Subject: Periodic Administrative Report for Award N00014-90-J1797
Liquid Collagen Wound Coverings

Dear Captain Lewis:

Attached is a brief summary of research progress since our last report of January 14, 1992.

Yours sincerely,

J. Peter Bentley, PhD
Professor of Biochemistry and Molecular Biology

cc: Administrative Grants Officer
Director, Naval Research Laboratory
Defense Technical Information Center
Office of Chief of Naval Operations
Bureau of Medicine and Surgery
Enclosed are copies of two patents which have been issued, one relating to Iodine Caused Gelation of Collagen and another to the development of a wound healing kit. Both of these patents directly result from work supported by the project.

As reported in January, 1992, a recruitment was undertaken to find an individual with light and electron microscopic technique skills. Such an individual has been identified and on April 15, 1992, Sandra Watt, PhD, was employed as a Senior Research Associate with direct responsibility for design and evaluating animal experiments involving the use of growth factors in our pourable wound dressing.

Freeze Drying of Collagen

A previously mentioned liaison with Oregon Freeze Dry has developed further and freeze dried collagen preparations have been made and incorporated into wound healing kits. This collagen can be rapidly hydrated and when iodine is added a gel results. Large numbers of these kits have now been made up for use of investigators at hospitals distant from OHSU, specifically the Oregon Burn Center at Emanuel Hospital and Medical Center in Portland. In these materials, when reconstituted with water, the bioburden of microorganisms was found to be 250 colony forming units of gram positive bacilli and 17 CFUs of gram positive cocci per 20 ml units of collagen. This is well below the accepted critical value of $10^5$ organisms per gram of tissue (US Army Surgical Research Unit). After the iodine gelling solution was added to this collagen it was not possible to demonstrate any CFUs at all, which is to be expected.

Evaluating a New Technique of Freeze Drying Collagen in Tissue Culture Flasks

These newly available flasks have a 0.2μ filter incorporated into the cap. Therefore, prefiltered, sterile solutions of collagen may be added to the sterile freeze dry flasks, which are frozen, and freeze dried with the caps in place, thus preventing any contamination, but permitting egress of water vapor during the lyophilization procedure.

Vehicle for Growth Factors

Collagen crosslinked with DOPA was molded in plastic syringes into firm gelatinous plugs. Platelet derived growth factor (PDGF) was incorporated into these plugs, which were implanted subcutaneously in rabbits. After being in place 15 days and 45 days the plugs were removed and studied histologically.
Abstract

A stabilized collagen gel is disclosed as are methods of making this collagen gel which is useful as a wound dressing to prevent dehydration of the subject being treated and infection of the wound. The collagen gel of the invention is stabilized by combining collagen (preferably a pharmaceutical grade collagen, which is atelopeptide collagen), with iodine or a composition capable of generating iodine. The collagen is flowable on first mixing and undergoes a phase transition to form a stable gel with sufficient structural integrity to form a wound dressing.

17 Claims, No Drawings
WOUND DRESSING PROTOCOL UTILIZING COLLAGEN GELATIN FORMED WITH IODINE

GOVERNMENTAL INTEREST

The present invention was developed in part under Federal Government support via contract N00014-84-K-0402 awarded by the Department of the Navy. The United States Government may have certain rights in this invention.

FIELD OF THE INVENTION

The present invention relates generally to wound dressings of the type which include collagen. More particularly, the invention relates to a stabilized form of collagen material which can be used to form a wound dressing composition and to methods of making and using such collagen materials and wound dressings.

BACKGROUND OF THE INVENTION

The ability to maintain an intact outer layer of skin is essential to life itself in that the skin is critical to preventing infection (keeping out unwanted organisms) and preventing dehydration (keeping in desired water and/or bodily fluids). Accordingly, when the skin of an individual is damaged over a large percentage of the individual’s body, a life threatening situation arises. The mortality following large burn wounds or other wounds which cause the removal of a large amount of skin are brought about by infection and/or dehydration caused by exposure of large areas of uncovered tissues i.e., tissue material not covered by an outer layer of skin.

In order to prevent dehydration and infection, a primary treatment regime involves the use of dressings which are designed to prevent loss of water and thus alleviate the dehydration problem and simultaneously prevent the proliferation of organisms and thus prevent infections. The dressings may include different forms of antiseptic compounds and may be comprised of a variety of materials capable of preventing the escape of substantial amounts of water.

