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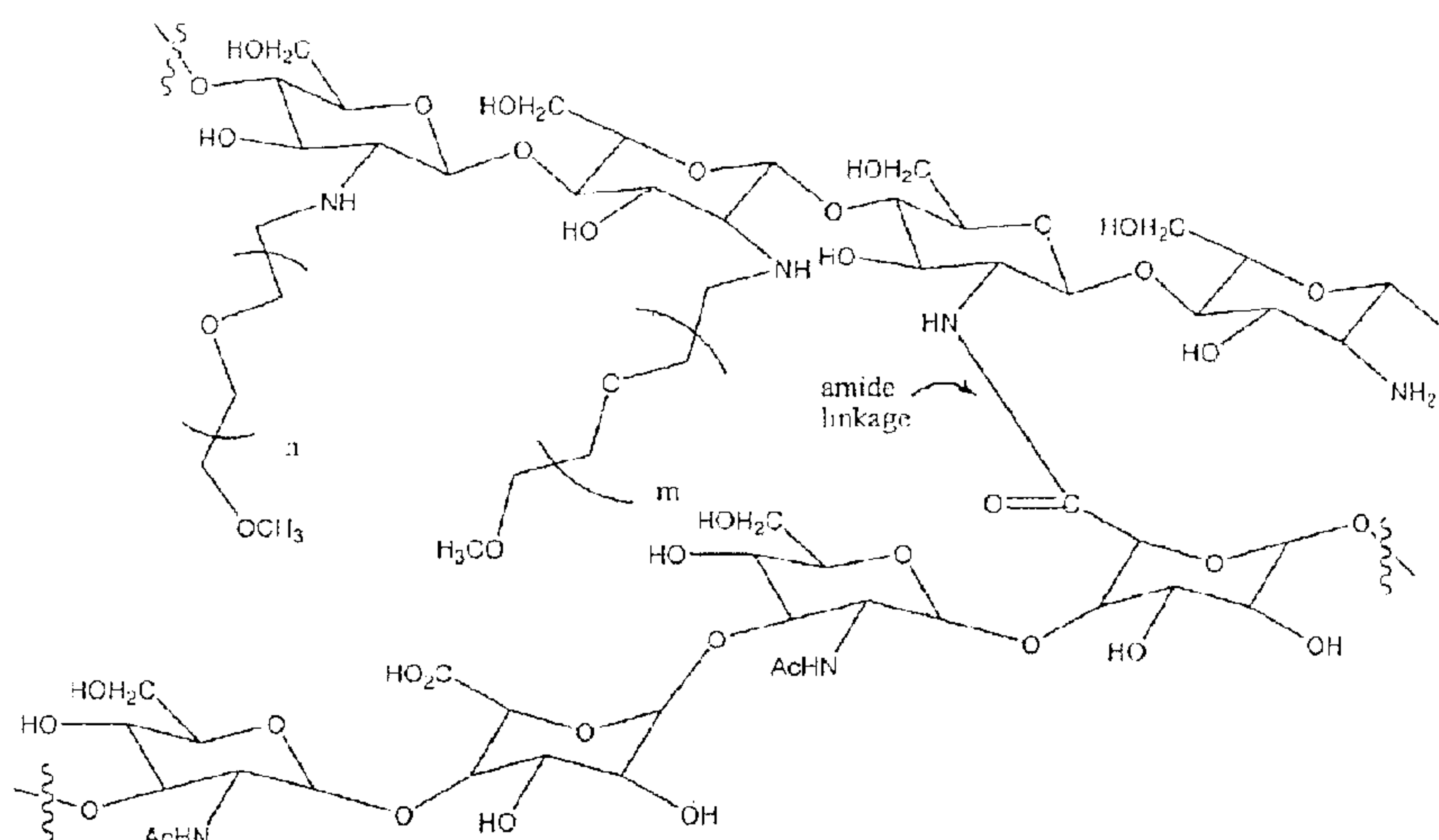
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(54) **Titre : SYSTEME DE BIOPOLYMERES POUR REPARER DES TISSUS**

(54) **Title: BIOPOLYMER SYSTEM FOR TISSUE SEALING**



(57) **Abrégé/Abstract:**

A tissue sealant for use in surgical and medical procedures for sealing the tissues of a living mammal is provided. The tissue sealant comprises a hydrogel which is formed by gelation of a premix disposed on the tissue to be sealed. The premix comprises an alkylated chitosan or a gelatin, and a polybasic carboxylic acid or an oxidized polysaccharide or both, in an aqueous medium. The premix can also include a dehydrating reagent, a carboxyl activating reagent, or both. A specific use of the tissue sealant is in the repair of the dura mater after brain surgery to prevent leakage of cerebrospinal fluid. The tissue sealant may include a therapeutic or protective agent such as an antibiotic or an anti-inflammatory drug.



ABSTRACT

A tissue sealant for use in surgical and medical procedures for sealing the tissues of a living mammal is provided. The tissue sealant comprises a hydrogel which is formed by gelation of a premix disposed on the tissue to be sealed. The premix comprises an alkylated chitosan or a gelatin, and a polybasic carboxylic acid or an oxidized polysaccharide or both, in an aqueous medium. The premix can also include a dehydrating reagent, a carboxyl activating reagent, or both. A specific use of the tissue sealant is in the repair of the dura mater after brain surgery to prevent leakage of cerebrospinal fluid. The tissue sealant may include a therapeutic or protective agent such as an antibiotic or an anti-inflammatory drug.

BIOPOLYMER SYSTEM FOR TISSUE SEALING

This application is a division of application number 2,808,235, filed in Canada on March 4, 2013 which is a divisional of application number 2,686,132, filed in Canada on October 16, 2009 (International Filing Date: February 19, 2007, PCT/US2007/062393).

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Field of the Invention

The invention relates to tissue sealants for medical or veterinary use, methods of preparing the sealants, and methods of using them in medical procedures.

15

Background of the Invention

Tissue sealants are increasingly important adjuncts in surgical procedures, being used in fields such as vascular surgery, cardiac surgery, spine surgery and brain surgery as well as in general surgery. Uses for tissue sealants include, among others, augmenting or replacing sutures to join tissues or place
 20 them in proximity, closing perforations in biological membranes to prevent leakage of fluids, incorporating medicinal substances at the location of emplacement for localized release, and filling areas of tissue removal. One commonly used tissue sealant is fibrin glue, a material analogous to clotted blood, which is obtained from reaction of fibrinogen and thrombin isolated from
 25 blood plasma. For example, see "Fibrin Glue from Stored Human Plasma: An Inexpensive and Efficient Method for Local Blood Bank Preparation," William D. Spotnitz, M.D., Paul D. Mintz, M.D., Nancy Avery, M.T., Thomas C. Bithell, M.D., Sanjiv Kaul, M.D., Stanton P. Nolan, M.D. (1987), The American Surgeon, 53, 460-62. However, concern about possible viral or prion
 30 contamination of human blood-derived protein products, and dissatisfaction with the relatively long times often required for fibrin gelation or "setting" to occur, have resulted in a search for tissue sealants with more advantageous properties.

There have been systems developed that use fibrin glues as part of a more complex assembly with more favorable properties. U.S. Patent No. 6,699,484 discusses the use of fibrinogen in mixtures with polysaccharides such as hyaluronan and chitosan to form surgical adhesives, wherein the fibrinogen and thrombin components are distributed in dry form on a support comprising the polysaccharide, which is activated by water when emplaced on a wound to form a sealant.

In an attempt to avoid the use of human blood products, other mammalian sources of proteins have been studied. A tissue sealant has been prepared using bovine serum albumin that is crosslinked with glutaraldehyde. An example is BioGlue Surgical Adhesive® produced by CryoLife, Inc. of Kennesaw, Georgia. However, bovine tissues are also a source of concern in terms of the possible presence of pathogenic entities such as viruses or prions. The types of processing required to destroy viruses or prions also tend to denature the desired proteins and make them intractable as sealants.

A tissue sealant that does not use proteins isolated from mammalian blood, such as Duraseal® produced by Confluent Surgical Inc. of Waltham, MA, comprises tri-lysine-amine and an activated polyethyleneglycol. A similar product, termed CoSeal® and produced by Baxter of Deerfield, IL, is likewise composed of synthetic functionalized polyethyleneglycol derivatives, also avoiding the use of blood-derived materials. However, both of these synthetic hydrogels are dimensionally unstable in the presence of water, undergoing considerable swelling. For example, see "Evaluation of Absorbable Surgical Sealants: In vitro Testing," Patrick K. Campbell, PhD, Steven L. Bennett, PhD, Art Driscoll, and Amar S. Sawhney, PhD, at www.duralsealant.com/duralsealant/literature.htm (as of Aug. 24, 2006). This tendency to swell can be highly disadvantageous in certain applications, such as neurosurgery, where the resulting pressure on nerve or brain tissue can produce serious side-effects.

Chitin, a biopolymer that is abundant in the shells of arthropods, is a β -1,4 polymer of 2-acetamido-2-deoxyglucose. During its isolation, it is freed from proteinaceous and mineral components of the shell. Purified chitin can be further processed by chemical treatment resulting in deacetylation to yield chitosan, (poly-(2-amino-2-deoxyglucose)), which is a basic (alkaline) substance

due to its free amino groups. From the perspective of medical uses, chitosan offers several desirable properties. The material is known to be non-toxic and biocompatible, and since chitin is not derived from vertebrates and is processed under rather harsh conditions such as exposure to alkali during its

5 transformation into chitosan, the possibility of contamination with viruses or prions that are pathogenic to mammals is very low. The utility of biocompatible chitosan derivatives in medical applications has received attention. For example, U.S. Patent No. 5,093,319 discusses the use of films prepared from carboxymethylated chitosan for use in surgery to prevent post-operative
10 adhesion of injured soft tissues upon healing. The chitosan derivatives are described to be formed into a biodegradable "sheet" that during surgery is emplaced between soft tissues for which adherence during healing is not desired. In another type of use, U.S. Patent No. 4,532,134 discusses the use of chitosan in promoting blood coagulation in wounds.

