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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :		(11) International Publication Number:
A61L 27/00, C08H 1/06	A1	WO 97/32615
		(43) International Publication Date: 12 September 1997 (12.09.97)

(21) International Application Number: PCT/US97/03249

(22) International Filing Date: 3 March 1997 (03.03.97)

(30) Priority Data:
08/608,014 4 March 1996 (04.03.96) US

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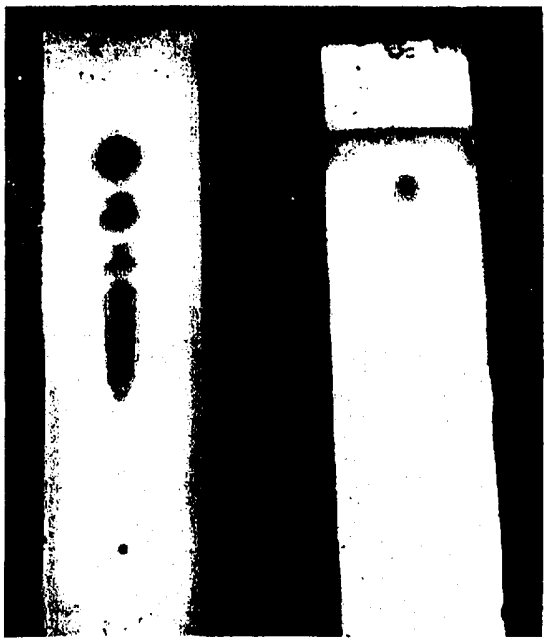
(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: NONPOLYMERIC EPOXY COMPOUNDS FOR CROSS-LINKING BIOLOGICAL TISSUE AND BIOPROSTHETIC GRAFTS PREPARED THEREBY

(57) Abstract

Nonpolymeric epoxy compounds for cross-linking biological tissues and bioprosthetic materials prepared thereby. The nonpolymeric epoxy compounds of the present invention have the general structural formula: R_1-CH_2-O-X-O-CH_2-R_2 wherein, X is either (a) a straight chain aliphatic hydrocarbon having at least four (4) and no more than five (5) carbon atoms bonded directly to one another, said straight chain aliphatic hydrocarbon being devoid of side branches and having terminal carbon atoms at either end therof, the terminal carbon atoms at the ends of said straight chain aliphatic hydrocarbon being bonded to the oxygen atoms shown in the foregoing general formula, (b) a substituted aromatic hydrocarbon, or (c) a substituted or unsubstituted cycloaliphatic hydrocarbon; and, wherein at least one of the terminal groups R_1, or R_2 is an epoxy group and the other of said terminal groups R_1 or R_2 is either (a) an epoxy group, (b) an aldehyde group, (c) an isocyanate group, or (d) a thiocyanate group. One preferred cross-linking agent of the above general formula is 1,4, butanediol diglycidyl ether. The cross-linking agents of the present invention are preferably utilized in the absence of other chemical compounds (e.g., congeners, molecular fragments, impurities) which would react with either collagen or the cross-linking agent of the present invention.



Denacol - 810 1.4 butanediol diglycidyl ether

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NONPOLYMERIC EPOXY COMPOUNDS FOR CROSS LINKING BIOLOGICAL TISSUE AND BIOPROSTHETIC GRAFTS PREPARED THEREBY

Field of the Invention

The present invention pertains generally to chemical fixatives which may be used to preserve biological tissue, and more particularly to a group of nonpolymeric difunctional epoxy compounds capable of cross linking biological tissues and the preserved bioprosthetic grafts which are prepared thereby.

Background of the Invention

i. Collagenous Biological Tissues Used For Prosthetic Grafting

Various tissues of biological origin have heretofore been used as prosthetic grafts for surgical implantation in or attachment to the body of a human being. As used herein, the term "graft" shall refer to any type of tissue or organ used for subsequent implantation or transplantation, including for example, certain cardiovascular tissues (e.g., segments of blood vessels, heart valves, pericardium), integumentary tissues (e.g., skin), tendons, or other tissues which have been harvested from human or other mammalian sources.

Prior to surgical implantation or transplantation of a graft of biological origin, the graft tissue is typically subjected to a chemical tanning or preservation treatment. The preserved tissue is then stored until it is needed for surgical implantation or grafting into the body of a human patient.

Biological tissues of the type used for allogenic or xenogeneic grafting in human beings (e.g., heart valves, pericardium, blood vessel, skin, etc...) typically contain a connective tissue matrix. Such connective tissue matrix acts as the supportive framework for the tissue. The cellular parenchyma of the living tissue is

disposed within, and supported by, such connective tissue framework. Collagen and elastin are two substances which make up the connective tissue framework of most biological tissues. The flexibility or rigidity of biological tissue is determined largely by the relative amounts of collagen and elastin present therewithin and/or the physical structure and configuration of the connective tissue framework.

Collagen is a naturally occurring substance which, on a molecular level, consists of three polypeptide chains intertwined in a coiled helical confirmation. The individual amino acid constituents of each polypeptide chain are connected, by way of carbon bonds, to the adjacent amino acids of a neighboring polypeptide chain. Such amino acid bonding serves to hold the polypeptide chains in the triple helical confirmation of the collagen molecule.

Collagenous biological tissues may be tanned or preserved for subsequent surgical grafting and/or implantation by a chemical "fixing" process wherein the collagen network of the graft tissue is exposed to one or more chemical cross linking agents capable of forming chemical cross linkages between the amine groups of the collagen molecules.