A second step in a treatment regime requires the application of surgical debridement procedures. In these procedures, badly damaged and dead tissue is removed from the wound area along with any foreign substances which may have become implanted in the exposed tissue. Any such surgical procedures, of course, leave exposed wound areas. Accordingly, a third step in the treatment regime often involves the placement of an auto-graft of the patient’s own skin onto the wound bed. Although this procedure can give very desirable results, it is generally not immediately possible in patients where a large percentage of the skin has been burned or removed. Insufficient skin is available in such circumstances and in other situations, the patient may be too ill to undergo the required transplant procedures.

If insufficient skin is available for transplant procedures or the patient is too ill to undergo such procedures, other treatment regimes are available which involve the placement of temporary dressings. Such dressings are comprised of materials such as pig skin, skin taken from human cadavers, various artificial skin-like membranes and various artificial skin-like preparations. These dressings must generally be removed prior to grafting. Further, since they often involve the use of foreign tissue material, they may generate an immune reaction and be rejected. The present invention is an attempt to alleviate and/or eliminate deficiencies of such prior art dressings and thus provide an alternative protocol for the treatment of large surface wounds such as burn wounds.

SUMMARY OF THE INVENTION

The present invention provides a stabilized collagen gel composition containing an anti-bacterial agent. Methods of producing such a stable gel composition as well as methods of forming the composition into wound dressings and using such wound dressings in order to treat large skin wounds such as burn wounds are taught. The wound dressings are useful in preventing dehydration and infection.

The collagen compositions of the invention include a modified form of collagen which is both stabilized and sterilized simultaneously to degrees not possible with other collagen-containing compositions. The collagen is stabilized by combining an atelopeptide or pharmaceutical grade collagen with a reagent capable of generating a stable collagen gel. The reagent must be pharmaceutically acceptable with respect to the wound and compatible with the collagen. The reagent is preferably an iodine generating reagent and most preferably a combination of potassium iodide and potassium iodate in an acidic milieu.

A primary object of the invention is to provide a stabilized form of collagen gel.

Another object of the present invention is to provide a method for producing the stabilized collagen gel material.

Another object of the present invention is to provide a convenient method of stabilizing the collagen composition of the invention by combining water with lyophilized collagen in the presence of an iodine generating system.

An important feature of the present invention is that the stabilized collagen gel compositions can be readily formed into wound dressings useful in the treatment of wounds such as skin wounds such as large burn wounds.

An advantage of the present invention is that after the lyophilized collagen is combined with an iodine generating system, the collagen remains liquid and can be placed and formed into a particular shape on a wound where it begins to set into a firm stable gel. The gelling is accelerated at temperatures of mammalian tissues although it will occur at room temperature if additional time is provided.

A feature of the present invention is that the liberation of iodine in a solution containing atelopeptide collagen causes the collagen to form a firm, stable gel which can be readily utilized in connection with wound healing.

Yet another advantage of the present invention is that the collagen forms a gel which includes the iodine which, in turn, acts as a bactericide when the gel is used in connection with wound healing.

These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and usage as more fully set forth below, reference being made to the accompanying examples forming a part hereof.
Before the present stabilized collagen gel compositions and processes for making such are described, it is to be understood that this invention is not limited to the particular collagens, reagents, or process steps described as such compounds and methods may, of course, vary. It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only and is not intended to be limiting since the scope of this invention is limited only by the appended claims.

It must be noted that as used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a collagen" includes mixtures of collagen materials of the type described herein. Reference to "the method of treatment" includes a plurality of different types of treatment protocols of the type which will be generally known to those skilled in the art or become apparent to them upon reading this disclosure.