15 Hydrogels are gels in which water is the dispersion medium. A common example of a hydrogel is a gel formed from the protein gelatin in water. Other hydrogels are formed by polysaccharides such as agar dispersed in water. Hydrogels in the form of sheets are used as wound dressings, where they are favored for their ability to help maintain a moist environment to facilitate healing
20 of the wound without drying and cracking of tissues. For example, see www.medicaledu.com/hydrogellsheet.htm. Chemical derivatives of chitosan have also been used to form hydrogels for use as surgical sealants and in drug delivery devices. U.S. Patent No. 6,602,952, assigned to Shearwater Corp., describes the preparation of poly(alkyleneoxide)chitosan derivatives and their use in the
25 formation of hydrogels. The addition of these hydrophilic but non-ionic groups to the chitosan molecule alters its physical properties. Poly(alkyleneoxides) such as poly(ethyleneoxide), also known (somewhat inaccurately) as poly-ethyleneglycols or PEGs, are formed by the polymerization of alkylene oxides (epoxides) such as ethylene oxide. They may be obtained in a wide variety of molecular weights,
30 with various structural features such as activated end groups, hydrolysable linkages, and others. For example, see the Nektar PEG catalog that lists a wide variety of the Shearwater functionalized PEGs, at www.nektar.com/pdf/nektar_catalog.pdf (as of Aug. 24, 2006).

Other methods have been described for the preparation of hydrogels from chitosan. The published PCT application WO2005/113608 and the published U.S. patent application no. 2005/0271729, both by the same inventor, discuss the crosslinking of chitosan and hyaluronan, also known as hyaluronic acid.

5 Hyaluronan is an acidic linear polysaccharide formed of β -1,3 linked dimeric units, the dimeric units consisting of an 2-acetamido-2-deoxyglucose and D-gluconic acid linked in a β -1,4 configuration. These published applications discuss crosslinking the two types of polysaccharides using a carbodiimide reagent.

10 Hydrogels comprising chitosan derivatives and polybasic carboxylic acids or oxidized polysaccharides, for use in vascular occlusion, are also disclosed in copending U.S. Patent Publication No. 2007/0031467, filed February 8, 2007, by the same inventors as in the present application.

15 There is an ongoing need for a hydrogel tissue sealant that is not blood or animal protein derived, that consists of biocompatible materials, is dimensionally stable after emplacement in the patient's body, has good sealant and tissue adhesive properties, is of sufficient strength and elasticity to effectively seal biological tissues, that can be readily prepared and used during surgery, and that forms the tissue seal on a timescale compatible with surgery on living patients.

20

Summary of the Invention

The present invention provides a tissue sealant for medical or veterinary use in repair of physical damage to living mammalian tissues such as cuts, tears, holes, bone breaks and other unintentional injury.

25 The invention further provides a tissue sealant for medical or veterinary use in repair of physical damage resulting from surgical procedures, such as in closing a suture line, reinforcing a suture line, tissue approximation using the sealant instead of a suture, filling a disused dead space or void, or sealing a vascular defect.

30 The invention further provides a tissue sealant useful in medical procedures such as in preventing adhesions such as post-surgical adhesions, as a mechanism of drug delivery, or in coating implanted medical devices.

The invention further provides a tissue sealant that is well-suited for the repair and sealing of membranous biological tissues, in particular the dura mater and other membranes surrounding neural tissue.

The invention further provides a tissue sealant that due to its exceptional
5 dimensional stability may be used in situations where swelling and the resulting pressure are undesirable and produce unwanted side effects.

The invention further provides a tissue sealant that offers a very low risk of contamination by pathogens such as viruses and prions.

The invention further provides a tissue sealant that is not prepared from
10 human blood products, which is desirable because human blood products carry a risk of contamination with pathogens and are also objectionable to certain patients on religious and moral grounds.

The invention further provides a premix, a flowable, substantially liquid sol, that on standing forms a hydrogel that seals biological tissues, preferably
15 adhering to the tissues.

A composition of the invention can comprise a chitosan derivative that has been modified by the introduction of covalently bound moieties onto the polymer chain. The chitosan derivative, and a polybasic carboxylic acid or an oxidized polysaccharide or both, upon dissolution in an aqueous medium can
20 initially form a flowable, substantially liquid sol, a premix, that over a period of time, typically in the order of minutes, gels to form a hydrogel of the invention. The hydrogel, which is biocompatible and can be biodegradable, when formed serves to seal tissues that have been cut or torn, for example from a surgical procedure or from an injury. The hydrogel can adhesively seal tissues, holding
25 the tissues with sufficient strength to hold the tissues in the relative positions in which they are initially sealed, either together or separated.

The invention further provides an embodiment of a premix comprising a poly(oxyalkylene)chitosan and a hyaluronan. In another embodiment according to the present invention, the premix comprises an acrylated chitosan and a
30 dibasic carboxylic acid.

The invention further provides a premix for a hydrogel tissue sealant comprising an alkylated chitosan, a polybasic carboxylic acid, a carboxylic acid activating reagent, a dehydrating reagent, and an aqueous medium. The carboxyl activating reagent can be an N-hydroxy compound that can form an

ester with the carboxyl group, preferably N-hydroxysuccinimide (NHS) or N-hydroxybenztriazole. The dehydrating reagent can be a carbodiimide that removes the elements of water from reactants by thermodynamically driving the reaction, through formation of a urea compound. In one embodiment, the
5 specific carbodiimide is 1-ethyl-3-(N,N-dimethylpropyl)carbodiimide (EDCI).

The invention also provides an embodiment of a flowable aqueous sol comprising a gelatin and a polybasic carboxylic acid or an oxidized polysaccharide or both. A specific example is a composition comprising gelatin and oxidized hyaluronan.

10 The invention further provides methods for preparing the tissue sealants as are described herein for medical or veterinary use. The tissue sealant comprises a hydrogel that preferably adheres to the biological tissue of a living mammal. A specific method of preparation of a tissue sealant comprising a hydrogel according to the present invention is through combination of an
15 alkylated chitosan, and a polybasic carboxylic acid or an oxidized polysaccharide or both in an aqueous medium. Optionally, a dehydrating reagent, a carboxyl-activating reagent, or both, may be included.

The invention further provides methods for using a hydrogel according to the present invention in tissue repair and other medical procedures. A specific
20 embodiment of a hydrogel according to the present invention is used to reinforce a suture line, or to seal cut, torn, or perforated tissues. It is also used to prevent leakage of biological fluids, such as cerebrospinal fluid, through repair of biological membranes that when intact contain the fluids. It can be used to bring tissues into approximation and hold them in place after a surgical procedure has
25 been carried out, to fill a tissue void, or to act as a barrier to prevent tissue adhesion.

In another specific embodiment of a use of a hydrogel according to the present invention, the hydrogel may further comprise a protective or therapeutic material or substance. The substance may be an antibiotic, an anticancer agent, a
30 peptide, a protein, a nucleic acid or a nucleic acid analog, a radioactive material, or another protective or therapeutic substance where it is advantageous to provide the substance at the location within the body where the hydrogel is emplaced.

For example, the protein may be a growth factor, such as a vascular growth factor or a factor that induces a particular kind of tissue growth, such as bone morphogenic factor. In another specific embodiment, the protein may be an inhibitory factor, such as a receptor antagonist such as for a growth factor,
 5 when supply of an inhibitory factor is desirable, for example after removal of a tumor or cancerous tissue.

In yet another specific embodiment, the nucleic acid may be an antisense nucleic acid, or a small interfering nucleic acid analog, wherein it is advantageous to securely emplace the material at a particular site within a living
 10 mammal undergoing treatment for a condition responsive to such therapy.

In another specific embodiment, the therapeutic agent may be an antibiotic to inhibit bacterial infection after repair of a wound or after damage to tissues caused by surgery. Or, a protective agent may be an anti-inflammatory substance wherein it is advantageous to supply the substance directly at the site
 15 of damage that is repaired with the tissue sealant, such as to reduce swelling and resulting pressure on surrounding tissues.

In another specific embodiment, the hydrogel comprises a dye, such as a visible dye or a radio-opaque dye, to enable visualization of the position of localization of the hydrogel in the body.

20 In another specific embodiment, the hydrogel comprises a microsphere or a nanosphere, preferably a large number of microspheres or nanospheres dispersed in the hydrogel. Preferably the microsphere or nanosphere contains a therapeutic agent or a protective agent.

The invention further provides a kit adapted for preparing and using the
 25 tissue sealant of the invention, the kit comprising a first container and a second container, wherein the first container comprises an alkylated chitosan or a gelatin, the first container further comprising an aqueous medium or being adapted for addition thereto of an aqueous medium; and the second container comprising a polybasic carboxylic acid, an oxidized polysaccharide, or both, the
 30 second container further comprising an aqueous medium or being adapted for addition thereto of an aqueous medium. The kit can contain instructional material. The kit can also contain a mixing apparatus for the contents of the two containers, such as a pair of coupled syringes wherein the alkylated chitosan or gelatin and the polybasic carboxylic acid, the oxidized polysaccharide, or both,

can be mixed in an aqueous medium to prepare a premix suitable for
emplacement on tissue to form the hydrogel tissue sealant of the invention.

Thus, the hydrogel of the present invention, and the use of the hydrogel
as a tissue sealant, which can further contain therapeutic or protective agents,
5 offers outstanding advantages of ease of use, biocompatibility and
biodegradability, suitability for use in conjunction with other surgical
procedures, strength, adhesivity, and versatility.

