0163-0169). It is disclosed that delivery rate varies depending on the critical total amount of hydrogen peroxide, the surface area and the time of exposure; for example for a 9% hydrogen peroxide device at 5 minutes the rate was 0.78 mg/cm²-min, at 30 minutes the rate was 0.43 mg/cm²-min and at 60 minutes the rate was 0.33 mg/cm²-min (Paragraphs 0176-0179). It is disclosed that the length of time the device is left on can be varied in order to achieve the desired results and treatment times longer than about four hours are contemplated (Paragraph 0147).

The difference between the prior art and the claimed invention is that the prior art does not expressly disclose at least about a 12 hour delivery period. However, the prior art simply suggests the same as the prior art discloses that periods of longer than about 4 hours is contemplated. As such, it would have been well within the skill of and one of ordinary skill in the art would have been motivated to modify the prior art with the expectation that the time period could be varied as desired depending on desired effect including those periods falling within the scope of the claimed invention.

Claims 1-37 lack an inventive step under PCT Article 33(3) as being obvious over Wikstrom et al. (US 4,162,800) in view of DeBock et al. (US 2004/0001873) and Segal et al. (US 2003/0119855).

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International
PCT/ISA/237

Supplemental Box

In case the report in any of the preceding boxes is not sufficient.

Wickert et al. disclose a method of treating skin lesions, such as lacerations and ulcers, with hydrogen peroxide, which comprises from about 1% to about 15% of the composition (Column 1, lines 29-33, Column 4, lines 27-31, Column 5, lines 4-13). It is disclosed that the typical usage rate is about 0.001 gram/2 to about 0.5 gram/2 skin per application, but that this can vary with the user, the severity of the affliction and the concentration of peroxide present (Column 6, lines 64-68, column 7, lines 1-5). A method of treating some lesions by topically applying a composition containing 3% hydrogen peroxide, acetic acid and water twice a day at 0.05 gram/2 skin is disclosed (Column 8, lines 13-24).

Ullrich et al. disclose that low concentrations of hydrogen peroxide have been shown to stimulate fibroblast proliferation which is particularly desirable during the later stages of wound healing (Paragraph 0071). A dressing is disclosed which is suitable for healing of skin wounds, such as lacerations, trophic ulcer, trophic lesion, nerve cuts and abrasions (Paragraph 0051). It is disclosed that the dressing can be a hydrocolloid dressing containing primarily gel-forming polymers, gums and adhesive of film or foam backing, for example CAC, PVP and polyacrylates (Paragraphs 0044-0050). It is disclosed that the dressing can be in sheet form, aqueous gel form, wafer and can contain collagen or gelatin etc. (Paragraphs 0037-0039). It is disclosed that gauze, semipermeable or tape can also be used (Paragraph 0163).

Schiff et al. disclose a patch for repair of damaged tissues which can contain nonabsorbable materials such as polyester cloth, polyurethane sheeting, nylon mesh and absorbable materials such as collagen, polyglycolic acid and polyglycerol (paragraphs 0040-0062, 0067).

The difference between the prior art and the claimed invention is that the prior art does not expressly disclose the use of a dressing having a specific structure or the treatment of specific types of wounds or ulcers as claimed. However, the prior art simply suggests the same as the prior art discloses the treatment of lesions and ulcers with low doses of hydrogen peroxide, the treatment of wounds with low doses of hydrogen peroxide, and the use of various carriers including bandages, dressings, gels containing one of more active ingredients or mixtures of active ingredients, synthetic and/or non-synthetic polymeric components. As such, it would have been well within the skill of one of ordinary skill in the art would have been motivated to modify the prior art as above with the expectation that the products would be effective in healing lesions, wounds and ulcers.

NOTES TO FORM PCT/IS-A/201

These Notes are intended to give the basic instructions concerning the filing of amendments under Article 19. The Notes are based on the provisions of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and these requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule" and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions, respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant may, after having received the international search report, and, accordingly, to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where the applicant wants the claims to be published for the purposes of provisional protection or any another reason for amending the claims before international publication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19 only the claims may be amended.

During the international phase, the claims may also be amended to further amend under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Preliminary Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 19 or, where applicable, Article 41.

When? Within 2 months from the date of issuance of the international search report or 15 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where and to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.1).

Where a demand for international preliminary examination has been filed, see below.

How? Either by cancelling one or more claims (Rule 46.2) adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or cancellation, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered as Article numbers. Where a claim is cancelled, so renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 303.5).