The chemical cross linkages formed by the fixative agent include both "intramolecular" and "intermolecular" cross linkages. Intramolecular cross linkages are formed between the amine groups on neighboring polypeptide chains within a particular collagen molecule, while intermolecular cross linkages are formed between amine groups located on different collagen molecules. In general, it is desirable to accomplish substantially complete intramolecular cross linking of collagen within a biological graft material, with only minimal formation of intermolecular cross linkages within such material. Indeed, a high intramolecular cross link density and low intermolecular cross link density is typically associated

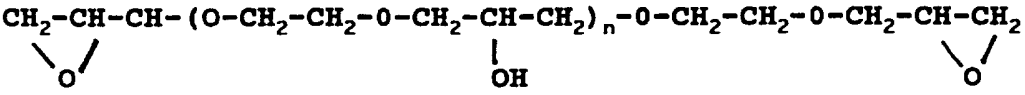
with the most desirable preservation and physical properties of the resultant biological graft.

ii. **Fixative Agents Used to Cross Link Collagenous Tissues**

Chemical compounds which are known to be useable as fixatives for cross linking collagen include formaldehyde, glutaraldehyde, dialdehyde starch, hexamethylene diisocyanate and certain polyepoxy compounds.

Polyepoxy compounds which have heretofore been known for use as collagen cross linking agents are described in United States Patent Nos. 4,806,959 (Noishiki et al.) and 5,080,670 (Imamura et al.). At least some of these heretofore-known polyepoxy fixatives are commercially available under the trademark Denacol™ from Nagase Chemicals, Ltd., Osaka, Japan. In particular, one difunctional epoxy compound which has been disclosed for use as a collagen cross linking agent is an ethylene glycol diglycidyl ether based compound commercially available from Nagase Chemicals, Ltd. of Osaka, Japan under the designation Denacol Ex-810. The chemical structure of Denacol Ex-810 is as follows:

Denacol Ex-810

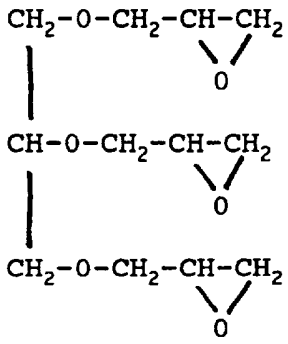


(Denacol Ex-810 is a mixture of congeners wherein n equals 0,1,2 and 3)

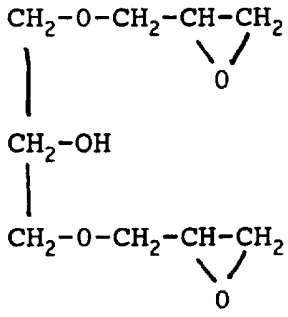
As noted, Denacol Ex-810 is actually a mixture of several molecular congeners, each of which has a different molecular weight based on the number (n) of repeating molecular subunits (represented in the above-shown structural formula) being equal to 0, 1, 2 and 3.

Other epoxy compounds which have been disclosed for use as collagen cross linking agents include those which are commercially available as Denacol Ex-313 and Dencacol Ex-314 from Nagase Chemicals, Ltd. of Osaka, Japan. Denacol Ex-313 and Ex-314 are specifically described in United States Patent No. 5,080,670 (Imamura et al.). Denacol Ex-313 and Denacol Ex-314 are blends of different relative amounts of the following molecular congeners (A-D) as follows:

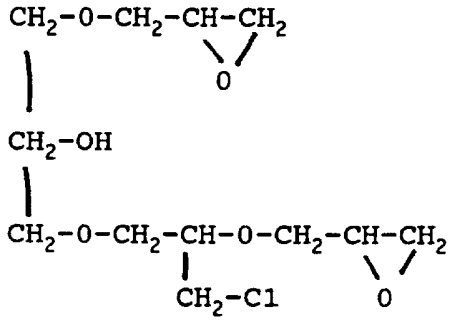
(A).



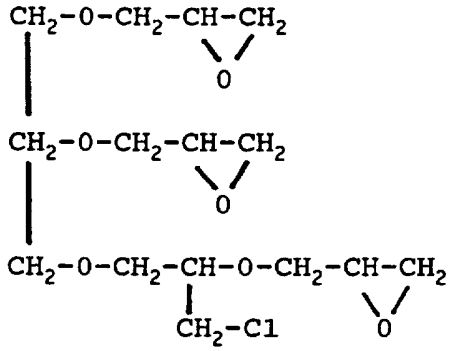
(B).



(C).



(D).



Because Denacol Ex-313 and Ex-314 contain different relative amount of these four (4) molecular congeners (A-D), the average molecular weights and epoxy functionalities of Denacol Ex-313 and Ex-314 differ. The published average molecular weight of Denacol Ex-313 is 270 and its published average epoxy functionality is 2.0. The published average molecular weight of Denacol Ex-314 is 320 and its published average epoxy functionality is 2.3.

In general, cross linking agents of low molecular weight cause relatively fast cross linking of collagen while cross linking agents of high molecular weight are relatively slow acting in this regard. Thus, at a given temperature and pressure, the cross link density or

number of cross linkages formed may be affected by both time of exposure of the fixative (i.e., cross linking agent) solution and the molecular weight (or molecular weight distribution) of the particular cross linking agent(s) being used. Additionally, the cross link density or number of cross linkages formed in the collagen network may be affected by other factors including a) the concentration of the cross linking agent in the fixative solution, b) the pH of the fixative solution, and c) any alteration or change in the physical conditions such as temperature and pressure.