In one aspect, there is provided a biocompatible, biodegradable
composition for use in sealing a biological tissue of a living mammal, the
10 composition comprising a hydrogel produced by mixing a first solution
comprising an acrylated chitosan with a second solution comprising oxidized
dextran to produce a mixture, in an aqueous medium, comprising from about 1%
to about 10% by weight of the acrylated chitosan that forms the hydrogel, wherein
the acrylated chitosan has a degree of substitution of a chitosan backbone with
15 acrylate groups in the range of from about 0.25 to about 0.5, and wherein the
mixture, when applied to a biological tissue in need of sealing, forms a hydrogel
within a minute at around 37°C.

In another aspect, there is provided use of a premix comprising an
acrylated chitosan and an oxidized dextran in an aqueous medium for the
20 manufacture of a medicament for forming a hydrogel for sealing biological tissue,
wherein the hydrogel is formed by gelation of the premix.

In another aspect, there is provided use of the composition as described
herein for sealing a biological tissue of a living mammal.

In another aspect, there is provided use of the hydrogel as described herein
25 for sealing a biological tissue of a living mammal.

In another aspect, there is provided use of the wound closure device as
described herein for sealing a biological tissue of a living mammal.

10

Brief Description of the Drawings

Figure 1 shows the chemical structure of a segment of a PEG-chitosan molecule wherein the degree of substitution with the PEG group is 0.5.

15

Figure 2 shows the chemical structure of a segment of a hyaluronan molecule.

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Figure 3 shows the chemical structure of a segment of the PEG-chitosan of Figure 1 and a segment of the hyaluronan of Figure 2 crosslinked by amide linkages between a hyaluronan carboxylate moiety and a PEG-chitosan amino moiety.

Figure 4 shows a segment of an acrylated chitosan polymer.

25

Figure 5 shows a segment of an acrylated chitosan polymer crosslinked by amide linkages formed with a adipic acid.

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Figure 6 shows a segment of an acrylated chitosan polymer crosslinked with an oxidized polysaccharide by imine and hemiaminal linkages.

Detailed Description of the Invention

Definitions

5 As used herein, “tissue” refers to the material forming the solid or semi-solid structures that make up any of the organs or components of a living organism. Thus, liquids such as blood are not “tissue” according to the definition used herein, but the term “tissue” encompasses membranes, skin, muscles, bones, cartilage, nerves and nerve sheathes, meninges, connective
10 tissue, blood vessels, the sclera or iris of the eye, the solid materials constituting internal organs such as liver, stomach, pancreas, intestine, kidney, thymus, uterus, testes, bladder, lung, heart and any other internal structures that are solid or semi-solid in texture.

 As used herein, the term “to seal” or “sealing” refers to the act wherein
15 two physically noncontiguous tissues or portions thereof are joined together with a sealant material, or “sealant,” where a hole, tear, cut, perforation or other discontinuity is repaired with the material so as to close the hole, tear, cut or perforation. A sealant can also hold adjacent tissue masses in close proximity such that they are in physical contact with each other, for example when it
20 desired for the adjacent tissue masses to heal together. Sealing implies at least some degree of adhesion of the material used to the tissue to which it is applied, such that the sealed tissue is secured against at least a moderate displacing force. The discontinuity in the tissue that is being sealed may be an incision made as part of a surgical procedure, or it may be a wound. The sealant adheres, at least
25 to some degree, to the tissue which is being sealed, such that the sealant material is unlikely in the short term to detach from the repaired or sealed tissue under the influence of at least a moderate force, such as may be experienced when a patient to whom the sealant has been applied moves in a normal fashion. The sealant may strongly adhere to the tissue. The sealant may be biodegradable and
30 eventually dissolve or be absorbed into the patient’s body without departing from the principles of the invention. The sealant can be used for filling a tissue void, for example, where a hydrogel mass is formed within the void and adherence of the hydrogel to the surrounding tissues prevents movement of the hydrogel out of the tissue void. The sealant can also be used for holding two

adjacent tissue masses apart such that tissue adhesion does not take place during healing. For example, if it is desired to prevent two nearby tissue masses that are healing from an incision from rejoining during healing, the hydrogel can seal the tissues such that they are prevented from touching or forming tissue connecting
 5 the two masses.

The degree of force that is needed to disrupt a seal formed according to the invention may vary. If the tissue is “sealed”, as the term is used herein, the degree of adhesivity may be relatively low, such that the sealant serves to fill a void in the tissue or to keep the tissue portions in conjunction when they are not
 10 subject to a high degree of strain. If a tissue is “adhesively sealed,” as the term is used herein, a higher degree of strain is necessary to disrupt the bond between the tissue portions, such that rupture of the seal only takes place when a relatively high degree of force is applied.

Thus, a tissue may be sealed such that the joined tissue portions are held
 15 in proximity by a sealant but application of a relatively high degree of strain would tend to separate the portions and rupture the seal, or the tissue may be adhesively sealed such that a relatively high degree of strain is needed to rupture the seal.

“Adhere” or “adherence” refers to the creation of a physical bond
 20 between the material and tissue such that a moderate motion or force does not cause separation of the material from the tissue on which it is disposed. Thus, a tissue sealant serves to glue together living tissue, at least temporarily, such as for the amount of time it takes healing to occur. However, sealing may take place for a more prolonged period. The physical bond that is created between
 25 the material and the tissue that is being sealed may have one or several bases including electrostatic bonding and covalent bonding, but any mechanism by which the adherence takes place falls within the definition herein.

The terms “adhesive” and “adhesivity” similarly refer to the existence of a physical bond between two materials such as a tissue sealant and the tissue to
 30 which the sealant is applied. An adhesive is a material which adheres to tissue or other material and which may be used to constrain the separation of two tissue masses. Adhesivity is the property or degree to which a material adheres to a tissue or other material. As used herein, adhesive tissue sealants are those

sealants of the invention that are adapted to hold the tissue portions being sealed together against a relatively high degree of rupturing strain.

As used herein, a “hydrogel” refers to a material of solid or semi-solid texture that comprises water. Hydrogels are formed by a three-dimensional network of molecular structures within which water, among other substances, may be held. The three-dimensional molecular network may be held together by covalent chemical bonds, or by ionic bonds, or by any combination thereof. A common example of a hydrogel is gelatin, a protein, that “sets up” or forms a gel from a sol upon heating and subsequent cooling. Not all substances that form hydrogels are proteins; polysaccharides such as starches may also form hydrogels. Still other hydrogels may be formed through the mixture of two or more materials that undergo chemical reactions with each other to create the three-dimensional molecular network that provides the hydrogel with a degree of dimensional stability. Such mixtures of materials that interact or react with each other to form a hydrogel are referred to herein as a “premix.” Thus, a “premix” as used herein refers to a mixture of materials that after mixing will gel, or “set up,” to form the hydrogel. A premix may be of a liquid or semi-liquid texture such that it can be pumped or transferred by the methods usually used for liquids, such as flow through tubes.

The act of “gelation” refers to the formation of a gel from a sol. In some cases, the sol may consist of a single material dispersed in a solvent, typically water, as in the case of gelatin. In other cases, the sol may consist of more than a single material dispersed in a solvent wherein the several materials will eventually react with each other to form a gel, and when the solvent in which they are dispersed comprises water, the gel is a hydrogel. The hydrogels claimed herein are of the type that are formed by the mixture of more than a single component.

A “saccharide” as used herein refers to a carbohydrate. The term “carbohydrate” includes the class of compounds commonly known as sugars, in addition to compounds that are chemically related to sugars. The term thus includes simple “monosaccharide” sugars, “disaccharide” sugars as well as polymeric “polysaccharides.”. The term encompasses a group of compounds including sugars, starches, gums, cellulose and hemicelluloses. The term further encompasses sugar derivatives such as amino-sugars, for example, 2-amino-2-

deoxyglucose, as well as their oligomers and polymers; sulfated sugars; and sugars with hydroxyl, amino, carboxyl and other groups.

A carbohydrate as defined herein comprises sugars or sugar derivatives with beta (β) or alpha (α) anomeric stereochemistry; moreover, the sugars can have (R) or (S) absolute configurations, can exist as the (+) or (-) isomer, and can exist in the D or L configuration. The terms “anomer” and “anomeric” refer to the stereochemical configuration at the acetal, hemiacetal, or ketal carbon atom, as is well known in the art.

As used herein, “chitosan” refers to a polysaccharide polymer, either obtained from a natural source such as chitin, or synthetically prepared. Chemically, chitosan is predominantly a polymer of β -1,4-linked 2-amino-2-deoxyglucose monomers. When prepared from a natural source, the usual natural source is chitin, a major constituent of the shells of crabs, shrimp and other arthropods. Chitin is chemically a polymer comprising β -1,4-linked 2-acetamino-2-deoxyglucose monomers. After isolation of chitin from its natural source, it is treated in a manner as to cause hydrolysis of the acetamido group without cleavage of the sugar-sugar bonds, typically through alkaline hydrolysis. A sample of chitosan is typically not composed of a single molecular entity, but of a set of polymeric chains of various lengths.

As used herein, an “alkylated chitosan” is a material formed of chitosan molecules to which carbon-containing molecules have been bonded. The term “alkylated chitosan” thus comprises a large number of possible chemical structures, but they all share the unifying feature that chemical bonds have been formed between the components of the chitosan molecules and at least one carbon atom in each of the molecules that are bonded to the chitosan. For example, methylation of chitosan, in which bonds are formed between methyl radicals or groups and atoms within the chitosan molecule such as nitrogen, oxygen or carbon atoms, provides an alkylated chitosan within the definition used herein. Other carbon-containing groups may likewise be chemically bonded to chitosan molecules to produce an alkylated chitosan. Specific examples include poly(oxyalkylene)chitosan, wherein poly(oxyethylene), or polyethyleneglycol, chains are covalently bonded to the chitosan backbone, and acrylated chitosans, formed by alkylation of chitosan with acrylates, for example with sodium acrylate.

