TITLE: HONEY BASED GEL FORMULATIONS

ABSTRACT: Honey based gel compositions containing protein growth factors and/or debriding enzymes are provided. These compositions of the present inventions comprise honey and a water soluble, pharmaceutically compatible polymer. The preparations are stable and the biologically active components of the gel retain greater than 90% of its activity. Such gel formulations are useful in wound healing.
HONEY BASED GEL FORMULATIONS.

The following Complete Specification describes the nature of the invention and the manner in which it is to be performed:-

Field of Invention:

The invention relates to the formulation of Honey based gel compositions containing protein growth factors and/or debriding enzymes are provided.

Background of the Invention

The use of honey as a wound dressing material is an 8000 year old ancient remedy used by early Egyptians as far back as 6000 BC. It was a popular remedy even in ancient India and evidence of this can be derived from early scriptures, the Vedas, dating back to 5000 years ago.

It popular use may have been due to various reasons. The combination of honey and animal fat may have prevented the bandages from drying out and adhering to the wound. Perhaps more importantly, the honey could have been used for its antibacterial effects. The early civilizations could have known this empirically, but this is a scientifically proven fact that honey is antibacterial. Honey has, in a way been rediscovered in the 20th century and is becoming of increasing interest as more and more reports of its effectiveness in wound healing are being published.

The antibacterial effects of honey are due to both physical and chemical characteristics. Honey with its very high osmotic pressure will draw water from another source, such as tissues or bacteria cells.
When it draws the water out of bacteria, it kills them. These antibacterial properties of honey have been compared to those of saturated sugar solutions with comparable osmotic pressures. However, it was found that the saturated sugar solutions exhibited less antibacterial activity than natural honey, indicating that the antibacterial effect was related to factors other than just mere removal of water. The low water activity also starves the bacteria and yeast of water crucial for their survival and support. The antibacterial effect is also partially attributed to its acidic pH that inhibits bacterial growth. Honey has been found to have antibacterial properties against many gram negative bacteria and higher fungi while sugar solutions did not have the anti-microbial activity.

As far as it chemical characteristics are concerned, honey contains a number of ingredients that possess antibacterial activity. One such ingredient is a particular enzyme called glucose oxidase. This enzyme produces hydrogen peroxide, which kills bacteria when it breaks down to form oxygen radicals. The levels of hydrogen peroxide produced are not sufficient to damage tissues. Besides this it is also believed to contain some non-peroxide agents that also contribute to its antibacterial properties. Many of these antibacterial agents in the honey are still to be identified, but some phytochemicals and antioxidants present in Honey have known protective effect. Both enzymatic and non enzymatic antioxidants like catalase, ascorbic acid, flavonoids and alkaloids are believed to give Honey its unique properties.

Honey is composed primarily of sugars and very small quantity of acids, proteins and minerals. The average honey is about 80% sugar containing fructose (40%), glucose (32%) and maltose (7.3%) and other sugars. It is also has phytochemicals, antioxidants, organic acids
and a few amino acids. Sugars are well known stabilizers of therapeutic proteins and other biological molecules and a number of drugs and biological therapeutic medicines present in the market include different sugars in different compositions either in liquid form or dried form.

A skin wound is defined as a breach in the continuity of any body tissue caused by a minimal direct injury to the skin. Wounds are normally classified into two groups- acute wounds like bruises, incisions, cuts etc and chronic wounds like pressure ulcers, diabetic ulcers, venous and arterial ulcers etc.

Acute wounds usually heal by primary intention with minimal scarring while the chronic wounds heal by secondary intention with granulation and scar tissue formation. Pathophysiology of chronic wounds has been attributed to delayed healing process probably due to an impaired ability of the wound to harness the growth factors responsible for healing. This process of chemotaxis of growth factors to chronic wound is a complex process the details of which still being unraveled. However it is now known that wound healing is promoted by a number of growth factors like platelet derived growth factors (PDGF), Vascular endothelial growth factor (VEGF), Fibroblast growth factors (FGF) Keratinocyte Growth factors (KGF), Transforming growth factor (TGF), Insulin like growth factor (IGF) and Epidermal growth factors (EGF).

Chronic, non healing wounds have plagued healthcare practitioners for decades. There are many instances where a quick closure of the wounded skin will promote a beneficial response. The current invention relates to the usage of a honey based formulation with these any of
these growth factors and/or debridging enzymes to achieve rapid and optimal wound healing.

References

Ross et. All PNAS 71; 1207, 1974
Kohler and Lipton, Exp. Cell res. 87:297, 1974
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Thomason et al EPO 282,317 A2
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Honey-Health and therapeutic qualities, National Honey Board, USA

Summary of the invention

Honey is known to be very effective as a dressing for wounds, burns and skin ulcers: inflammation, swelling and pain are quickly reduced, malodour is reduced, shedding of dead tissue is induced so surgical removal is unnecessary, healing occurs rapidly with minimal scarring. Honey creates a moist environment which promotes the growth of new skin tissue and antimicrobial property of honey prevents infection. But unlike other antiseptics, honey is not harmful to tissues, and actually speeds up the growth of new tissue to heal the wound. In many of the reports the effectiveness of honey as a dressing on infected wounds is
attributed in part to its antibacterial properties. Honey can also be used for sore eyes and applied as ointment.