One method of assessing the cross link density or relative number of cross linkages formed in a collagenous tissue is by a chemical assay known as the ninhydrin assay. The ninhydrin assay measures the number of unbound amine (NH_2) groups present in the collagenous tissue. Since the cross linking agents bind to the amine groups of the collagen molecules, the present number of unbound amine groups is directly indicative of the completeness of the cross linking which has occurred. In this regard, high ninhydrin assay values indicate a relatively incomplete state of cross linking of the collagenous tissue while lower ninhydrin assay values indicate relatively complete cross linking of the collagenous tissue.

iii. Problems and Limitations Associated With Cross Linked Collagenous Grafts

One drawback associated with chemically cross linked collagenous biograft materials is that residual chemical cross linking agent may remain within the graft and may adversely affect the biocompatability and/or tissue affinity of the graft material.

Prior investigators have attempted to deal with this problem by utilizing chemical neuteraliting agents which act to chemically neutralize or deactivate residual or unreacted cross linking agent which is present within the

graft. Examples of prior United States patents which describe methods whereby collagenous graft materials are treated with fixative deactivating or neutralizing chemical agents include U.S. Patent No. 3,974,526 (Dardik) entitled VASCULAR PROSTHESES AND PROCESS FOR PRODUCING THE SAME; U.S. Patent No. 3,988,782 (Dardik) entitled NON-ANTIGENIC, NON-THROMBOGENIC INFECTION-RESISTANT GRAFTS FROM UMBILICAL CORD VESSELS AND PROCESSES FOR PREPARING AND USING SAME and U.S. Patent No. 4,553,974 (Dewanjee) entitled TREATMENT OF COLLAGENOUS TISSUE WITH GLUTARALDEHYDE AND AMINODIPHOSPHONATE CALCIFICATION INHIBITOR.

In many applications, fixed biograft materials are grafted within the host body in a manner which results in direct contact between specific regions or portions of the graft and certain host tissues. Thus, noncompatability between the graft and the host tissue may give rise to problems with graft biocompatability or graft-host reactions. The amount of fixative chemical present in a particular region, portion or surface of a graft may affect the biocompatability of that portion or surface of the graft with the adjacent host tissue.

In many applications sufficient bio-affinity is required to enable the tissue graft to undergo endothelialization, (e.g., in situ endothelialization of a vascular graft by way of blood stream regeneration or in vitro endothelialization of a graft surface prior to its surgical implantation). Vascular grafts of biological origin are typically implanted to a host blood vessel by way of end-to-end anastomosis of such that blood will flow directly through the lumen of the graft.

Another problem associated with chemically cross linked collagenous grafts is that the chemical cross linking process may result in stiffening or rigidification of the graft tissue. Such stiffening or rigidification of the graft tissue can cause difficulty in subsequent handling of the tissue to form the desired

graft material and/or in the surgical implantation and anastomosis of the tissue to the recipient host.

Yet another problem associated with chemically cross linked collagenous grafts is calcification of the graft following implantation thereof. Such calcification of the implanted graft can be particularly problematic in bioprosthetic heart valve grafts, as such calcification may cause the valve leaflets to become rigid and incapable of performing their intended hemodynamic valving function.

In view of the forgoing problems associated with the chemically cross linked collagenous bioprosthetic graft materials, there remains a need in the art for the development of improved chemical fixative agents which do not cause unacceptable graft-host reactivity and/or which do not undergo unacceptable post-implantation calcification having and/or which do not cause unacceptable stiffening of the cross linked tissue.

Summary of the Invention

The present invention provides certain nonpolymeric epoxy compounds which are usable to cross link collagenous biological materials (e.g., heart valves, pericardium, blood vessels, skin, etc.).

In accordance with the invention, there are provided epoxy compounds for cross linking biological materials, said compounds having the general structural formula:

$$R_1\text{-}CH_2\text{-}O\text{-}X\text{-}O\text{-}CH_2\text{-}R_2$$

wherein, the molecular backbone X is either a) a straight chain aliphatic hydrocarbon having at least four (4) and no more than five (5) carbon atoms bonded directly to one another, said straight chain aliphatic hydrocarbon being devoid of side branches and having terminal carbon atoms at either end thereof, the terminal carbon atoms at the ends of said straight chain aliphatic hydrocarbon being bonded to the oxygen atoms shown in the foregoing general formula, b) a substituted aromatic hydrocarbon or c) a

substituted or unsubstituted cycloaliphatic hydrocarbon; and, wherein at least one of the terminal groups R_1, or R_2 is an epoxy group and the other of said terminal groups R_1 or R_2 is either a) an epoxy group, b) an aldehyde group, c) an isocyanate group, or d) a thiocyanate group.

Still further in accordance with the invention, there are provided methods for cross linking collagenous tissues wherein one or more compounds of the general formula described hereabove are dissolved in a liquid solvent, and a collagenous biograft material is then immersed emersed within, or otherwise placed in contact with, such solution for a sufficient period of time to cause the collagenous tissue to become cross linked to a desired cross link density. Examples of the types of collagenous tissues which may be utilized include heart valves, pericardium, blood vessels, tendons, skin, etc.

Still further in accordance with the invention, there are provided fixed biograft articles prepared in accordance with the foregoing method, such articles including but not necessarily limited to cross linked heart valves, pericardium, blood vessels, tendons, skin, etc.

Further objects and advantages of the present invention will become apparent to those skilled in the art upon reading and understanding of the following detailed description of preferred embodiments.