The present invention requires the use of collagen material. Initially, the collagen is obtained from a natural source. In that the present invention uses the collagen in combination with a reagent such as an iodine generating reagent which has an antibacterial effect "native" or "natural" collagen can be used in connection with the present invention. The present invention allows the formation of gels useful in wound dressing by starting with all types of collagen material. Accordingly, all types of collagen materials can be used in connection with the present invention. However, the collagen used in connection with the present invention is preferably not "native" or "natural" collagen. It has been modified to some extent in order to purify the collagen material and change its structure in an attempt to eliminate the generation of an immune response when the collagen comes into contact with living tissue. In connection with the present invention, such collagen is generally referred to as "pharmaceutical grade collagen" or "atelopeptide collagen". A general description of collagen and how "native" or "natural" collagen is modified to obtain a pharmaceutical grade collagen is put forth below. Accordingly, the term "pharmaceutical grade collagen" as used in connection with this invention is intended to encompass all types of collagen material which have been modified to some extent in order to purify the collagen material and change its structure in some manner in an attempt to eliminate the generation of an immune response when the collagen comes in contact with living tissue.

In its broadest sense, the present invention is a stabilized collagen gel. The stabilized gel is obtained by combining collagen with iodine or an iodine generating composition and allowing the components to interact until the stable gel is formed. Details with respect to iodine generating compositions, collagens and other components which can be included in the composition are given below. The relative amounts of these components will affect the final composition and the gelling time as further explained below. The following explanation is given with respect to stabilized gels which are most preferably used in connection with wound dressings. However, the stabilized gel composition of the present invention is a novel chemical composition and such a composition is considered to be an important aspect of the present invention apart from any use of the composition as a wound dressing.

Native collagen consists in large part of a triple helical structure containing repeating triplet sequences composed of glycine linked to two additional amino acids, commonly proline and hydroxyproline; thus, glycine appears in every third position in the chain. In addition, all collagen chains contain regions at each end which do not have the triple glycine sequence and are thus not helical. These regions are thought to be responsible for the immunogenicity associated with most collagen preparations. Immunogenicity can, in large part, be mitigated by removal of these regions to produce "atelopeptide" collagen. This can be accomplished by digestion with proteinolytic enzyme such as trypsin, chymotrypsin or pepsin. Because of differing specificities of these proteases, the degree of completeness of removal of the atelopeptides varies. Thus certain proteases, which effect the most complete removal, are preferred. Included among these is pepsin, which results in removal of substantially all of the telopeptide portions.

In native collagen the non-helical telopeptide regions are also responsible for forming the cross-links which aid in stability of the fibrillar structure. These regions contain aldehydes capable of cross-linkage to lysine residues. Atelopeptide collagen does not include any significant degree of cross-linking. Collagen has been subclassified into more than ten types depending on the precise amino acid sequence in the individual chains of the triple helix, the carbohydrate content, and the presence or absence of disulfide cross-linking and other differences. The most common subtypes are Type I which is present in skin, tendon, and bone, and which is made by fibroblasts, and Type III which is found primarily in skin. Other types reside in specialized membranes or cartilage or at cell surfaces. Types I and III contain similar numbers of amino acids in their helices; however, Type III (but not Type I) contains two adjacent cysteines at the C-terminal ends of the triple helix which are capable of forming interchain cross-links. As indicated above, the present invention can be used in connection with all different types of collagen. However, it is most preferably used in connection with atelopeptide Type I collagen which is a "pharmaceutical grade" collagen material which has been purified and modified with respect to the telopeptides in order to eliminate or reduce the generation of an immune response when the collagen comes in contact with living tissue.

Although methods for obtaining collagen from natural sources and treating the collagen to obtain a pharmaceutical grade collagen are not part of this invention, this methodology is described in Example 1 which involves the isolation of collagen from bovine skin. However, the present invention is not limited to this specific example and includes any "Type" of collagen which would be encompassed by the general description of collagen materials given above. Preferred collagens include materials described as "pharmaceutical grade collagen", "atelopeptide collagen" or simply "collagen" throughout this disclosure and the attached claims. A pharmaceutical grade collagen material sold under the tradename "Zyderm" (sold by the Collagen Corporation of Palo Alto, Calif.) could also be used in connection with this invention.

When the collagen is combined with the iodine, it provides a modified collagen composition which is liquid and thus flowable (for a given period of time...
possibility that the dressing will act as an appropriate Compacted lyophilized collagen is considerably more
tissue. The ability to leave the dressing in the gel preparations stops the bacterial invasion of the into the collagen compartment in order to prevent com-
been seen. It has been found that the presence of iodine
gel wherein animals have had applied thereto dressings of concentrations of Collagen, KI and KI03 which will
order to form a stabilzied gel.