When referring to the “molecular weight” of a polymeric species such as an alkylated chitosan, a weight-average molecular weight is being referred to herein, as is well known in the art.

A “degree of substitution” of a polymeric species refers to the ratio of the average number of substituent groups, for example an alkyl substituent, per monomeric unit of the polymer as defined.

A “degree of polymerization” of a polymeric species refers to the number of monomeric units in a given polymer molecule, or the average of such numbers for a set of polymer molecules.

A “poly(oxyalkylene)chitosan” is a variety of alkylated chitosan as defined herein. A “poly(oxyalkylene)” group is a polymeric chain of atoms wherein two carbon atoms, an ethylene group, are bonded at either end to oxygen atoms. The carbon atoms of the ethylene group may themselves bear additional radicals. For example, if each ethylene group bears a single methyl group, the resulting poly(oxyalkylene) group is a poly(oxypropylene) group. If the ethylene groups are unsubstituted, the poly(oxyalkylene) group is a poly(oxyethylene) group. A poly(oxyethylene) group may be of a wide range of lengths, or degrees of polymerization, but is of the general molecular formula of the structure $[-CH_2-CH_2-O-CH_2-CH_2-O-]_n$, where n may range from about 3 upwards to 10,000 or more. Commonly referred to as “polyethyleneglycol” or “PEG” derivatives, these polymeric chains are of a hydrophilic, or water-soluble, nature. Thus, a poly(oxyalkylene)chitosan is a chitosan derivative to which poly(oxyalkylene) groups are covalently attached. A terminal carbon atom of the poly(oxyalkylene) group forms a covalent bond with an atom of the chitosan chain, likely a nitrogen atom, although bonds to oxygen or even carbon atoms of the chitosan chain may exist. Poly(oxyethylene)chitosan is often referred to as “polyethyleneglycol-grafted chitosan” or “PEG-chitosan” or “PEG-g-chitosan” or “PEG-grafted-chitosan.”

The end of the poly(oxyethylene) chain that is not bonded to the chitosan backbone may be a free hydroxyl group, or may comprise a capping group such as methyl. Thus, “polyethylene glycol” or “poly(oxyethylene)” or “poly(oxyalkylene)” as used herein includes polymers of this class wherein one, but not both, of the terminal hydroxyl groups is capped, such as with a methyl group. In a specific method of preparation of the poly(oxyethylene)chitosan,

use of a polyethyleneglycol capped at one end, such as MPEG (methyl polyethyleneglycol) may be advantageous in that if the PEG is first oxidized to provide a terminal aldehyde group, which is then used to alkylate the chitosan via a reductive amination method, blocking of one end of the PEG assures that
 5 no difunctional PEG that may crosslink two independent chitosan chains is present in the alkylation reaction. It is preferred to avoid crosslinking in preparation of the poly(oxyethylene)chitosans of the present invention.

Figure 1 provides an example of the chemical structure of a segment of a poly(oxyethylene)chitosan polymer.

10 An alkylated chitosan is also a chitosan to which other carbon-containing molecules are linked. An "acrylated chitosan" as the term is used herein is an alkylated chitosan wherein acrylates have been allowed to react with, and form chemical bonds to, the chitosan molecule. An acrylate is a molecule containing an α,β -unsaturated carbonyl group; thus, acrylic acid is prop-2-enoic acid. An
 15 acrylated chitosan is a chitosan wherein a reaction with acrylates has taken place. The acrylate may bond to the chitosan through a Michael addition of the chitosan nitrogen atoms with the acrylate. Figure 4 provides an example of the chemical structure of a segment of an acrylated chitosan polymer.

As used herein, a "polybasic carboxylic acid" means a carboxylic acid
 20 with more than one ionizable carboxylate residue per molecule. The carboxylic acid may be in an ionized or salt form within the meaning of the term herein. A dibasic carboxylic acid is a polybasic carboxylic acid within the meaning herein. Thus, adipic acid is a polybasic carboxylic acid, having two ionizable carboxylate residues per molecule. Disodium adipate is a polybasic carboxylic
 25 acid within the meaning of the term herein. Alternatively, the polybasic carboxylic acid may have hundreds or thousands of ionizable carboxylate groups per molecule; for example, hyaluronan, also known as hyaluronic acid, which is an acidic polysaccharide, is a polybasic carboxylic acid within the meaning assigned herein. The hyaluronan or hyaluronic acid may be in an ionized or salt
 30 form within the meaning used herein. Thus sodium hyaluronate is a polybasic carboxylic acid within the meaning of the term as used herein. An example of the chemical structure of a segment of a hyaluronan polymer is shown in Figure 2.

As used herein, the term “acidic polysaccharide” refers to polymeric carbohydrates comprising carboxylic acid groups. The polymeric carbohydrate can be naturally occurring, or can be synthetic or semi-synthetic. Examples of acidic polysaccharides are hyaluronan and carboxymethyl cellulose.

5 Carboxymethylcellulose, as is well-known in the art, is prepared by reaction of cellulose with sodium chloroacetate, and the product is believed to contain acidic carboxymethyl groups covalently linked to the primary hydroxyl groups of the anhydroglucose monomeric units that make up the cellulose molecule. An oxidized hyaluronan, that is, hyaluronan that has been treated with an oxidizing agent, such as sodium periodate, that cleaves vicinal diol moieties and provides aldehyde groups, is an acidic polysaccharide within the meaning herein because it contains carboxylic acid groups, and is also an oxidized polysaccharide within the meaning herein because it contains aldehyde groups.

The term “oxidized polysaccharide” refers to a polymeric carbohydrate, acidic or non-acidic, that has undergone treatment with an oxidizing reagent, such as sodium periodate, that cleaves vicinal diol moieties of the carbohydrate to yield aldehyde groups. An oxidized hyaluronan, that is, hyaluronan that has been treated with an oxidizing agent, such as sodium periodate, that cleaves vicinal diol moieties and provides aldehyde groups, is an example of an acidic polysaccharide within the meaning herein. An oxidized dextran, that is, dextran that has been treated with an oxidizing agent, such as sodium periodate, that cleaves vicinal diol moieties and provides aldehyde groups, is another example of an oxidized polysaccharide within the meaning herein. Another example of an oxidized polysaccharide is an oxidized starch, that is, a starch that has been treated with an oxidizing agent, such as sodium periodate, that provides aldehyde groups. It is believed that the aldehyde groups of oxidized polysaccharides interact with the amino groups of an alkylated chitosan in such a way as to markedly increase the viscosity of the mixture and cause gelation. While not wishing to be bound by theory, it is believed that this intermolecular interaction takes place through the formation of imines (also known as Schiff bases) or of hemiaminals, between the amino groups and the aldehyde groups, such as are shown in Figure 6.

A “dehydrating reagent” as used herein refers to a molecular species that takes up the elements of water from a reaction, serving to drive a coupling

reaction by thermodynamic factors. A dehydrating reagent is a compound that undergoes reaction of covalent bonds upon taking up the elements of water, as opposed to merely absorbing water into physical particles or the like. Preferably a dehydrating reagent is an organic compound. A specific example of a
 5 dehydrating reagent is a carbodiimide, that takes up the elements of water and undergoes changes in covalent bonds to ultimately yield a urea derivative.

As used herein, a "carbodiimide" is a class of organic substances comprising
 a $R-N=C=N-R'$ moiety. The R and R' groups may be any organic radicals. For
 10 example, when R and R' are cyclohexyl groups, the carbodiimide is 1,3-dicyclohexylcarbodiimide, a dehydrating reagent well known in the art. A water-soluble carbodiimide is a carbodiimide that has sufficient solubility in water to form a homogeneous solution at concentrations suitable to carry out the gelation reaction as described herein. Typically, a water-soluble carbodiimide
 15 contains an ionic group, such as an ammonium salt, to confer water-solubility upon the molecule. The water-soluble carbodiimide EDCI is 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.

A "carboxyl activating reagent" as used herein refers to a molecular species that interacts with a carboxyl group in such a way as to render the
 20 carbonyl of the carboxyl group more susceptible to nucleophilic attack, as by an amine to yield an amide. This activation may take place by formation of a complex or by formation of a covalent intermediate. A specific example of a carboxyl activating reagent is an N-hydroxy compound that can form an N-hydroxy ester of the carboxylic acid group, increasing the reactivity of the
 25 carbonyl moiety to nucleophilic addition of a molecular species such as an amine.

The term "N-hydroxy compound" refers to an organic compound comprising a chemical bond between a hydroxyl group and a nitrogen atom. Specific N-hydroxy compounds such as N-hydroxysuccinimide and N-
 30 hydroxybenzotriazole (1-hydroxy benzotriazole) are well known in the art as reagents that form esters with carboxylic acid groups and serve to activate the carboxylic acid group in reactions with nucleophiles.

"Gelatin," as the term is used herein, is a collagen-derived material that is about 98–99% protein by dry weight. The approximate amino acid composition

of gelatin is: glycine 21 %, proline 12 %, hydroxyproline 12 %, glutamate 10 %, alanine 9 %, arginine 8 %, aspartate 6 %, lysine 4 %, serine 4 %, leucine 3 %, valine 2 %, phenylalanine 2 %, threonine 2 %, isoleucine 1 %, hydroxylysine 1 %, methionine and histidine <1% and tyrosine < 0.5 %.

5 An “aqueous medium,” as the term is used herein, refers to a liquid medium composed largely, but not necessarily exclusively, of water. Other components may also be present, such as salts, co-solvents, buffers, stabilizers, dispersants, colorants and the like.

10 As used herein, the act of “mixing between mutually coupled syringes” refers to a procedure wherein one syringe is partially filled with one ingredient, a second syringe is partially filled with a second ingredient, and the two syringes are coupled together as with a luer connector such that the contents of the syringes are mixed by drawing the contents of one syringe through the connector into the second syringe, then reciprocally expelling the contents of the second
15 syringe back into the first syringe. This process may be repeated until adequate mixing is achieved.