The amendment must be made in the language in which the international application is to be published.

What documents may accompany the amendments?

Letter (Section 305(h)).

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below) under "Statement under Article 19(1)".

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

PCT

REQUEST

The undersigned requests that the present
international application be processed
according to the Patent Cooperation Treaty

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Applicant"

Applicant's or agent's file reference
(if checked) (73 characters maximum) 22727/04329

Box No. I TITLE OF INVENTION
METHODS AND COMPOSITIONS FOR WOUND HEALING

Box No. II APPLICANT ☐ This person is also inventor

Name and address: (Priority must be claimed by this name for a legal entity, full official designation.
The address must include postal code and name of country. The country of the address indicated in this
form is the applicant's State (check, country of residence if no State of residence is indicated below)

THE OHIO STATE UNIVERSITY RESEARCH
FOUNDATION
1960 Kenny Road
Columbus, OH 43210
US

Telephone No.

Facsimile No.

Telex/Internet No.

Applicant's registration No. with the Office

State (check, country) of nationality:

US

State (check, country) of residence:

US

This person is applicant
for the purposes of:☐ all designated
States☒ all designated States except
the United States of America☐ the United States
of America only☐ the States indicated in
the Supplemental Form

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Priority must be claimed by this name for a legal entity, full official designation.
The address must include postal code and name of country. The country of the address indicated in this
form is the applicant's State (check, country of residence if no State of residence is indicated below)

SEN, Chandan K. (Chandan K. Sen)
4804 Sapwood Drive
New Albany, OH 43054
US

This person is:

☐ applicant only☒ applicant and inventor☐ inventor only (if this check box is
marked, do not fill in below)

Applicant's registration No. with the Office

State (check, country) of nationality:

India

State (check, country) of residence:

US

This person is applicant
for the purposes of:☐ all designated
States☐ all designated States except
the United States of America☒ the United States
of America only☐ the States indicated in
the Supplemental Form☐ Further applicants and/or (further) inventors are indicated on a supplementary sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf
of the applicant(s) before the competent International Authorities as:

☒ agent☐ common
representative

Name and address: (Priority must be claimed by this name for a legal entity, full official designation.
The address must include postal code and name of country)

MYERS-PAYNE, Sean C. (Sean C. Myers-Payne)
Calfee, Halter & Griswold LLP
1100 Fifth Third Center
21 East State Street
Columbus, OH 43216
US

Telephone No.

(614) 621-7754

Facsimile No.

(614) 621-0010

Telex/Internet No.

Agent's registration No. with the Office

42,920

☐ Address for correspondence: Mark this check-box when an agent or common representative has been appointed and the
space above is used instead to indicate a special address to which correspondence should be sent.

95

Box No. V DESIGNATIONS				
The filing of this request constitutes under Article 4.9(a), the designation of all Contracting States bound by the PCT on the international filing date, for the grant of every kind of protection available and, where applicable, for the grant of both regional and national patents. However, ☐ The filer hereby is not designating for any kind of national protection ☐ The Republic of Korea is not designated for any kind of national protection ☐ The Russian Federation is not designated for any kind of national protection (The filer should also be aware to include (wherever possible) the designations concerned in order to avoid the loss of the effect under the national law, of an earlier national application from which priority is claimed, by the failure to Box No. V or to the consequences of such national law provisions in those and certain other States.)				
Box No. VI PRIORITY CLAIM				
The priority of the following earlier application(s) is hereby claimed:				
Filing date of earlier application (date of priority)	Number of earlier application	Where earlier application is:		
		national application country or countries of WTO	regional application regional Office	international application PCT/EA/Paris
Item (1) 18 June 2004	103671,168	US		
Item (2)				
Item (3)				
☐ Further priority claims are indicated in the Supplemental Box. The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) only if the earlier application was filed with the Office which for the purposes of this international application is the receiving Office(s) identified above as: ☒ all cases ☐ Item (1) ☐ Item (2) ☐ Item (3) ☐ other, see Supplemental Box * Where the earlier application is an EPO application, inclusion of Item one country party to the Paris Convention for the Protection of Industrial Property or one Member of the World Trade Organization for which the earlier application was filed (Article 4.10(a)(ii)).				
Box No. VII INTERNATIONAL SEARCHING AUTHORITY				
Choice of International Searching Authority (ISA) (if two or more international searching authorities are requested to carry out the international search, indicate the authority chosen; the two-letter code may be used): ISA / USPTO Request to use results of earlier search: Indicate in the search of an earlier search has been carried out by or requested from the International Searching Authority: Date (day/month/year) Number Country for regional Office				
Box No. VIII DECLARATIONS				
The following declarations are contained in Boxes Nos. VIII (i) to (v); check the applicable check boxes below and indicate in the right column the number of each type of declaration:				Number of declarations
☐ Box No. VIII (i)	Declaration as to the identity of the inventor	:		
☐ Box No. VIII (ii)	Declaration as to the applicant's entitlement, as to the international filing date, to apply for and be granted a patent	:		
☐ Box No. VIII (iii)	Declaration as to the applicant's entitlement, as to the international filing date, to claim the priority of this earlier application	:		
☐ Box No. VIII (iv)	Declaration of inventorship (only for the purposes of the designation of the national States of America)	:		
☐ Box No. VIII (v)	Declaration as to non-prejudicial disclosure or exception to lack of novelty	:		

