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(54) SKIN DRESSINGS

(71) We, PIKOK INTERNATIONAL TRADING CO. LTD., a Hong Kong Company of Room 1006-8 American International Tower, 16 Queen's Road, Central, Hong Kong, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:-

This invention relates to skin dressings of chemically modified collagen in gel form.

According to one aspect the invention provides a skin dressing of petroleum jelly consistency comprising enzyme-solubilized acylated, preferably succinylated, atelocollagen gel and at least one substance selected from antibiotics and bactericides.

According to another aspect, the invention provides a method for the preparation of a collagen skin dressing in gel form which comprises:

a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor atelocollagen extract,

b) precipitating atelocollagen from the extract,

c) reacting an aqueous dispersion of the atelocollagen extract with an acylating agent,

d) separating acylated atelocollagen from the reaction mixture, and

e) converting the acylated atelocollagen to a gel of petroleum jelly consistency.

The acylated atelocollagen is preferably purified by solution and reprecipitation prior to its conversion to a gel.

A number of investigators including the present inventors have suggested the use of collagen material as a skin, burn or wound dressing. The feature of this invention, however, consists in the form of the collagen dressing and in the method of producing such desired types.

The U.S. National Fire Protection Association reported in 1962 that approximately 1,800,000 persons sustain burns yearly and occupy over 11,000 hospital beds per day. There is a great need for a readily available, easily stored and temporary substitute for human skin for the effective treatment of thermal burns and other forms of skin loss. It is common practice to cover skin loss area with split-thickness autografts, homografts and heterografts. Such treatments protect against infection, the loss of protein, fluid and electrolytes from exposed tissue. These treatments, however, have the following drawbacks. Grafts are difficult to obtain, and to store for any prolonged period of time and also are quite expensive. These difficulties could be reduced by the development of artificial skin dressings which are inexpensive and readily available to use.

Collagen is a major protein of connecting tissue such as skin and cornea, and can be solubilized, separated and purified by the treatment with proteolytic enzymes (other than collagenase), e.g., proctase, pepsin, trypsin and pronase. Solubilized collagen is telopeptides-poor, relatively inexpensive and ideal as a material for development into a skin wound dressing.

Solubilized collagen has many NH_2 and COOH groups in its structure, and chemical modifications of the molecule can be readily made, e.g. all or some of the amino groups may be acylated by reaction with a mixture of acetic anhydride and acetic acid. Similarly, succinic anhydride reacts with collagen replacing amino groups by carboxyl groups.

The gel type skin dressings of this invention are especially suitable for application to irregular body surfaces e.g., areas of the joints, elbows, knees, etc. The gel dressing is preferably used in the form of a viscous paste of petroleum jelly consistency which contains 1 - 10% by weight, preferably 2 - 5% by weight of collagen.

In the preferred preparation of the col-

lagen gel, skin or hide is solubilized in an enzyme solution at acidic pH. The resulting gel is a viscous material which is recovered by filtering, e.g., through cheese cloth and/or a Millipore (Trade Mark) filter. The viscous solution is made alkaline by addition of caustic to a pH of about 10. At this stage the material is permitted to stand in order to inactivate any remaining enzyme. The material is thereafter neutralized, the collagen collected by centrifuge and washed with water. A second purification step follows, namely, redissolving in aqueous acid (pH 2.0 - 5.0), reprecipitation by neutralization to a pH of 6 to 7, and purification to remove acid by dialysis against water. The neutral gel is recovered and at this stage antibiotics or bactericides or both may be added before storage of the gel material.

Collagen material used in the preparation of gel is preferably not a multimer and, therefore, the material is not subjected to tanning during its preparation.

Succinylated collagen is preferably used for making the viscous gel skin dressing since it can be redissolved in water at physiologic pH (6.8 - 7.4) without requiring dialysis against water to remove acid durings its preparation. The collagen concentration of the preferred gel skin dressing is from 0.5% to 7% by weight and it contains the following bactericidal agents: Silver nitrate solution (0.5 g/100ml) or silver lactate solution (0.5 g/100ml), 25 mg/ml Lincomycin, 5 mg/ml Amphotericin B and 25 mg/ml Gentamicin, or one or more thereof.

An effective skin dressing should have the following properties:

1. good adherence to the wound surface,
2. prevention of loss of protein, fluid and electrolytes,
3. prevention of infection,
4. reduction of pain,
5. no stimulation of local tissue response.

Collagen skin dressings as hereinafter described in detail have been found to satisfy the above properties and to be easy to use and relatively inexpensive. In particular, the viscous gel-like paste and gels of petroleum jelly consistency show excellent adherence to wound surfaces and effective protection against infection and good wound healing.