Brief Description of the Drawings

Figure 1 is a comparison of thin layer chromatographs of the 1,4-butanedoil diglycidyl ether compound of the present invention and Denacol™ 810.

Figure 2 is a gel permeation chromatogram comparing the 1,4-butanedoil diglycidyl ether of the present invention and Denacol™ 810.

Detailed Description of the Preferred Embodiments

The following detailed description, and the examples articulated therein, is provided for the purpose of

describing and illustrating presently preferred embodiments of the invention only, and is not intended to limit the scope of the invention in any way.

The compounds of the present invention have the general structural formula:

$$R_1-CH_2-O-X-O-CH_2-R_2$$

In accordance with this general structural formula, each compound of the present invention has a molecular backbone X, and two terminal groups R, and R_2.

The molecular backbone X preferably comprises either a) an aliphatic hydrocarbon consisting of a straight carbon chain having at least four (4) and no more than five (5) carbon atoms bonded directly to one another, said straight chain aliphatic hydrocarbon being devoid of side branches and having terminal carbon atoms at either end thereof, the terminal carbon atoms at the ends of said straight chain aliphatic hydrocarbon being bonded to the oxygen atoms of the foregoing general formula; b) a substituted aromatic hydrocarbon or, c) a substituted or unsubstituting cycloaliphatic hydrocarbon. Irrespective of the specific composition of the molecular backbone X, the opposite termini or ends of such molecular backbone X are preferably bonded directly to the oxygen atoms of the molecule, as shown in the above-set-forth general formula.

Examples of specific aliphatic hydrocarbons which consist of straight carbon chains of at least four (4) carbon atoms bonded directly to one another which may be utilized as the molecular backbone X include, but are not necessarily limited to, n-butyl ($-CH_2-CH_2-CH_2-CH_2-$) and n-pentyl ($-CH_2-CH_2-CH_2-CH_2-CH_2-$). It is preferable that this straight-chain aliphatic hydrocarbon be either 4 or 5 carbon atoms in length, so as to provide a molecule of optimal size for cross linking of collagen. It is further preferable that the straight carbon chain of the aliphatic hydrocarbon be devoid of any side branches

which would sterically or otherwise hinder or interfere with the collagen cross linking function of the molecule.

Examples of substituted aromatic hydrocarbons which may be utilized to form the molecular backbone X include, but are not necessarily limited to chlorophenyl, dichlorophenyl, fluorophenyl or difluorophenyl.

Examples of substituted and unsubstituted cycloaliphatic hydrocarbons which may be utilized to form the molecular backbone X include, but are not necessarily limited to, cyclohexane and chlorocyclohexane.

Preferably, the molecular backbone X of the nonpolymeric epoxy compounds of the present invention will be of a size which results in the functional groups R_1 and R_2 being spaced apart by a distance which results in intramolecular cross linking between collagen molecules.

At least one of the terminal groups R_1, R_2, is an epoxy group having the structure  .

The other of such terminal groups R_1, R_2, may be either:

a) an epoxy group having the structure  ;

b) and aldehyde group having the structure  ;

c) an isocyanate group having the structure -N=C=O; or,

d) a thiocyanate group having the structure -N=C=S.

It is further preferable that each collagen cross linking compound of the present invention having the above-set-forth general formula, be formulated in a preparation which consists essentially of that particular collagen crosslinking compound and which is substantially devoid of other congeners, molecular fragments, other chemical compounds or impurities which would affect the rate and/or completeness of collagen cross linking by

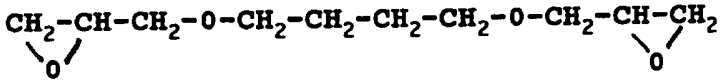
said agent either by reacting directly with the collagen or by reacting with the compound of the present invention.

In many applications, it will be preferable to select collagen crosslinking compounds of the present invention which are soluble in aqueous solution up to at least 4% (w/w) and preferably up to about 10% (w/w).

EXAMPLE I

(Preferred Collagen Cross linking Compound of the Present Invention)

The following presently preferred compound of the present invention is soluble in water and usable, in aqueous solution, as a fixative (i.e., cross linking) agent for collagenous biological materials:



(Chemical Name: 1,4-butanediol diglycidyl ether)

In accordance with this presently preferred compound, the molecular backbone X is an n-butyl group ($-CH_2-CH_2-CH_2-CH_2-$) and the terminal groups R_1, and R_2, are both epoxy groups

This preferred compound is prepared in a fixative solution which is devoid of any other amine-reactive compounds or congeners or fragments of the above-shown preferred compound having molecular weights or molecular structures which differ from that shown in the above chemical formula for this preferred compound.

This presently preferred compound of the present invention is soluble in water and may be prepared in aqueous solution for fixing of collagenous tissue by

immersion of the collagenous tissue within such access solution. The concentration of the 1,4-butanedoil diglycidyl ether within such access solution may differ depending of the intended application and/or the type of collagenous tissue to be cross linked. In many applications, concentrations of approximately 4% (w/w) will be usable.

The following are examples of methods by which the presently preferred compound of the present invention may be utilized to chemically cross link certain types of collagenous biomaterial, to thereby form preserved, surgical implantable bioprostheses.

Example II

(Preparation of a Pericardial Heart Valve Prosthesis)

A. Harvesting and Preparation of Pericardial Tissue

Bovine pericardial sacs are removed from donor animals and are cut into pericardial tissue segments of desired size and shape. Each pericardial tissue segment is thoroughly cleaned with sterile saline solution and any excess or surrounding connective tissue or fat is trimmed away.