96

Box No. IX CHECK LIST: LANGUAGE OF FILING																																																								
This international application contains: (i) on paper, the following number of sheets: <table border="0"> <tr> <td>request (including declaration sheets)</td> <td>: 3</td> </tr> <tr> <td>description (including sequence listing and/or tables related thereto)</td> <td>: 23</td> </tr> <tr> <td>claims</td> <td>: 4</td> </tr> <tr> <td>abstract</td> <td>: 1</td> </tr> <tr> <td>drawings</td> <td>: 6</td> </tr> <tr> <td>Sub-total number of sheets</td> <td>: 37</td> </tr> <tr> <td>sequence listing</td> <td>:</td> </tr> <tr> <td>tables related thereto</td> <td>:</td> </tr> <tr> <td>(for each, exact number of sheets if filed on paper, whether or not also filed in electronic form; see (j) below)</td> <td></td> </tr> <tr> <td>Total number of sheets</td> <td>: 37</td> </tr> </table> (ii) ☐ only in electronic form (Section 801(a)(1)) (i) ☐ sequence listing (ii) ☐ tables related thereto (iii) ☐ also in electronic form (Section 801(a)(2)) (i) ☐ sequence listing (ii) ☐ tables related thereto Type and number of carriers (diskette, CD-ROM, CD-R or other) on which are contained the: ☐ sequence listing: ☐ tables related thereto: (Additional copies to be indicated under items 9(i) and/or 10(i), in right column)		request (including declaration sheets)	: 3	description (including sequence listing and/or tables related thereto)	: 23	claims	: 4	abstract	: 1	drawings	: 6	Sub-total number of sheets	: 37	sequence listing	:	tables related thereto	:	(for each, exact number of sheets if filed on paper, whether or not also filed in electronic form; see (j) below)		Total number of sheets	: 37	This international application is accompanied by the following items (i) insert the applicable check-boxes below and indicate in right column the number of each item: <table border="0"> <tr> <td>1. ☒ fee calculation sheet</td> <td>: 1</td> </tr> <tr> <td>2. ☐ original separate power of attorney</td> <td>:</td> </tr> <tr> <td>3. ☐ original general power of attorney</td> <td>:</td> </tr> <tr> <td>4. ☐ copy of general power of attorney; reference number, if any:</td> <td>:</td> </tr> <tr> <td>5. ☐ statement explaining lack of signature</td> <td>:</td> </tr> <tr> <td>6. ☐ priority document(s) identified in Box No. VI as item(s):</td> <td>:</td> </tr> <tr> <td>7. ☐ translation of international application into (language):</td> <td>:</td> </tr> <tr> <td>8. ☐ separate indication concerning deposited information or other biological material</td> <td>:</td> </tr> <tr> <td>9. ☐ sequence listing in electronic form (indicate type and number of carriers)</td> <td>:</td> </tr> <tr> <td>(i) ☐ copy submitted for the purposes of international search under Rule 1.1ter only (and not as part of the international application):</td> <td>:</td> </tr> <tr> <td>(ii) ☐ copy where check-box (i) or (i)(ii) is marked in left column; additional copies indicating, where applicable, the copy for the purposes of international search under Rule 1.1ter</td> <td>:</td> </tr> <tr> <td>(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column</td> <td>:</td> </tr> <tr> <td>10. ☐ tables in electronic form related to sequence listing (indicate type and number of carriers)</td> <td>:</td> </tr> <tr> <td>(i) ☐ copy submitted for the purposes of international search under Section 802(b-querer) only (and not as part of the international application)</td> <td>:</td> </tr> <tr> <td>(ii) ☐ copy where check-box (i) or (i)(ii) is marked in left column; additional copies including, where applicable, the copy for the purposes of international search under Section 802(b-querer)</td> <td>:</td> </tr> <tr> <td>(iii) ☐ together with relevant statement as to the identity of the copy or copies with the tables mentioned in left column</td> <td>:</td> </tr> <tr> <td>11. ☒ notes (specify): <i>Notes regarding documents</i></td> <td>: 1</td> </tr> </table>	1. ☒ fee calculation sheet	: 1	2. ☐ original separate power of attorney	:	3. ☐ original general power of attorney	:	4. ☐ copy of general power of attorney; reference number, if any:	:	5. ☐ statement explaining lack of signature	:	6. ☐ priority document(s) identified in Box No. VI as item(s):	:	7. ☐ translation of international application into (language):	:	8. ☐ separate indication concerning deposited information or other biological material	:	9. ☐ sequence listing in electronic form (indicate type and number of carriers)	:	(i) ☐ copy submitted for the purposes of international search under Rule 1.1ter only (and not as part of the international application):	:	(ii) ☐ copy where check-box (i) or (i)(ii) is marked in left column; additional copies indicating, where applicable, the copy for the purposes of international search under Rule 1.1ter	:	(iii) ☐ together with relevant statement as to the identity of the copy or copies with the sequence listing mentioned in left column	:	10. ☐ tables in electronic form related to sequence listing (indicate type and number of carriers)	:	(i) ☐ copy submitted for the purposes of international search under Section 802(b-querer) only (and not as part of the international application)	:	(ii) ☐ copy where check-box (i) or (i)(ii) is marked in left column; 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Figure of the drawings which should accompany the abstract: 1		Language of filing of the international application: English																																																						
Box No. X SIGNATURE OF APPLICANT, AGENT OR COMMON REPRESENTATIVE Must be with signature, followed by name of the person acting and the capacity in which the person signs (if such capacity is not obvious from reading the request). <i>Sean C. Myers-Payne</i> Sean C. Myers-Payne Reg. No. 42,828																																																								