 A “suture” or the act of “suturing” refers to the physical joining of two separate masses of tissue with thread or fiber, or alternatively with solid materials such as fabrics or plastic films on which an adhesive is disposed,
20 whereby the physical joining serves to hold the separate tissue masses in close physical proximity at least temporarily, such as for the period of time required for biological healing to occur. A “suture line” is a line of, for example, stitches of thread as is used to close an incision at the end of a surgical procedure.

 A “therapeutic agent” is any agent which serves to repair damage to a
25 living organism to heal the organism, to cure a malcondition, to combat an infection by a microorganism or a virus, to assist the body of the living mammal to return to a healthy state. A “protective agent” is any agent which serves to prevent the occurrence of damage to an organism, such as by preventing the establishment of an infection by a microorganism, to prevent the establishment
30 of a malcondition, to preserve an otherwise healthy body in the state of health. Therapeutic and protective agents comprise pharmaceuticals, radiopharmaceuticals, hormones or their analogs, enzymes, materials for genetic therapy such as antisense nucleotides or their analogs, macroscopic ingredients such as bone powder as is used to induce bone growth, growth factors as may be

used to stimulate tissue growth such as by angiogenesis, or any other such agents as are medically advantageous for use to treat a pathological condition. As used herein, “treating” or “treat” includes (i) preventing a pathologic condition from occurring (e.g. prophylaxis); (ii) inhibiting the pathologic condition or arresting
 5 its development; (iii) relieving the pathologic condition; and/or (iv) diminishing symptoms associated with the pathologic condition.

A therapeutic agent or a protective agent may comprise a “drug.” As used herein, a “drug” refers to a therapeutic agent or a diagnostic agent and includes any substance, other than food, used in the prevention, diagnosis,
 10 alleviation, treatment, or cure of a disease. Stedman’s Medical Dictionary, 25th Edition (1990). The drug can include any substance disclosed in at least one of: The Merck Index, 12th Edition (1996); Pei-Show Juo, Concise Dictionary of Biomedicine and Molecular Biology, (1996); U.S. Pharmacopeia Dictionary, 2000 Edition; and Physician’s Desk Reference, 2001 Edition.

15 Specifically, the drug can include, but is not limited to, one or more polynucleotides, polypeptides, oligonucleotides, gene therapy agents, nucleotide analogs, nucleoside analogs, polynucleic acid decoys, therapeutic antibodies, anti-inflammatory agents, blood modifiers, anti-platelet agents, anti-coagulation agents, immune suppressive agents, anti-neoplastic agents, anti-cancer agents,
 20 anti-cell proliferation agents, and nitric oxide releasing agents.

The polynucleotide can include deoxyribonucleic acid (DNA), ribonucleic acid (RNA), double stranded DNA, double stranded RNA, duplex DNA/RNA, antisense polynucleotides, functional RNA or a combination thereof. In one embodiment, the polynucleotide can be RNA. In another
 25 embodiment, the polynucleotide can be DNA. In another embodiment, the polynucleotide can be an antisense polynucleotide.

The polynucleotide can be a single-stranded polynucleotide or a double-stranded polynucleotide. The polynucleotide can have any suitable length. Specifically, the polynucleotide can be about 2 to about 5,000 nucleotides in
 30 length, inclusive; about 2 to about 1000 nucleotides in length, inclusive; about 2 to about 100 nucleotides in length, inclusive; or about 2 to about 10 nucleotides in length, inclusive.

An antisense polynucleotide is typically a polynucleotide that is complimentary to an mRNA, which encodes a target protein. For example, the

mRNA can encode a cancer promoting protein e.g., the product of an oncogene. The antisense polynucleotide is complimentary to the single stranded mRNA and will form a duplex and thereby inhibit expression of the target gene, i.e., will inhibit expression of the oncogene. The antisense polynucleotides of the invention can form a duplex with the mRNA encoding a target protein and will disallow expression of the target protein.

A “gene therapy agent” refers to an agent that causes expression of a gene product in a target cell through introduction of a gene into the target cell followed by expression. An example of such a gene therapy agent would be a genetic construct that causes expression of a protein, such as insulin, when introduced into a cell. Alternatively, a gene therapy agent can decrease expression of a gene in a target cell. An example of such a gene therapy agent would be the introduction of a polynucleic acid segment into a cell that would integrate into a target gene and disrupt expression of the gene. Examples of such agents include viruses and polynucleotides that are able to disrupt a gene through homologous recombination. Methods of introducing and disrupting genes within cells are well known to those of skill in the art.

Nucleotide and nucleoside analogues are well known on the art. Examples of such nucleoside analogs include, but are not limited to, Cytovene® (Roche Laboratories), Epivir® (Glaxo Wellcome), Gemzar® (Lilly), Hivid® (Roche Laboratories), Rebetrone® (Schering), Videx® (Bristol-Myers Squibb), Zeret® (Bristol-Myers Squibb), and Zovirax® (Glaxo Wellcome). See, Physician’s Desk Reference, 2001 Edition.

As used herein, a “peptide” and a “protein” refer to polypeptides, linear polymers of amino acids, the difference between the terms “peptide” and “protein” largely being in the length of the polymer. In one embodiment, the polypeptide can be an antibody. Examples of such antibodies include single-chain antibodies, chimeric antibodies, monoclonal antibodies, polyclonal antibodies, antibody fragments, Fab fragments, IgA, IgG, IgM, IgD, IgE and humanized antibodies. In one embodiment, the antibody can bind to a cell adhesion molecule, such as a cadherin, integrin or selectin. In another embodiment, the antibody can bind to an extracellular matrix molecule, such as collagen, elastin, fibronectin or laminin. In still another embodiment, the antibody can bind to a receptor, such as an adrenergic receptor, B-cell receptor,

complement receptor, cholinergic receptor, estrogen receptor, insulin receptor, low-density lipoprotein receptor, growth factor receptor or T-cell receptor. Antibodies of the invention can also bind to platelet aggregation factors (e.g., fibrinogen), cell proliferation factors (e.g., growth factors and cytokines), and blood clotting factors (e.g., fibrinogen). In another embodiment, an antibody can be conjugated to an active agent, such as a toxin or a radionuclide.

An “anti-cancer agent” means an agent that either inhibits the growth of cancerous cells, or causes the death of cancerous cells. Anti-cancer agents include, e.g., nucleotide and nucleoside analogs, such as 2-chloro-deoxyadenosine, adjunct antineoplastic agents, alkylating agents, nitrogen mustards, nitrosoureas, antibiotics, antimetabolites, hormonal agonists/antagonists, androgens, antiandrogens, antiestrogens, estrogen & nitrogen mustard combinations, gonadotropin releasing hormone (GNRH) analogues, progestins, immunomodulators, miscellaneous antineoplastics, photosensitizing agents, and skin & mucous membrane agents. See, Physician’s Desk Reference, 2001 Edition.

An “antimicrobial,” as used herein, refers to a molecular entity that is effective as a therapeutic agent or as a protective agent against an infection by a microorganism, which could be a bacterium, a protozoan, a fungus, a virus, or another pathogenic living organism. An antimicrobial may be an antibiotic, effective against bacteria, including aminoglycoside antibiotics such as gentamicin or streptomycin, a cephalosporin such as cephalexin or cephtriaxone, a carbacephem such as loracarbef, a glycopeptide such as vancomycin, a macrolide such as erythromycin, a penicillin such as amoxicillin or ampicillin, a polypeptide such as bacitracin or polymyxin B, a quinolone such as ciprofloxacin, a tetracycline such as oxytetracycline, a sulfonamide, or any other medically approved agent for treatment of bacterial infections. Alternatively the antimicrobial may be an antifungal agent such as ketoconazole, miconazole or amphotericin B, or an antiviral agent such as acyclovir or AZT.

A “radioactive material” as used herein refers to any naturally occurring or manmade substance that emits ionizing radiation such as gamma rays, beta particles, Auger electrons, X-rays, or alpha particles. A radioactive material may be used for diagnostic purposes, such as for imaging as in positron emission tomography (PET). A radionuclide commonly used for imaging diagnostics is

fluorine-18. Alternatively a radioactive material may be used for therapeutic purposes, as in treating tumors. Radionuclides used therapeutically include technetium-99m, iodine-123, -125, and -131, and gallium-67, among others.

In the claims provided herein, the steps specified to be taken in a claimed method or process may be carried out in any order without departing from the principles of the invention, except when a temporal or operational sequence is explicitly defined by claim language. Recitation in a claim to the effect that first a step is performed then several other steps are performed shall be taken to mean that the first step is performed before any of the other steps, but the other steps may be performed in any sequence unless a sequence is further specified within the other steps. For example, claim elements that recite “first A, then B, C, and D, and lastly E” shall be construed to mean step A must be first, step E must be last, but steps B, C, and D may be carried out in any sequence between steps A and E and the process of that sequence will still fall within the four corners of the claim.

Furthermore, in the claims provided herein, specified steps may be carried out concurrently unless explicit claim language requires that they be carried out separately or as parts of different processing operations. For example, a claimed step of doing X and a claimed step of doing Y may be conducted simultaneously within a single operation, and the resulting process will be covered by the claim. Thus, a step of doing X, a step of doing Y, and a step of doing Z may be conducted simultaneously within a single process step, or in two separate process steps, or in three separate process steps, and that process will still fall within the four corners of a claim that recites those three steps.

Similarly, except as explicitly required by claim language, a single substance or component may meet more than a single functional requirement, provided that the single substance fulfills the more than one functional requirement as specified by claim language.