B. Preparation of Test Solutions

Four (4) percent (w/w) aqueous solutions of 1,4-butanediol diglycidyl ether, Denacol EX 313 (Nagase Chemicals Ltd., Osaka, Japan) and Denacol EX 810 (Nagase Chemicals Ltd., Osaka, Japan are prepared, and each of these test solutions is placed in a separate container.

C. Fixing of the Pericardial Tissue

The segments of pericardial tissue are mounted on suitable tissue-holding fixtures to maintain their desired shape or orientation during the fixation process. The pericardial tissue segments, with their accompanying mounting fixtures, are then separated into groups, and each group of tissue segments is immersed in one of the test solutions (i.e., 1,4-butanediol diglycidyl ether,

Denacol Ex-313 or Denacol Ex-810). The test solutions are maintained at room temperature. Tissue segments are then removed from the Denacol Ex-810 bath after 72 and 144 hours of exposure, and form the 1,4-butanediol diglycidyl ether and Denacol Ex-313 baths after 24, 48 and 144 hours of exposure.

Following their removal from the test solution baths, the pericardial tissue segments are subjected to ninhydrin assays to determine the concentration of free amino groups present in each. The results of these ninhydrin assays are as follows:

TEST SOLUTION (4% w/w)	EXPOSURE TIME (Hrs. @ room temp.)	FREE AMINE GROUPS DETERMINED BY NINHYDRIN ASSAY (mole NH_2/mole collagen)
1,4-butanedoil diglycidyl ether	24 48 144	7.4 6.5 2.2
Denacol Ex-313	24 48 144	14.1 10.8 4.0
Denacol Ex-810	72 144	7.6 4.6

The results of these ninhydrin assays indicate that, after 144 hours of exposure, the completeness of collagen cross linking accomplished by the 1,4-butanediol diglycidyl ether of the present invention was significantly greater than that accomplished by either Denacol Ex-313 or Denacol Ex-810.

D. Fabrication of Pericardial Heart Valve Prostheses

The fixed segments of pericardial tissue are removed from their fixtures, dicut into heart valve leaflet shapes, and attached by way of sutures, to pericardial aortic valve stents, in accordance with known methodology for manufacturing of such bovine pericardial heart valves.

It has been subjectively noted that the pericardial tissue cross linked with the 1,4-butanediol diglycidyl ether compound of the present invention is easier to handle and more easily sutured to the valve stent than are the pericardial tissues which had been cross linked by either Denacol Ex-313 or Denacol Ex-810.

E. Storage of Pericardial Heart Valve Prostheses

The pericardial heart valve prostheses which incorporate leaflets formed from the tissue which had been fixed in the 1,4-butanediol diglycidyl ether of the present invention were subsequently stored by immersion in the 4% (w/w) aqueous 1,4-butanediol diglycidyl ether for periods of 1, 2 and 6 days. The shrinkage temperatures of these fixed pericardial tissue leaflets were determined after 1, 2 and 6 days of storage in the 1,4-butanediol diglycidyl ether solution. The shrinkage temperatures of these fixed pericardial tissue leaflets were 77° after 1 day of storage, 77°c after 2 days of storage and 76°c after 6 days of storage. These relatively stable shrinkage temperatures indicate that the properties of the fixed pericardial tissue have remained substantially unchanged during 6 days of storage in the 4% 1,4-butanediol diglycidyl ether solution of the present invention.

Example III

(Comparison of the 1,4-butanediol diglycidyl ether fixative of the Present Invention to Denacol Ex-810)

The preferred fixative of the present invention as described in Example I hereabove, is advantageous in that it has a simple molecular structure, which enables such compound to be synthesized in a highly pure form. Furthermore, the molecular weight, size, and reactivity of the 1,4-butanediol diglycidyl ether compound promotes rapid intramolecular cross linking of collagenous tissues in a manner which imparts desirable physical and chemical

properties to the cross linked tissue graft. Also, the 1,4-butanediol diglycidyl ether compound of the present invention is highly soluble in an aqueous environment, thereby avoiding the need for the addition of potentially toxic organic solvents to the fixative solution.

In this example, the 1,4-butanediol diglycidyl ether described in Example 1 hereabove and Denacol™ 810 (Nagase Chemicals, Ltd., Osaka, Japan) were subjected to thin layer chromatography using a Whatman K6 60Å Silica TLC Plate of 250μm thickness. A chloroform-methanol mixture (95%/5% v/v) was used as the carrier solvent and iodine vapor was used as the visualization reagent.

Figure 1 shows a comparison of the thin layer of chromatographs of these compounds, indicating that the 1,4-butanedoil diglycidyl ether compound of the present invention exhibits high resolution and purity compared to that of Denacol™ 810.

Also, in this example, the 1,4-butanedoil diglycidyl ether of Example I was compared to Denacol™ 810 (Nagase Chemicals, Ltd., Osaka, Japan) by gel permeation chromatography (GPC) (also known as size exclusion chromatography (SEC)). A Perkin Elmer 250 binary L.C. pump, equipped with a Hewlett Packard Series 1050 Autosampler was used in this example, with four (4) 30cm ultrastyragel columns, 500 Å, 100 Å & 50 Å connected in series to increase the resolution of the gel permeation chromatogram. Quantities of 1,4-butanedoil diglycidyl ether and Denacol™ 810 were dissolved in tetrahydrofuran (THF) to concentrations of 0.5%. The mobil phase was THF. The flow rate was 1.0ml/min. The injection volume was 100μl. The temperature of each column was controlled at 35°c during this experiment.