The present invention may be further understood from the following examples in which all percentages are by weight unless otherwise indicated:

Example 1

Fresh calfskin (about 5 kg) was dehaired, cleaned by shaving and cut into small pieces. The skin was washed repeatedly with 10% NaCl containing a 0.2% sodium azide bactericide and with sterilized water. The skin was solubilized in 10 liters of water (pH 2.5 HCl) containing 30 mg/ml Gentamicin by addition of 1 g. of pepsin (approximate ratio of enzyme to collagen was 1/400) at 20°C for 4 days with intermittent stirring. The resulting viscous solubilized collagen was filtered through cheesecloth, its pH raised to 10 by NaOH and allowed to stand for 24 hours at 20°C to inactivate the pepsin. The pH of collagen was then adjusted to 7-8 (HCL) and collagen precipitate was collected by centrifuging and washed with sterilized water. The washed precipitate was redissolved in acidic solution and reprecipitated at pH 7-8 for further purification.

Succinylation of solubilized collagen was performed as follows:

Ten grams (dry basis) of solubilized collagen precipitate was resuspended in 4 liters of water and its pH adjusted to 9.0 by NaOH. Acetone solution (100 ml) containing 2.0 g of succinic anhydride was gradually added to the collagen suspension. During the addition of succinic anhydride the pH of collagen suspension was maintained at about 9.0 by NaOH solution. Succinylated collagen was precipitated by acidification to about pH 4.2, washed with water and freeze-dried. This freeze-dried collagen (sponge-like in form) was sterilized to remove micro-organisms. Five grams of the sterilized collagen sponge was dissolved in acidified water. Upon addition of NaOH to a pH of about 7.4 the collagen emerges in a highly viscous form. It was preserved by addition of silver nitrate (0.5% solution). This gel skin dressing was excellent in adhesion to the wound, in wound healing, and in protection against infection.

Example 2

Succinylated solubilized collagen was prepared by the method described in Example 1. Sterilized succinylated collagen (10 g) was treated in the same manner as in Example 1 but preserved by Silver Lactate (0.5% solution). This gel skin dressing again displayed excellent properties as above.

Example 3

Sterilized succinylated collagen was prepared by the method described in Example 1 and treated in the same manner as in Example 1 but was preserved in sterile lactated Ringer's solution (0.6% NaCl, 0.31% sodium lactate, 0.03% potassium chloride, 0.02% calcium chloride, pH adjusted to 7.4) containing the following antibiotics: 25 mg/ml Lincomycin, 5 mg/ml Amphotericin B, and 25 mg/ml Gentamicin. This gel skin dressing once again displayed excellent properties.

WHAT WE CLAIM IS:-

1. A skin dressing of petroleum jelly consistency comprising enzyme-solubilized acylated atelocollagen gel and at least one

lagen gel, skin or hide is solubilized in an enzyme solution at acidic pH. The resulting gel is a viscous material which is recovered by filtering, e.g., through cheese cloth and/or a Millipore (Trade Mark) filter. The viscous solution is made alkaline by addition of caustic to a pH of about 10. At this stage the material is permitted to stand in order to inactivate any remaining enzyme. The material is thereafter neutralized, the collagen collected by centrifuge and washed with water. A second purification step follows, namely, redissolving in aqueous acid (pH 2.0 - 5.0), reprecipitation by neutralization to a pH of 6 to 7, and purification to remove acid by dialysis against water. The neutral gel is recovered and at this stage antibiotics or bactericides or both may be added before storage of the gel material.

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WHAT WE CLAIM IS:-

1. A skin dressing of petroleum jelly consistency comprising enzyme-solubilized acylated atelocollagen gel and at least one

substance selected from antibiotics and bactericides.

2. A skin dressing according to claim 1 in which the acylated atelocollagen is succinylated atelocollagen.

3. A method for the preparation of a collagen skin dressing in gel form which comprises:

a) treating a source of collagen with a proteolytic enzyme (other than collagenase) to form a telopeptide-poor atelocollagen extract,

b) precipitating atelocollagen from the extract,

c) reacting an aqueous dispersion of the atelocollagen extract with an acylating agent,

d) separating acylated atelocollagen from the reaction mixture, and

e) converting the acylated atelocollagen to a gel of petroleum jelly consistency.

4. A method according to claim 3 in which the acylating agent is succinic anhydride.

5. A method according to claim 3 or 4 in which the acylated atelocollagen is purified by solution and re-precipitation prior to its conversion to a gel.

6. A method according to claim 3, 4 or 5 in which there is incorporated into the gel at least one substance selected from antibiotics and bactericides.

7. A method according to claim 3, substantially as described in any of the Examples.

8. A collagen skin dressing in gel from when prepared by a method according to any one of Claims 3 to 7.

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