30

Detailed Description

A hydrogel for use as a tissue sealant according to the present invention is a hydrogel that achieves a gelled state after formation by gelation from a premix composed of more than a single molecular component in an aqueous

medium. The hydrogel, which may be used to seal the tissues of a living mammal such as a human patient, is formed upon gelation of the premix, the premix being in the physical form of a substantially liquid sol. Mixing of the components that make up the premix, such as by reciprocating transfer of the solutions between a pair of syringes, provides a liquid or semi-liquid sol that may be pumped or transferred by any technique suitable for handling somewhat viscous liquid materials, such as syringes, pipettes, tubing and the like. Upon standing, the premix sol after a period of time sets up into the hydrogel of the present invention.

The premix sol and the resulting hydrogel that forms from the sol are suitable for contact with living biological tissue, being biocompatible and preferably biodegradable. Thus, the hydrogel can remain in contact with living biological tissue within a human patient for an extended period of time without damaging the tissue on which it is disposed. In an embodiment, the hydrogel has strongly adhesive properties towards living tissues on which it is disposed. In an embodiment, the hydrogel contains therapeutic or protective agents that are released into the surrounding tissues on which the hydrogel is disposed. In an embodiment, the hydrogel has both adhesive properties towards the tissue on which it is disposed and also contains therapeutic or protective agents that are released into the surrounding tissues on which the hydrogel is disposed. In another embodiment the hydrogel contains microspheres or nanospheres containing therapeutic agents or protective agents that further control the release of the agents from the hydrogel.

A specific embodiment of a premix that forms a hydrogel according to the present invention comprises an alkylated chitosan. Referring to Figure 1, in a specific embodiment an alkylated chitosan comprises a poly(oxyethylene)chitosan. The poly(oxyethylene)chitosan is a polymer formed of 2-amino-2-deoxyglucose monomeric units. Each monomeric unit comprises a single free amino group and two free hydroxyl groups. In Figure 1, one amino group is alkylated on the nitrogen atom with a poly(oxyethylene) chain, also known as a polyethyleneglycol or a PEG chain. In the example provided in Figure 1, the chitosan has a degree of substitution of 0.5, because two of the four amino groups in the tetrameric unit shown bears the PEG substituent. However, a poly(oxyethylene)chitosan according to the present invention may have a

degree of amino group substitution ranging down to about 0.1 (wherein only one in about every ten monomeric units is alkylated). Furthermore, a poly(oxyethylene)chitosan according to the present invention may also bear the poly(oxyethylene) derivative on one of the two free hydroxyl groups in a given monomeric unit, or may comprises a mixture of N- and O-alkylated chitosan monomeric units, or be di-alkylated or tri-alkylated on a single monomer unit. Thus, a fully alkylated chitosan monomeric unit has a degree of substitution of 3.0, and a poly(oxyethylene)chitosan according to the present invention may have a degree of substitution ranging up to 3.0. However, a preferred degree of substitution for a poly(oxyethylene)chitosan of the invention is about 0.35 to about 0.95. A particularly preferred degree of substitution is about 0.5.

It should be understood that other poly(oxyalkylene) groups may be substituted for the poly(oxyethylene) group shown in Figure 1. For example, a poly(oxypropylene)chitosan may be used in place of, or in addition to, the poly(oxyethylene)chitosan. A poly(oxypropylene) group is the structure that would be obtained if the poly(oxyethylene) group as shown in Figure 1 bore a methyl group on every ethylene unit ($-O-CH_2CH(CH_3)-O$), or alternatively, every ethylene unit shown in Figure 1 were a 3-carbon linear propylene group ($-O-CH_2CH_2CH_2-O$).

The number of monomeric units that make up a chitosan according to the present invention may vary widely without departing from the principles of the invention. Any sample that contains more than a single molecule of a chitosan derivative will almost inevitably contain a distribution of molecules of different molecular weights. A preferred poly(oxyethylene)chitosan according to the present invention has a molecular weight of about 200 kD to about 600 kD.

In a specific embodiment, a premix for a hydrogel contains a polybasic carboxylic acid comprising a hyaluronan. A member of the class of acidic polysaccharides, a hyaluronan bears an ionizable carboxylic acid group on every other monosaccharide residue. The hyaluronan can be in the form of a hyaluronate, that is, with at least most of the carboxylic acid groups being in the ionized or salt form. Sodium hyaluronate is a specific example. Referring to Figure 2, a hyaluronan or a hyaluronic acid is a polybasic carboxylic acid, and the number of ionizable carboxylate groups per hyaluronan molecule is dependent on the degree of polymerization of the hyaluronan. The degree of

substitution of carboxylic acid groups on the polymer backbone, assuming a monomeric unit comprising the disaccharide formed of one glucuronic acid monosaccharide and one 2-acetamido-2-deoxyglucose monosaccharide, is 1.0. Every monomeric unit (disaccharide unit) bears a single ionizable carboxylic acid group. A hyaluronan may be of any of a wide range of degrees of polymerization (molecular weights), but a preferred hyaluronan has a molecular weight of about 2,000 kD to about 3,000 kD. An example of a premix comprising a hyaluronan further comprises a poly(oxyethylene)chitosan, synonymously a PEG-chitosan.

Another specific embodiment of a premix that forms a hydrogel according to the present invention comprises an acrylated chitosan. Referring to Figure 4, in a specific embodiment an alkylated chitosan comprises a acrylated chitosan wherein at least some of the free amino groups of the 2-amino-2-deoxyglycose monosaccharide monomeric units are substituted with acrylate groups. It is believed that acrylate groups are bonded to free amino groups of the chitosan via a Michael type conjugate addition wherein the nucleophilic amino group forms a bond to the β -carbon of the α,β -unsaturated acrylate, but the acrylate may be bonded to the chitosan in a different manner without departing from the principles of the invention. Furthermore, as is illustrated in Figure 4, acrylates may themselves oligomerize after initial alkylation of the chitosan backbone. The three-carbon carboxylic acid substituent on the left in Figure 4 illustrates the alkylation of chitosan with a single molecule of acrylate, whereas the six-carbon dicarboxylic acid substituent on the right in Figure 4 illustrates the product resulting from addition of a second acrylate molecule to the first acrylate molecule.

A preferred degree of substitution of the chitosan backbone with acrylate groups according to the present invention is about 0.25 to about 0.45. The number of monomeric units that make up a acrylated chitosan according to the present invention may vary widely without departing from the principles of the invention. Any sample that contains more than a single molecule of a chitosan derivative will almost inevitably contain a distribution of molecules of different molecular weights. A preferred acrylated chitosan has a molecular weight of about 200 kD to about 600 kD.

A premix that includes an acrylated chitosan can also include a polybasic carboxylic acid comprising a dicarboxylic acid. A preferred dicarboxylic acid is a dicarboxylic acid wherein the two carboxylate groups are bonded to a moiety of about one to about twelve carbon atoms, which may comprise chains,
 5 aliphatic or aromatic rings, or heteroatoms such as nitrogen, oxygen or sulfur. Referring to Figure 5, a particularly preferred dicarboxylic acid is a linear alkyl α,ω -dicarboxylic acid, which can crosslink acrylated chitosan polymer chains through the intermolecular formation of amide bonds between the chitosan amino groups and the carboxylic acid groups of the dicarboxylic acid. Specific
 10 examples of linear alkyl α,ω -dicarboxylic acids are malonic, succinic, glutaric, adipic, pimelic, suberic, azaleic, and sebacic acid. A particularly preferred example is adipic acid. A specific example of a premix of the invention comprises acrylated chitosan, disodium adipate, a dehydrating reagent and a carboxyl activating reagent.

15 In another embodiment, a premix that includes an alkylated chitosan also includes a polybasic carboxylic acid comprising a carboxymethylcellulose. A carboxymethylcellulose is a derivative of cellulose (a β -1,4 linked polymer of glucose) wherein hydroxyl groups are substituted with carboxymethyl (-CH₂CO₂H) moieties. It is understood that the term carboxymethylcellulose
 20 comprises salts of carboxymethylcellulose, such as the sodium salt. A specific example of a premix comprises acrylated chitosan, carboxymethylcellulose sodium salt, a dehydrating reagent and a carboxyl activating reagent. Carboxymethylcellulose, as is well-known in the art, may have varying degrees of substitution, a "degree of substitution" referring to the number of derivatizing
 25 groups, herein carboxymethyl, per each monomer unit on the average. A particularly preferred carboxymethylcellulose according to the present invention has a degree of substitution of about 0.7 and a molecular weight of about 80 kD. Another polybasic carboxylic acid that can be used in a premix with an acrylated chitosan is hyaluronic acid, as described above.

30 In another embodiment, a premix that includes an alkylated chitosan also includes an oxidized polysaccharide. An oxidized polysaccharide, for example, an oxidized starch, an oxidized dextran, or an oxidized hyaluronan, can be prepared from the corresponding polysaccharide by oxidation, such as with sodium periodate, that brings about formation of aldehyde groups from the

vicinal 2,3-diol units of the polysaccharides. Thus, a premix can include an acrylated chitosan and an oxidized polysaccharide, such as an oxidized dextran. The degree of oxidation of the polysaccharide refers to the number of monomeric units wherein the vicinal 2,3-diol unit has been cleaved to form a
5 dialdehyde, in relation to the total number of monomeric units in the polymer. The degree of oxidation can be determined chemically, as is well known in the art and examples of which are provided below (e.g., Examples 6, 7, 9, and 10).

A premix can also include an alkylated chitosan, a polybasic carboxylic acid, and an oxidized polysaccharide. For example, a premix can include an
10 acrylated chitosan, a hyaluronic acid, and an oxidized dextran in an aqueous medium.

A premix according to the present invention comprises an aqueous medium. An aqueous medium necessarily includes water, and may include other components including salts, buffers, co-solvents, additional cross-linking
15 reagents, emulsifiers, dispersants, electrolytes, or the like.