For receiving Office use only	
1. Date of actual receipt of the purported international application:	2. Drawings: ☐ received ☐ not received
3. Corrected date of actual receipt due to later but clearly marked papers or drawings comprising the purported international application:	
4. Date of timely receipt of the required notifications under PCT Article 11(2):	
5. International Searching Authority (if two or more are competent): ISA /	6. ☐ Transmittal of search copy delayed until search fee is paid
For International Bureau use only Date of receipt of the record copy by the International Bureau:	

This sheet is not part of and does not count as a sheet of the international application.

PCT

FEE CALCULATION SHEET

Annex to the Request

For receiving Office use only

International Application No.

Date stamp of the receiving Office

Applicant's or agent's
file reference

22727/04329

Applicant

THE OHIO STATE UNIVERSITY RESEARCH FOUNDATION

CALCULATION OF PRESCRIBED FEES

1. TRANSMITTAL FEE 300.00 [F]

2. SEARCH FEE 1,000.00 [F]

International search to be carried out by USPTO
(If two or more International Searching Authorities are requested to carry out the international search, indicate the name of the Authority which is asked to carry out the international search.)

3. INTERNATIONAL FILING FEE

Where items (b) and/or (c) of Item No. 1X apply, enter Sub-total number of sheets } 37
Where items (b) and (c) of Item No. 1X do not apply, enter Total number of sheets }

[1] per 30 sheets 1,134.00 [G]

[2] $\frac{7}{\text{number of sheets in excess of 30}} \times \frac{12.00}{\text{fee per sheet}} = 84.00 [H]$

[3] additional computer (only if a computer listing and/or tables related thereto are filed in electronic form under Section 861(a)(3), or both to that form and on paper, under Section 801(a)(3)):

400 x $\frac{\text{fee per sheet}}{\text{fee per sheet}} = 400.00 [I]$

Add amounts entered at 1), 2) and 3) and enter total as: 1,218.00 [J]

(Applicant from certain States are entitled to a reduction of 75% of the international filing fee. Where the applicant is for an applicant who is entitled, the total to be entered at 1 to 3) of the international filing fee.)