Figure 2 shows the GPC comparison scan of a) 1,4-butanedoil diglycidyl ether b) Denacol™ 810 and c) the solvent used. As shown, multiple peaks were present at various molecular weights with Denacol™ 810, while only a single peak was observed with 1,4-butanedoil diglycidyl

ether. This confirms that the 1,4-butanedoil diglycidyl ether preparation of the present invention consists substantially of a single chemical compound and is devoid of impurities, congeners and/or other chemical compounds which could react with collagen, or which could autoreact with the 1,4-butanedoil diglycidyl ether itself.

Those skilled in the art will appreciate that the present invention has been described hereabove with reference to certain presently preferred embodiments or examples only, and no effort has been made to exhaustively describe or list all possible embodiments in which the invention may be practiced. Indeed, various additions, deletions, modifications and alterations may be made to the above-described specific embodiments and examples without departing from the intended spirit and scope of the invention. Accordingly, it is intended that all such additions, deletions, modifications and alterations be included within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A compound for cross linking collagen, said compound having the formula:

$$R_1\text{-}CH_2\text{-}O\text{-}X\text{-}O\text{-}CH_2\text{-}R_2$$

wherein, the molecular backbone X is either a) an aliphatic hydrocarbon having a straight carbon chain consisting of at least four and no more than five carbon atoms bonded directly to one another said straight carbon chain being devoid of side branches and having, the terminal carbon atoms at the ends of said straight carbon chain being bonded to the oxygen atoms of said formula, b) a substituted aromatic hydrocarbon having, or c) a substituted or unsubstituted cycloaliphatic hydrocarbon; and,

wherein at least one of the terminal groups R_1, R_2 is an epoxy group and the other of the such terminal groups R_1, R_2 is either a) an epoxy group, b) an aldehyde group, c) an isocyanate group, or d) a thiocyanate group.

2. The compound of Claim 1 wherein X is a straight chain aliphatic hydrocarbon of four carbon atoms bonded directly to one another.

3. The compound of Claim 1 wherein X is a straight chain aliphatic hydrocarbon selected from the group consisting of:

n-butyl; or

n-pentyl.

4. The compound of Claim 1 wherein X is a substituted aromatic hydrocarbon selected from the group consisting of:

chlorophenyl;

dichlorophenyl;

fluorophenyl; and

diflurophenyl.

5. The compound of Claim 1 wherein X is a cycloaliphatic hydrocarbon selected from the group consisting of:

cyclohexane; and,

chlorocyclohexane.

6. The compound of Claim 1 wherein both R_1 and R_2 are epoxy groups.

7. The compound of Claim 1 wherein one of R_1 and R_2 is an epoxy group and the other thereof is an aldehyde group.

8. The compound of Claim 1 wherein one of R_1 and R_2 is an epoxy group and the other thereof is an isocyanate group.

9. The compound of Claim 1 wherein one of R_1 and R_2 is an epoxy group and the other thereof is a thiocyanate group.

10. The compound of Claim 1 wherein said compound consists essentially of said general formula and is substantially devoid of any other impurities, congeners of said compound and other chemical compounds which react with either collagen or said compound.

11. The compound of Claim 1 wherein said compound is soluble in aqueous solution at concentrations of at least 4% (w/w).

12. The compound of Claim 1 wherein said compound is soluble in aqueous solution at concentrations of at least 10% (w/w).

13. The compound of Claim 1, wherein said compound is 1,4-butanenediol diglycidyl ether.

14. A biological tissue graft comprising a collagen- containing tissue which has been cross linked by a compound having the general formula:

$$R_1\text{-}CH_2\text{-}O\text{-}X\text{-}O\text{-}CH_2\text{-}R_2$$

wherein, the molecular backbone X is either a) an aliphatic hydrocarbon having a straight carbon chain consisting of at least four and no more than five carbon atoms bonded directly to one another, said straight carbon chain being devoid of side branches and having terminal carbon atoms at either end thereof, the terminal carbon atoms at the ends of said straight carbon chain being bonded to the oxygen atoms shown in general

formula, b) a substituted aromatic hydrocarbon having, or c) a substituted or substituted cycloaliphatic hydrocarbon, d) a fluorocarbon; and,

wherein at least one of the terminal groups R_1, R_2 is an epoxy group and the other of the such terminal groups R_1, R_2 is either a) an epoxy group, b) an aldehyde group, c) an isocyanate group, or d) a thiocyanate group.

15. The biological tissue graft of Claim 14 wherein said collagen containing tissue comprises a mammalian heart valve.

16. The biological tissue graft of Claim 14 wherein said collagen containing tissue comprises a mammalian cardiovascular valve.

17. The biological tissue graft of Claim 14 wherein said collagen containing tissue comprises a segment of blood vessel.

18. The biological tissue graft of Claim 14 wherein said collagen containing tissue comprises a tendon.

19. The biological tissue graft of Claim 14 wherein said collagen containing tissue comprises skin.

20. The biological tissue graft of Claim 14 wherein said collagen containing tissue is cross linked by said compound in the substantial absence of any other impurities, congeners molecular fragments and other chemical compounds which would affect the collagen crosslinking created by said compound, through either direct reaction with collagen or said compound.