A premix according to the present invention can comprise a dehydrating reagent. A preferred dehydrating reagent is a dehydrating reagent that is sufficiently stable when dissolved or dispersed in an aqueous medium to assist in driving the formation of the amide bonds before it is hydrolyzed by the water in
20 the aqueous medium. A particularly preferred type of dehydrating reagent is a carbodiimide, which is transformed to a urea compound through incorporation of the elements of water. An example of a water-soluble carbodiimide, is 1-ethyl-3-(N,N-dimethylpropyl)carbodiimide (EDCI), which is preferred as it is soluble in the aqueous medium and thus does not require a co-solvent or dispersant to
25 distribute it homogeneously throughout the premix. Other water-soluble carbodiimides are also preferred dehydrating reagents.

A premix according to the present invention can comprise a carboxyl activating reagent. A preferred carboxyl activating reagent is a reagent that serves to activate a carboxyl group towards formation of a new bond, such as an
30 amide or ester bond with an amine or a hydroxyl-bearing compound respectively. A specific embodiment of a carboxyl activating reagent reacts with the carboxyl group to form a new compound as an intermediate, which then further reacts with another substance such as an amine to form an amide, or a hydroxyl-bearing compound to form an ester. A preferred carboxyl activating

reagent is an N-hydroxy compound. An N-hydroxy compound reacts with a carboxyl group to form an N-hydroxy ester of the carboxylic acid, which may subsequently react with, for example, an amino group to form an amide. An example of an N-hydroxy compound is N-hydroxysuccinimide. Another
5 example of an N-hydroxy compound is N(1)-hydroxybenzotriazole.

Another preferred carboxyl activating reagent is a carbodiimide. A carbodiimide reacts with a carboxyl group to form an O-acylisourea, which may subsequently react with, for example, an amine to form an amide, releasing the carbodiimide transformed through covalent addition of the elements of water to a
10 urea compound. A preferred carbodiimide is a water-soluble carbodiimide, for example EDCI.

In a specific embodiment of the present invention, a carbodiimide may serve both as a dehydrating reagent and as a carboxyl activating reagent. Thus, a premix comprising an alkylated chitosan, a polybasic carboxylic acid, and a
15 carbodiimide is a specific embodiment according to the present invention. Another specific embodiment is a premix comprising an alkylated chitosan, a polybasic carboxylic acid, a carbodiimide, and another molecular species wherein that species is a carboxyl activating reagent. Another specific embodiment is a premix comprising an alkylated chitosan, a polybasic
20 carboxylic acid, a carbodiimide, and another molecular species wherein that species is a dehydrating reagent.

In an embodiment of a composition of the invention, no dehydrating reagent or carboxyl activating reagent is needed for formation of a hydrogel. For example, an acrylated chitosan forms a hydrogel after mixing with an oxidized
25 polysaccharide in an aqueous medium. The initial sol formed after mixing undergoes gelation over a period of time, typically a few minutes, to provide a hydrogel of the invention. A specific example of an oxidized polysaccharide is oxidized dextran. Additional examples are oxidized starch and oxidized hyaluronan. Another specific composition of the invention comprises acrylated
30 chitosan, oxidized dextran, and hyaluronic acid in an aqueous medium which likewise readily forms a hydrogel without addition of a dehydrating reagent or of a carboxyl activating reagent.

Upon standing at room temperature or at the temperature of the human body, a premix sol of the invention, after a period of time that is typically in the

order of a few minutes, for example about 0.5 minutes to about 20 minutes at about 37 °C, or about 1 minute to about 10 minutes at about 37 °C, undergoes gelation to form a hydrogel of the invention. A premix sol and a resulting hydrogel that forms from the sol are suitable for contact with living biological
 5 tissue, being biocompatible and preferably biodegradable. Thus, the hydrogel can remain in contact with living biological tissue within a human patient for an extended period of time without damaging the tissue on which it is disposed.

In an embodiment of the invention, the hydrogel contains therapeutic or protective bioactive agents that are released into the surrounding tissues.
 10 Bioactive agents can be combined with premix solutions, by simply blending commercially available solutions of polypeptides or other agents with the aqueous solutions, with gentle mixing. Cells can likewise be blended with the composition, preferably immediately prior to emplacement to enhance survival of living cells.

15 A radiopaque material that is optionally incorporated in the composition may be fine particles of a selected radiopaque metal, such as gold, platinum, tantalum, barium, or the like. Alternatively, a radiopaque agent can be an iodinated organic compound. A specific example is iohexol.

A variety of types of a bioactive agent can be incorporated into the
 20 composition of the invention. The agent can be a molecular entity, such as a regenerative agent such as one or more human growth modulating factors such as interleukins, transformation growth factor-b, fibroblast growth factor (FGF) or vascular endothelial growth factor (VEGF), a gene therapy agent, a cogener of platelet derived growth factor, a monoclonal antibody directed against growth
 25 factors; a drug, or a cell regeneration factor. A bioactive agent may also be a cellular entity such as a plurality of drug-producing cells or of regenerative cells such as stem cells.

In another embodiment of the invention, the hydrogel contains microspheres or nanospheres containing therapeutic agents or protective
 30 bioactive agents, the microspheres or nanospheres further controlling the release of the bioactive agents from the hydrogel into the surrounding tissues. A “microsphere” or a “nanosphere” as used herein is a particulate body of dimensions of the order of microns (micrometers) or nanometers respectively, wherein the particulate body may be hollow or solid. Microspheres and

nanospheres may be formed of organic or inorganic materials. For example, a nanosphere may comprise a buckminsterfullerene (buckyball), which is organic. Alternatively a nanosphere may comprise microporous glass, which is inorganic. It is understood that the terms encompass solid lipid nanoparticles, wherein the
 5 nanosphere particles are formed from a solid lipid. Preferably the microsphere or the nanosphere contains a drug or other substance, the timing of the release of which it is advantageous to control.

Due to the abundance of cationic amino groups in the chitosan structure, it is known that drugs with carboxyl groups can be conjugated thereto and
 10 sustained release can be achieved through the hydrolysis of the amide or ester bonds linking drugs to the chitosan molecule. Y.D. Sanzgiri, et al., Pharm. Res., 1, 418 (1990). As a polyelectrolyte, chitosan can also electrostatically conjugate sensitive bioactive agents while preserving their bioactivities and enhancing their stabilities. Such derivatives may be formed with the acrylated chitosan of the
 15 present invention, and will likewise serve to provide for sustained release and to preserve the bioactivity and to enhance the stability of the conjugated agent(s).

The abundance of positive charges on the alkylated chitosan enables the electrostatic binding of biologically active proteins such as rhVEGF. This is the most gentle mode of conjugating proteins and thus protecting and preserving the
 20 bioactivity of sensitive proteins like rhVEGF. The conjugation of proteins like rhVEGF to the alkylated chitosan also serves as a mechanism for modulating the biological activity of the growth factor, thereby limiting the potential for induction of uncontrolled tissue development.

The types of cells that may be incorporated into the composition include
 25 progenitor cells of the same type as those from the tissue site, and progenitor cells that are histologically different from those of the tissue site such as embryogenic or adult stem cells, that can act to accelerate the healing process. The therapeutic composition comprising cells can be administered in the form of a solution or a suspension of the cells mixed with the polymer solution, such that
 30 the cells are substantially immobilized within the application site upon gelation of the premix. This serves to concentrate the effect of the therapeutic agent or the cells at the site of the tissue damage and to provide for release of the agent or of the cells or of cellular products over a course of time.

A hydrogel of the invention adheres to the living tissues on which it is disposed such that it may be used as a tissue sealant. A tissue sealant of the invention has at least sufficient adhesivity such that after gelation, the hydrogel resists detachment from the tissue when subjected to a force at least as strong as
5 may be applied when a patient moves, or by the weight of an organ acted on by gravity, or by involuntary motions of surrounding tissues (heartbeat, peristalsis, etc.). For applications where the disrupting strain is likely to be more severe, for example in a limb where voluntary muscles exert a greater force, the tissue sealant preferably adhesively seals the tissue such that it can resist a relatively
10 large disruptive force. The hydrogel is of sufficient strength and elasticity such that the physical integrity of the mass of sealant as it is disposed on the tissue is maintained while the hydrogel adheres to the tissue. The inventive tissue sealant thus is suitable for at least temporarily holding the tissue surrounding a tear, hole, perforation, incision or any separation of tissue in physical proximity. For
15 example, the inventive tissue sealant is adapted to seal any laceration, cut, abrasion, or disruption of dermal tissue, i.e., skin.

A specific tissue sealant of the invention also preferably seals the tear, hole, perforation or incision in order to prevent leakage of any vital bodily fluids that are normally retained by the tissue in its undamaged state. For example, the
20 outer membrane surrounding the brain and the spinal cord, the dura mater, serves to contain the cerebrospinal fluid in which the nervous system tissue is normally immersed. A specific use for the tissue sealant according to the present invention is the repair or sealing of the dura, such as after brain surgery. To surgically reach the brain in order to carry out any of the operative procedures
25 that may be applied within the brain to treat or cure a malcondition, an incision must be made in the dura, and when the surgical procedures within the brain are complete, it is desirable to close the dura as tightly as possible to avoid leakage of cerebrospinal fluid to areas external to the dura as well as to allow healing of the tissue. A tissue sealant of the present invention is well suited to provide this
30 closure and sealing, either as a reinforcement of a suture line or without additional closure techniques being used.

The tissue sealant can be used in conjunction with a mechanical wound closure device, such as a suture strip or a tissue clip. For example, the premix can be applied to the wound closure device immediately prior to emplacement of

the device, or can be applied to the tissue following emplacement of the wound closure device, or both.

The tissue sealant can also be used to fill a tissue void, or to be used as a barrier layer to prevent adhesion of tissues during the healing process.