4. FEE FOR PRIORITY DOCUMENT (if applicable) 20.00 [F]

5. TOTAL FEES PAYABLE 2,638.00

Add amounts entered at 1, 2, 3, 4 and 5, and enter total in the TOTAL box

TOTAL

HOW TO PAY (Please fill in number of payment may be available at all receiving Offices)

☐ authorized to deposit ☐ postal money order ☐ cash ☐ cheque
☒ cheque ☐ bank draft ☐ revenue stamps ☐ other (specify):

AUTHORIZATION TO OPEN A/C OR CREDIT DEPOSIT ACCOUNT

(This mode of payment may not be available at all receiving Offices)

Receiving Office: USPTO

Deposit Account No.: 03-0172

☐ Authorization to charge the total (for credit deposit account)

Date: June 17, 2005

☒ (The checking Office is authorized to charge any deficiency or credit any overpayment in the total sum indicated above.)

Name: Sean C. Myers-Payne

☐ Authorization to charge the fee for priority document.

Signature: Sean C. Myers-Payne

78

**REQUISITOS MINIMOS PARA ADMISION A TRAMITE
PCT**

PATENTE DE INVENCION

[]

MODELO DE UTILIDAD []

- ◆ Indicación de que se solicita la concesión de una patente [✓]
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud [✓]
- ◆ Descripción de la invención [✓]
- ◆ Dibujos de ser estos pertinentes [✓]
- ◆ Comprobante de pago de las tasas establecidas [✓]

COMPLETA []

INCOMPLETA []

DISEÑO INDUSTRIAL []

- ◆ Indicación de que se solicita el registro de Diseño Industrial []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

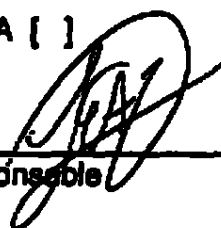
INCOMPLETA []

ESQUEMA DE TRAZADO []

- ◆ Indicación de que se solicita el registro de un Esquema de Trazado []
- ◆ Datos de identificación del solicitante o de la persona que presenta la solicitud []
- ◆ Representación gráfica del esquema trazado []
- ◆ Comprobante de pago de las tasas establecidas []

COMPLETA []

INCOMPLETA []


Firma Responsable

Fecha 10 JUN 2007

Carrera 13 No. 27-00 Piso 5, 7 y 10
Conmutador: 3 82 08 40
Fax: 350 52 20
E-mail: info@sic.gov.co
www.sic.gov.co
Bogotá, D.C., Colombia

REPUBLICA DE COLOMBIA
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

Bogotá,
2020

Doctor(a)
RODRIGUEZ DILIA MARIA

99

844

REFERENCIA	Radicación No.	07 1971
	Solicitud internacional	PCT/US2005/021447
	Fecha inicio fase nacional	18/01/2007
	Trámite	11
	Evento	379
	Actuación	430
	Oficio No.	1537

Como resultado del examen de forma, realizado con fundamento en el artículo 38 de la Decisión 486 de la Comisión de la Comunidad Andina, artículo 27 del Tratado de Cooperación en Materia de Patentes (PCT), regla 51 bis del Reglamento y Circular Único de la Superintendencia de Industria y Comercio, se le comunica que:

1. Si fuere el caso, el solicitante debe adjuntar la traducción de los anexos del reporte de examen preliminar internacional (Art. 38.2.b) del tratado PCT.
2. La solicitud no contiene la copia del documento en que consta la cesión del derecho a la patente del inventor al solicitante o a su causante. (Art. 26-k) Decisión 486.
3. La solicitud no contiene el poder debidamente otorgado, con el lleno de los requisitos exigidos por los artículos 63 y subsecuentes del Código de Procedimiento Civil. Adicionalmente, deberá presentar el documento que acredita la existencia y representación legal de la sociedad solicitante, cuando ésta fuere persona jurídica.

En cumplimiento de lo dispuesto por el artículo 38 de la Decisión 486, se le requiere para que dentro del término de dos meses siguientes a la fecha de notificación del presente oficio, complete los requisitos anteriormente enunciados. En caso de no dar cumplimiento al presente requerimiento, la solicitud se considerará abandonada y perderá su prelación.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal c), del capítulo quinto del título primero de la Circular Unica No.10 de 2001.

ALIX CARMEN CESPEDES DE VERGEL
Jefa de la división de Nuevas Creaciones

El anterior requerimiento fue notificado por fijación en lista de fecha 02 FEB 2007
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