A recombinant derived PDGF-BB (a PDGF homodimer similar to the one present in humans) has been shown to be active in promoting wound healing in several animal models and subsequently in humans as well. PDGF is useful in individuals who have substantially impaired wound healing capacity, and thereby lack the ability to provide to the wound site endogenous growth factors which are necessary for the process of wound healing. Human platelet-derived growth factor has been shown to be the major mitogenic protein in serum for mesenchymal derived cells. A number studies reported that platelet extracts or purified PDGF-BB induces either cell multiplication or DNA synthesis in cultured smooth muscle cells, fibroblasts and glial cells. Furthermore PDGF is a potent chemoattractant for cells that are responsive to it as a mitogen. This is also somewhat unusual in that mitogens generally do not also act as chemotactic agents.

PDGF-BB has therapeutic applications for the treatment of injuries which require the proliferation of fibroblasts or smooth muscle cells to heal. In this regard PDGF-BB has been shown to be active in promoting wound healing in several animal models. Lynch et al disclose the use of insulin like growth factor (IGF-1) and purified PDGF-BB to promote healing of dermal wounds in pigs. These two growth factors showed a synergistic effect in promoting the healing. Lynch et al also found that c combination PDGF-BB and IGF-1 promotes bone and cementum formation in a dog model of periodontitis. In addition Greenhalgh et al demonstrated enhanced healing of full-thickness skin wounds in genetically diabetic mice treated with recombinant PDGF-BB as compared to control animals.
Thomason et al disclose that recombinant PDGF-BB accelerates the gain in tensile strength of healing skin wounds in rats and promotes wound healing in diabetic rats.

The present invention describes honey based stable gel formulations for a topical application consisting of effective amount of a wound healing protein growth factors and/or debriding enzymes. This formulation contains water soluble, pharmaceutically accepted polymer providing viscosity within the range for topical applications like 1000-200,000 cps at room temperature. The present gel formulations have the advantage of having honey properties in wound healing and contribute to stabilizing effect on biological material. The percentage of honey can be varied in order to exploit its various properties ranging from wound healing to antibacterial properties. The stabilizing effect for the protein molecules seems to stem from sugar content of honey. Sugars like sucrose, mannitol, glucose, fructose, sorbitol, etc., have been used as good stabilizers of biological molecules since many years. Moreover honey has good antioxidant properties which will enhance the stabilization of the gel. The extraction of biological material is higher than wax based or other previous formulations which means higher accessibility of active component for the wound healing. The present gel formulation also provides an advantage of uniform spreading of gel on the wound which increases the contact time with active factor and delivers the drug over the time.

**Detailed description of invention**

The present invention provides honey based gel compositions comprising a therapeutically effective amount of a wound healing growth factor like but not limited to PDGF in a pharmaceutically
acceptable polymer like but not limited to carboxymethyl cellulose gel containing honey with an approximate viscosity ranging from 1000-200000 cps at room temperature. Recombinant PDGF has been shown to possess substantially the same biological activity as native PDGF. The basic biological activity of PDGF, particularly the induction of chemotaxis and mitogenesis in responsive cell types (including fibroblasts and smooth muscle cells), underlies many of the physiological roles of this protein, including its role in tissue repair.

An example is presented below to illustrate the subject invention. The examples are the actual tests carried out on the gels. It should be understood that the examples are neither exhaustive nor limiting and are presented only for a better understanding of the invention.

For those skilled in the art it is evident that similar formulations can be derived by using any of the known growth factors like platelet derived growth factors (PDGF), Vascular endothelial growth factor (VEGF), Fibroblast growth factors (FGF) Keratinocyte Growth factors (KGF), Transforming growth factor (TGF), Insulin like growth factor (IGF) and Epidermal growth factors (EGF) or debriding enzymes like collagenase or papain (alone or in combination with urea). Any of the known pharmaceutically acceptable polymers can be used to achieve the gel of required viscosity. Carboxy methyl cellulose is used in the example below.

**Example 1**

Briefly, carboxy methyl cellulose (CMC) is reconstituted in pure water for injection or aqueous buffer solution and honey to give desired viscosity. A preferred concentration is 1-10% of CMC or more
preferably 3-6%. Preferred buffers include but not limited to acetates with pH around 4.5-6.5 up to 100mM concentration preferably 50mM. Honey can be used in the range 20-80 % preferably 50%. After adding honey, sterile solution of PDGF-BB is added and mixed. The formulated gel can be sterilized by steam at 120 deg briefly or by gamma irradiation before adding PDGF-BB. The properties of honey do not change significantly. The gel was stored between 2-8 deg.c. Typically PDGF concentrations can be in the range 1µg– 1000µg /gm of gel or more preferably in the range of 10µg- 300µg/gm of gel. Other components like saline and preservatives (antimicrobial agents) like methyl and propyl paraben can be added to make the gel more biologically compatible and for long term storage respectively. The concentrations of these two are 0.15M saline, 0.02% to 0.2% methyl and propyl parabens.