21. A method of cross linking collagen containing biological tissue, said method comprising the step of:

A. contacting said collagen-containing tissue with a compound having the general formula:

$$R_1\text{-}CH_2\text{-}O\text{-}X\text{-}O\text{-}CH_2\text{-}R_2$$

wherein, X is either a) an aliphatic hydrocarbon having a straight carbon chain consisting of at least four and no more than five carbon atoms bonded directly to one another, said straight carbon chain being devoid of side branches and having terminal carbon atoms at

either end thereof, the terminal carbon atoms at the ends of said straight carbon chain being bonded to the oxygen atoms shown in said general formula, b) a substitute aromatic hydrocarbon having, or c) a substituted or unsubstituted cycloaliphatic hydrocarbon; and,

wherein at least one of the terminal groups R_1, R_2 is an epoxy group and the other of the such terminal groups R_1, R_2 is either a) an epoxy group, b) an aldehyde group, c) an isocyanate group, or d) a thiocyanate group.

22. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein X is a straight chain aliphatic hydrocarbon of four carbon atoms bonded directly to one another.

23. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein X is a straight chain aliphatic hydrocarbon selected from the group consisting of:

n-butyl; or

n-pentyl.

24. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein X is a substituted aromatic hydrocarbon selected from the group consisting of:

chlorophenyl;

dichlorophenyl;

fluorophenyl; and

diflurophenyl.

25. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein X is a cycloaliphatic hydrocarbon selected from the group consisting of:

cyclohexane; and,

chlorocyclohexane.

26. The method of Claim 21 step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein both R_1 and R_2 are epoxy groups.

27. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein one of R_1 and R_2 is an epoxy group and the other thereof is an aldehyde group.

28. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein one of R_1 and R_2 is an epoxy group and the other thereof is an isocyanate group.

29. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein one of R_1 and R_2 is an epoxy group and the other thereof is a thiocyanate group.

30. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein said compound consists essentially of material having said general formula and is substantially devoid of any other amine reactive compound.

31. The method of Claim 21 wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein said compound is dissolved in aqueous solution at a concentration of 4% by weight.

32. The method of Claim 21, wherein step A comprises contacting said collagen-containing tissue with a compound of said general formula wherein said compound is 1,4-butanenedoil diglycidyl ether.

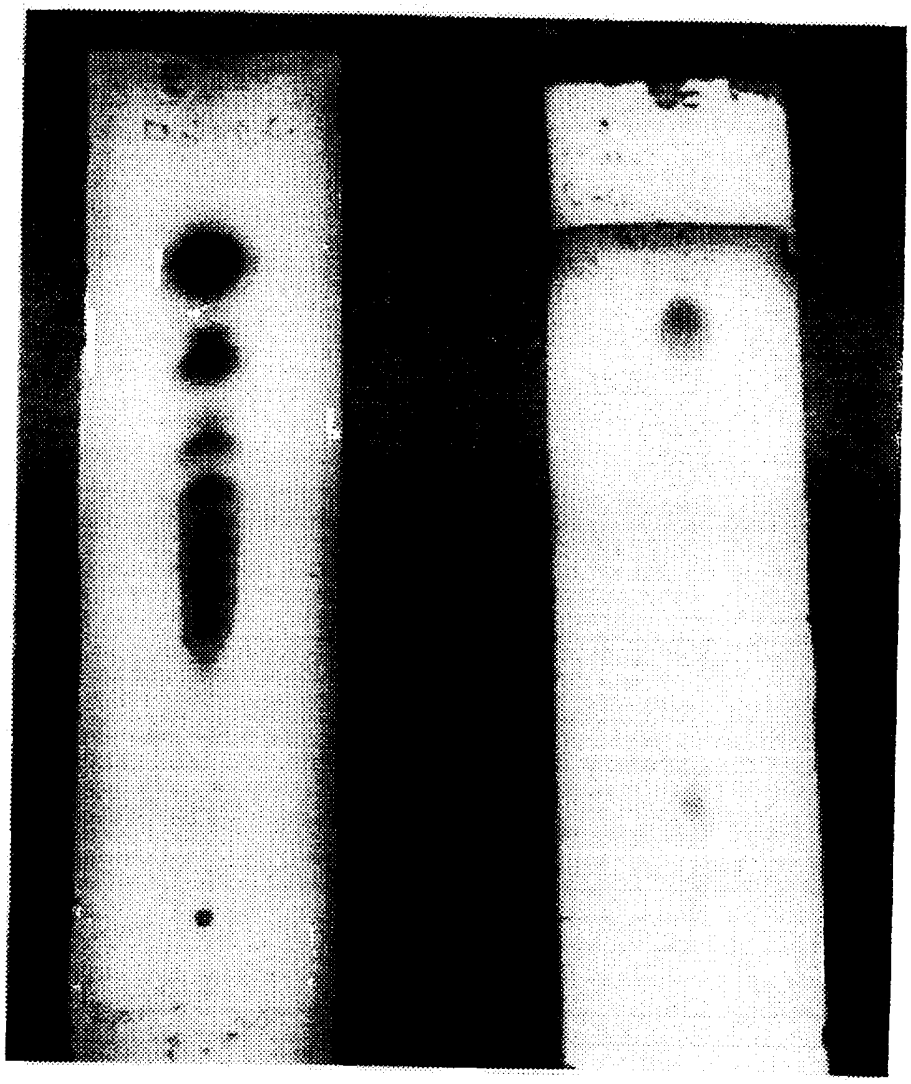
33. The method of Claim 21 wherein step A is carried out in the substantial absence of any other impurities, congeners, molecular fragments and other

chemical compounds which would affect the collagen crosslinking created by said compound through either direct reaction with collagen or through reaction with said compound.