The use of such an agent to one or more of a plurality (preferably 3) of separate compartments (preferably plastic
bag type compartments), each containing different components of the invention which components are combined to form the stabilized gel for treating wounds.

The three compartments will now be described. A first compartment of the kit will include a type I pepsin-
treated collagen material, such as the collagen material obtained in accordance with Example 1 below or a commercially available collagen such as Zyderm sold by
the Collagen Corporation of Palo Alto, Calif.

The Type I pepsin-treated collagen is dissolved in 0.005M acetic acid for storage in liquid nitrogen. A portion of the collagen solution is mixed with an equal
volume of a citrate buffer pH 7.2 and Lyophilized. The collagen is placed in one compartment of a 3-compartment
sterile blood bag type container which is then heat sealed. Accordingly, this first compartment includes the
collagen component and buffer.

A second compartment of the 3-compartment system contains potassium iodide (KI) and 0.005M citric acid.

The citric acid concentration is used to maintain the low pH required for iodine release. There are high concentrations of the buffer component within the collagen
compartment. Accordingly, the buffer of the collagen component will make it possible to obtain a substantial
lyophilized and this blood bag compartment is sealed.

The third compartment of the 3-compartment sterile blood bag container contains potassium iodate (KIO3).

Although the above description indicates that the 3 components are separately included, in a preferred
approach an alternative approach is possible. In accordance with the alternative, the three substances, collagen, KI and KIO3, are placed in three completely separate contain-
collagen compartment in order to prevent com-

compaction of the collagen during storage and shipment.

Compacted lyophilized collagen is considerably more
The composition of claim 5, wherein the buffer is a citrate/phosphate buffer.

7. The composition of claim 1, wherein said iodine generating composition is a reduced iodine in combination with an oxidizing agent and an additional compound which acts as a source of protons.

8. The composition as claimed in claim 7, wherein the reduced form of iodine is potassium iodide, and the oxidizing agent is selected from the group consisting of persulfates, perborates, hydrogen peroxide, tert-ary butyl peroxide, alkali metal periodate, hypochlorite salts and free hypochlorous acid and halogen amines.

9. The composition as claimed in claim 8, wherein the source of protons is citric acid.

10. A method of forming a collagen gel, comprising the steps of:

- combining collagen and water in relative amounts so as to form an aqueous collagen solution, and
- combining a composition capable of generating iodine in solution with the collagen solution in relative amounts so that the solution undergoes a phase transition from the solution to a gel, the phase transition occurring in ten minutes or less at a temperature in the range of 20°C to 28°C.

11. The method of claim 10, wherein said composition capable of generating iodine is comprised of an oxidized iodine in combination with a reducing agent.

12. The method of claim 10, wherein said oxidized iodine is selected from the group consisting of alkali metal iodate, periodate, iodine pentoxide.

13. The method of claim 10, wherein the composition capable of generating iodine is comprised of a reduced iodine in combination with an oxidizing agent.

14. The method as claimed in claim 13, wherein the reduced iodine is potassium iodide and the oxidizing agent is selecting form the group consisting of persulfates, perborates, hydrogen peroxide, tert-ary butyl peroxide, alkali metal periodate, hypochlorite salts and free hypochlorous acid and halogen amines.

15. The method as claimed in claim 14, wherein the composition capable of generating iodine is further comprised of a proton donor and the collagen is atelopeptide collagen.

16. The composition as claimed in claim 15, wherein the proton donor is citric acid and the atelopeptide collagen is a pharmaceutical grade Type I collagen.

17. A method of treating a wound, comprising the steps of:

- combining collagen and water in relative amounts so as to provide an aqueous collagen solution, forming a wound-healing composition by combining a composition capable of generating iodine in solution with the collagen solution in relative amounts so that the collagen solution will undergo a phase transition from the solution to a gel which phase transition would occur in ten minutes or less at a temperature in the range of 20°C to 28°C;
- applying the wound-healing composition to the wound while the composition is in a solution phase, allowing the solution to conform to the shape of the wound and undergo the phase transition from solution to gel on the wound.
I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231 on December 16, 1991

12/16/91
Date
Signature

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
J. PETER BENTLEY et al.
Serial No.: 07/787,556
Filing Date: 4 November 1991
Title: WOUND DRESSING PROTOCOL

PRELIMINARY AMENDMENT

The Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

Prior to the examination of this application on its merits, please amend the application as follows:

In the Title:

Please delete the present title and add therefore
--WOUND HEALING KIT COMPRised OF GELABLE COLLAGEN--.
In the Specification:

On page 1, beneath the title and above the subheading "Field of the Invention", delete any matter previously added via amendment and add therefore

--Cross-Reference

This application is a divisional application of our earlier filed U.S. application Serial No. 07/553,979, filed July 16, 1990, to which application we claim priority under 35 USC §120 and which application is incorporated herein by reference.