As noted above, the compositions of the present invention are typically administered topically in a therapeutically effective amount, depending of course on the size and characteristics of the wound. In general 0.2 to 0.6 g of the gel will be applied per / sq.cm of wound area. The gel will be formulated to provide about 10ug to 300ug of PDGF per sq.cm of wound area. The compositions may be reapplied at one to several day intervals until healing is complete.

**Stability of PDGF-BB in the Honey-CMC gels**

The stability of PDGF-BB in carboxymethyl cellulose gel was tested in an experiment described briefly here as follows. A carboxy methyl cellulose gel containing 100 ug/g of gel was prepared at 3% CMC and 50% honey, 0.15M NaCl at pH 6.5 using sodium acetate buffer. The gel also contained 0.2% of methyl paraben and .02% of propyl paraben
as microbial preservatives, up to five months. The stability of PDGF-BB was tested for its biological activity. The bioassay was performed on human fibroblasts cells and their proliferation by PDGF-BB. The structural integrity of PDGF-BB was determined by SDS-PAGE.

The results are as follows:

1. There was no change in the cell proliferation activity of PDGF-BB up to 5 months of storage. All the samples showed the activity 0.8-1.0x10^6 units/mg of protein.

2. SDS-PAGE pattern showed intact PDGF-BB dimer band and there were no aggregates or degraded bands in the gel pattern.

3. There was no apparent change in appearance or viscosity of the gel as observed visually.

The invention has been described herein with reference to certain preferred embodiment and example. It is apparent that obvious variations may appear to those skilled in the art. In particular, one skilled in the art may be able to vary the compositions of active and other ingredients to achieve desired gels for the specific purpose. Since obvious variations will appear to those skilled in the art, the invention is not to be considered limited thereto but only by the claims which follow.
Claims:

1. A pharmaceutical composition, comprising of wound healing growth factor in a mixture of honey and a pharmaceutically acceptable polymer as a carrier with a viscosity of about 1000-200000 cps.

2. A composition of claim 1, wherein the wound healing growth factor is but not limited to Platelet derived growth factor (PDGF), more preferably but not limited to PDGF-BB homodimer.

3. A composition in claim 1 wherein the PDGF-BB is at least 90% pure and is of, but not limited to, a recombinant origin.

4. A composition in claim 1 wherein the concentration of PDGF-BB is about 1-1000μg/gm of the said gel or more preferably between 10-300μg/gm of the said gel.

5. The composition of claim 1 wherein the pharmaceutically acceptable polymer is, but not limited to, carboxy methyl cellulose.

6. The composition of claim 1 wherein percentage of carboxymethyl cellulose is between 1-10%.

7. The composition of claim 1 wherein the honey composition in between 20-80%

8. The composition of claim 1 wherein the source of honey is preferably, but not limited to, a botanical source.
9. The composition of claim 1, wherein the wound healing growth factor may further comprise of individually or in various combinations of growth factors like Vascular endothelial growth factor (VEGF), Fibroblast growth factors (FGF) Keratinocyte Growth factors (KGF), Transforming growth factor (TGF), Insulin like growth factor (IGF) and Epidermal growth factors (EGF) or debriding enzymes like collagenase or papain (alone or in combination with urea).

10. A composition in claim 1 wherein the preservatives used is but not limited to methyl paraben or propyl paraben.

11. A composition in claim 1 wherein the concentration of methyl or propyl paraben is in the range of 0.02%- 0.2%

12. A composition in claim 1 wherein the viscosity of the formulation is in the range of 1000-20000cps when measured at room temperature.
# INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC*: A81K 38/18, 35/64
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC*: A61K

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)
WPI, EPODOC, PAJ, medline, CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 2002/030467 A2 (TRITICUM EXPLOITATIE B.V.) 18 April 2002 (18.04.2002) claims 1, 5.</td>
<td>1, 5, 7, 8</td>
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☐ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
**A** document defining the general state of the art which is not considered to be of particular relevance
**E** earlier application or patent but published on or after the international filing date
**L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
**O** document referred to in oral proceedings, use, exhibition or other means
**P** document published prior to the international filing date but later than the priority date claimed

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Form PCT/ISA/210 (second sheet) (January 2004)
Continuation of first sheet

Continuation No. II:

Observations where certain claims were found unsearchable

(Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 12 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Pharmaceutical compositions have to be characterized by their ingredients; any characterization merely by physical parameters is insufficient (claim 12).
## INTERNATIONAL SEARCH REPORT

### Information on patent family members

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