34. A collagen crosslinking solution comprising a compound according to Claim 1 dissolved in a solvent, said solution being devoid of other impurities, congeners, molecular fragments and other chemical compounds which would affect the collagen crosslinking created by said compound through either direct reaction with collagen or through reaction with said compound.

35. The collagen crosslinking solution of Claim 33 wherein said solution consists essentially of said compound and said solvent.

36. The collagen crosslinking solution of Claim 33 wherein said solvent is water.



Denacol - 810

1.4 butanediol diglycidyl ether

FIG. 1

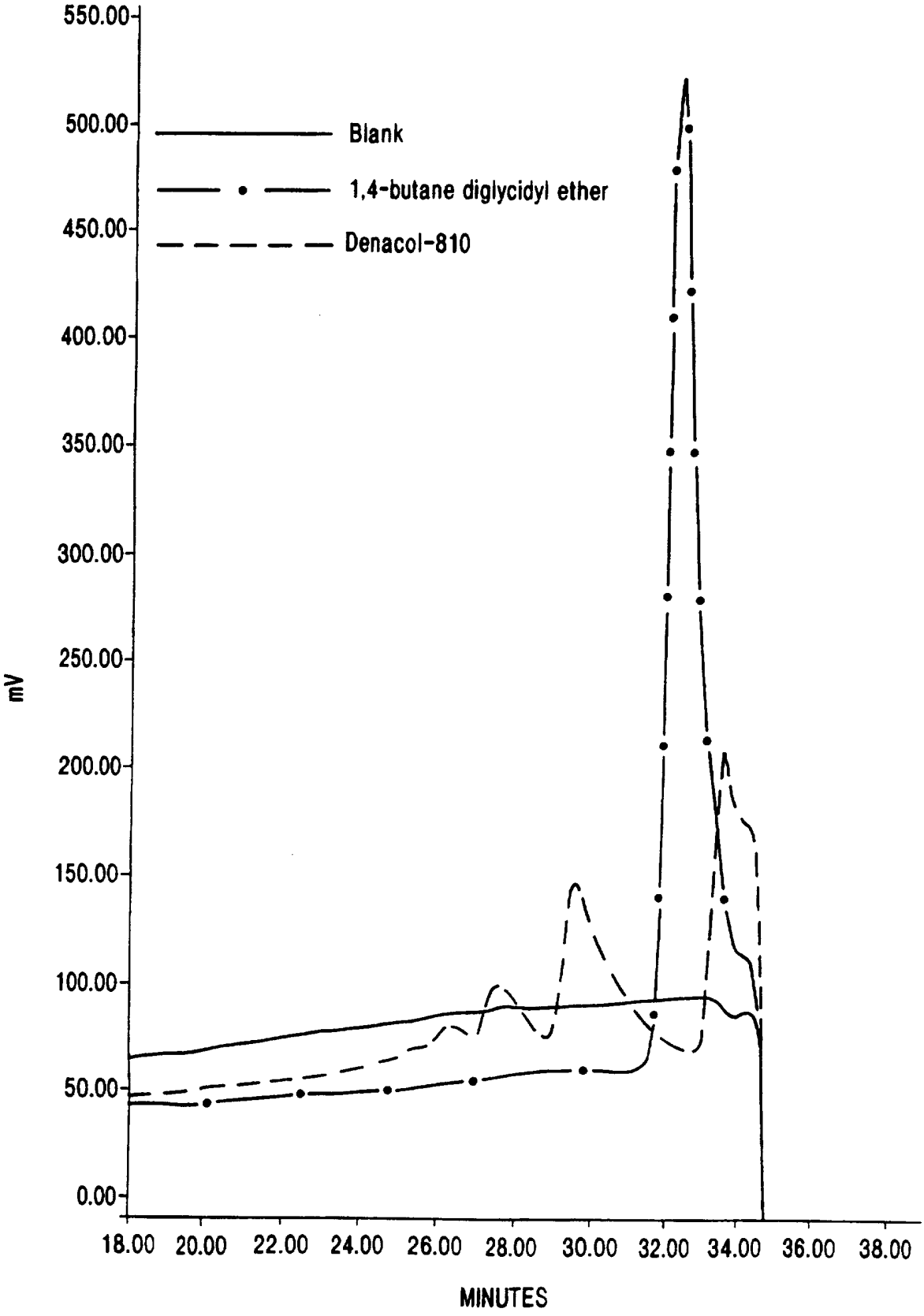

550.00
500.00
450.00
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
mV
Blank
1,4-butane diglycidyl ether
Denacol-810
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
38.00
MINUTES

FIG. 2

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/03249

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 C08H1/06

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 6 A61L C08H

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 practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	EP 0 097 907 A (FLOW GENERAL INC) 11 January 1984 see page 9, line 2 - page 9, line 19; claims 1-6,8; example 6 ---	1-3,6, 11-13 14,15, 17,19, 21-23, 26,32
Y	WO 94 17841 A (BAXTER INT) 18 August 1994 see abstract see examples 1,2 see claims 1-6,8-10,17 see page 7, line 1 - page 7, line 8 --- -/--	14,15, 17,19, 21-23, 26,32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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

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

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

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

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

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

"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
25 June 1997	0 8 -07- 1997

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/03249

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 336 964 A (TERUMO CORP) 18 October 1989 see abstract see page 2, line 23 - page 3, line 18 see page 6, line 18 - page 7, line 9 -----	1-3,6, 11-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]onal Application No
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