Acknowledgment of Government Support

As required under 37 CFR 401.14(f)(4), it is pointed out that the present invention was developed, in part, under Federal Government support via contract N00014-84-K-0402 awarded by the Department of Navy. The United States Government may have certain rights in this invention.--

In the Claims:

Please cancel presently pending claims 1-20 from the application and add the following new claims 21-30.

--21. (New) A wound healing kit, comprising:

a first container having therein a composition comprised of water soluble, lyophilized, atelopeptide collagen; and

a second container having therein a composition capable of generating iodine in the presence of water in an amount sufficient to gel the collagen.
22. (New) The wound healing kit as claimed in claim 21, wherein the composition capable of generating iodine is comprised of oxidized iodine, a reducing agent and a pH buffer.

23. (New) The wound healing kit as claim d in claim 22, wherein the oxidized iodine is selected from the group consisting of potassium iodate and iodine pentoxide.

24. (New) The wound healing kit as claimed in claim 21, wherein the composition capable of generating iodine is comprised of an alkali metal iodide, an oxidizing agent and a pH buffer.

25. (New) The wound healing kit as claimed in claim 24, wherein the oxidizing agent is selected from the group consisting of persulfate, perborate, citric acid, hydrogen peroxide, tertiary butyl peroxide, an alkali metal periodate, a hypochlorite salt, hypochlorous acid and a halogen amine.

26. (New) A wound healing kit, comprising:
a first container having therein a composition comprised of water soluble, lyophilized, atelopeptide collagen and a pH buffer;
a second container having therein an alkali metal iodide and a oxidizing agent; and
a third container having therein an alkali metal iodate.

27. (New) The wound healing kit as claimed in claim 26, wherein the alkali metal iodide is potassium iodide.
28. (New) The wound healing kit as claimed in claim 26, wherein the alkali metal iodate is potassium iodate.

29. (New) The wound healing kit as claimed in claim 26, wherein the oxidizing agent is citric acid.

30. (New) A wound healing kit in the form of three interconnected departmental containers including a first container having therein a composition comprised of water soluble, lyophilized, atelopeptide collagen and a pH buffer; a second container having therein an alkali metal iodide and an oxidizing agent; and a third container having therein an alkali metal iodate; wherein the first container, second container and third container are separated from each other by breakable seals which seals when broken allow intermixing of any contents in the containers.--

REMARKS

Claims 21-30 are now pending in this application.

Original claims 1-20 were canceled from the application and new claims 21-30 have been added in order to more particularly point out and distinctly claim the invention.

Newly added claims 21-30 are believed to be fully supported within the original filed application. All of these claims are directed to wound healing kits as were previously claimed within now canceled claims 19-20. Claims 19-20 were canceled during the prosecution of the parent application in response to a restriction requirement. The parent application has been allowed and the issue fee was paid on October 1, 1991.

Support for the newly added claims 21-30 can be found within the originally filed application such as within now canceled claims 19-20 and in the specification on page 9, line 6-page 10, line 2 and on page 13, lines 13-25.
In that the presently pending claims are directed to the wound healing kit, the title of the invention has been amended.

Applicants respectfully request that the Examiner consider and make of record the prior art which was cited during the prosecution of the parent application.

This Preliminary Amendment is being filed concurrently with a transmittal letter/fee sheet. In the unlikely event that such is separated from this document and the Patent Office determines that fees are due, the Commissioner is authorized to charge such fees to our Deposit Account No. 03-1952.

Respectfully submitted,

By: Karl Bozicevic
Registration No. 28,807

Morrison & Foerster
545 Middlefield Road, Suite 200
Menlo Park, California 94025
Telephone: (415) 677-6159
Fax: (415) 327-2951
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