- 5 Deposition of the sol within the void, or between the tissue masses where adhesion is to be forestalled, leads to formation of the hydrogel of the invention *in situ*. The at least moderate degree of adhesiveness of the hydrogel is adapted to prevent slippage of the hydrogel mass out of the void or from between the two tissue masses to be held separate during healing. The hydrogel can be
- 10 biodegradable within a suitable time period such that the healed tissues are freed after adhesion is no longer probable.

- The premix is prepared and is applied to the incision in the sol form. For aid to the surgeon in visualizing the application of the tissue sealant, the sealant may comprise a dye or a fluorescent material to better enable the surgeon to see
- 15 and thus control the distribution of the sealant on the tissue being repaired. The sealant may also comprise a radio-opaque agent to aid in visualization of the disposition of the sealant post-operatively by fluoroscopy or X-ray photography.

- A specific technique for preparing the premix is with the use of two syringes coupled with a Luer coupling. The fitting can contain a three-way T-
- 20 valve. A first syringe is partially filled with a solution comprising an alkylated chitosan dissolved in an aqueous medium. The first syringe can further comprise a polybasic carboxylic acid. A second syringe is partially filled with a solution comprising a polybasic carboxylic acid or an oxidized polysaccharide or both dissolved in an aqueous medium. Optionally, either solution may contain
- 25 additional components such as a co-solvent, dispersant, emulsifier or other additive. Optionally, either syringe may further contain a dehydrating reagent, a carboxyl activating reagent or both.

- Formation of the premix can take place by reciprocally exchanging the contents of the two syringes through the coupling fitting, first depressing the
- 30 plunger of one syringe to expel the contents into the second syringe, then depressing the plunger of the second syringe to expel the mixed contents back into the first syringe. This procedure is preferably repeated until substantial homogeneity is achieved. Optionally a dehydrating reagent, a carboxyl activating reagent, or both, may be added by replacing the empty syringe with a

third syringe charged with a solution of the reagent(s) and repeating the reciprocal exchange. Then, the charged syringe containing the homogeneous premix can be attached to a suitable application tip, which is used to transfer the premix such that it is disposed on the tissue to be sealed, for example an incision
 5 in the dura mater.

In a similar manner the premix may be applied to other tissue types in need of sealing, such as dermal tissue, musculature, and so forth. For example, the premix of a tissue sealant may be applied to seal the annulus of a ruptured inter-vertebral disc as part of a surgical procedure to repair a ruptured disc.

10 A premix may be mixed up in any suitable container, taken up in a syringe or pipette, and transferred to the tissue to be sealed, or it may be poured onto the tissue in a controlled manner. Preferably, complete application of the premix to the tissue takes place before significant gelling occurs, although the premix may undergo a certain amount of thickening prior to application.

15 The present invention also comprises a kit adapted for preparing and applying the tissue sealant of the invention to the tissue of a patient. The kit comprises two containers, which may be separate or may be contained within a single unit. Each container comprises a component, the mixing of which in an aqueous medium provides the premix that by gelation forms the tissue sealant of the invention. For example, a first container can comprise an alkylated chitosan
 20 or a gelatin. The first container can comprise an aqueous medium.

Alternatively, the first container can comprise the alkylated chitosan or gelatin either as a dry (e.g. lyophilized) solid, or in concentrated form, and the container be adapted for addition of an aqueous medium which enables preparation of the
 25 first solution. Similarly, the second container, which can comprise a polybasic carboxylic acid, an oxidized polysaccharide, or both, can comprise an aqueous medium or can be adapted for addition of an aqueous medium, which enable preparation of the second solution. The kit may also include means for mixing the two solutions to provide the substantially liquid sol that upon gelation forms
 30 the tissue sealant hydrogel of the invention. For instance, the kit can comprise a pair of syringes and a coupling that enables the two solutions to be mixed by reciprocally drawing them between the two syringes. A kit can comprise additional components, such as a solution containing a radiopaque agent to be mixed with the premix. Following mixing, a syringe with a suitable tip can be

used to emplace the sol on the tissue such that gelation and sealing can take place. A kit can comprise a plurality of application tips adapted for particular uses. A kit can also comprise instructional material for the care-provider, including, for example, mixing instructions and expected gelation times.

5 The premix, in the form of a substantially liquid sol, preferably gels within a relatively short time frame, but not instantly, to enable the premix to be applied to the tissue to be sealed before substantial gelling takes place. Nevertheless, the premix preferably forms the hydrogel within a timeframe of minutes, such that surgical procedures are not unduly delayed or interrupted by
10 periods of time wherein the gelling is taking place. Preferably gelling takes place within about 1 minute to about 12 minutes at the body temperature of the patient, around 37°C. It is understood that even after the premix gels to a sufficient degree that the hydrogel ceases to be flowable, additional gelling or hardening may take place. It is preferred that gelation has occurred to a
15 sufficient degree within the 1 to 12 minute time window to permit surgical procedures to continue. Thus, it is preferred that both gelling and adhesion of the hydrogel be sufficiently achieved in this time window that the tissue and its surroundings may be at least gently manipulated by the end of the time window without causing flow of un-gelled premix or detachment of adhered hydrogel
20 from the tissue on which the hydrogel is disposed.

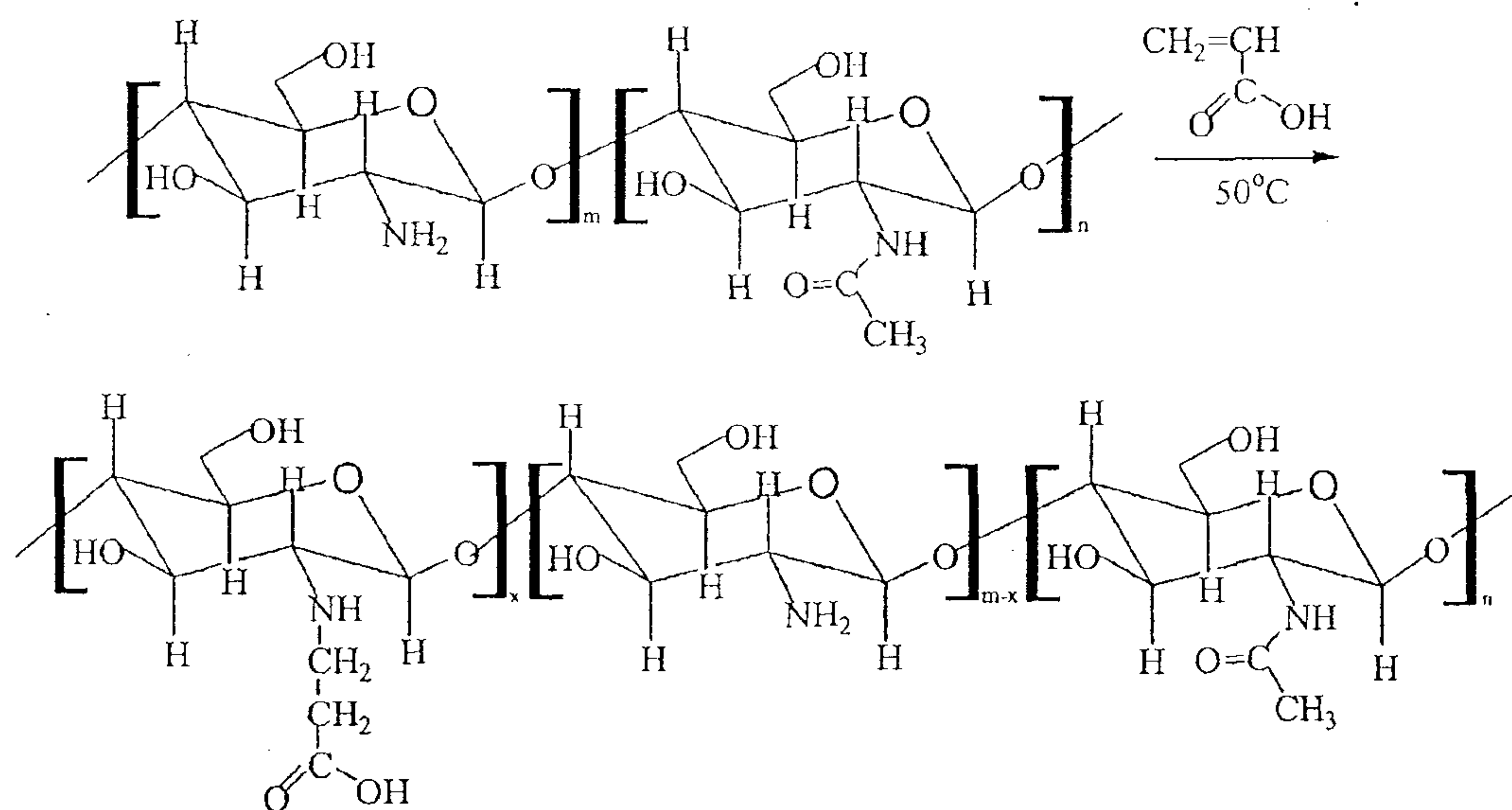
 The tissue sealant of the present invention preferably is dimensionally stable after gelling in the presence of aqueous media, such as in a living human body. The tissue sealant does not absorb water or swell greatly in the presence of water. This is particularly preferable when the tissue sealant is disposed in
25 proximity to nerves, where swelling and the resulting pressure applied to the nerves is particularly disadvantageous. The dimensional stability of a tissue sealant comprising a hydrogel is an outstanding feature according to the present invention. This dimensional stability enables a tissue sealant of the present invention to be particularly well-suited for use in proximity to neural tissue, for
30 example in sealing of dura mater in brain neurosurgery. In brain neurosurgery, avoidance of post-surgical swelling is especially critical, thus the dimensional stability of a tissue sealant of the present invention is highly advantageous in such use.

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the claims. Other aspects, advantages, and modifications are
5 within the scope of the claims and will doubtless be apparent to persons of ordinary skill in the art.

Examples

Example 1

5 Preparation of acrylated chitosan



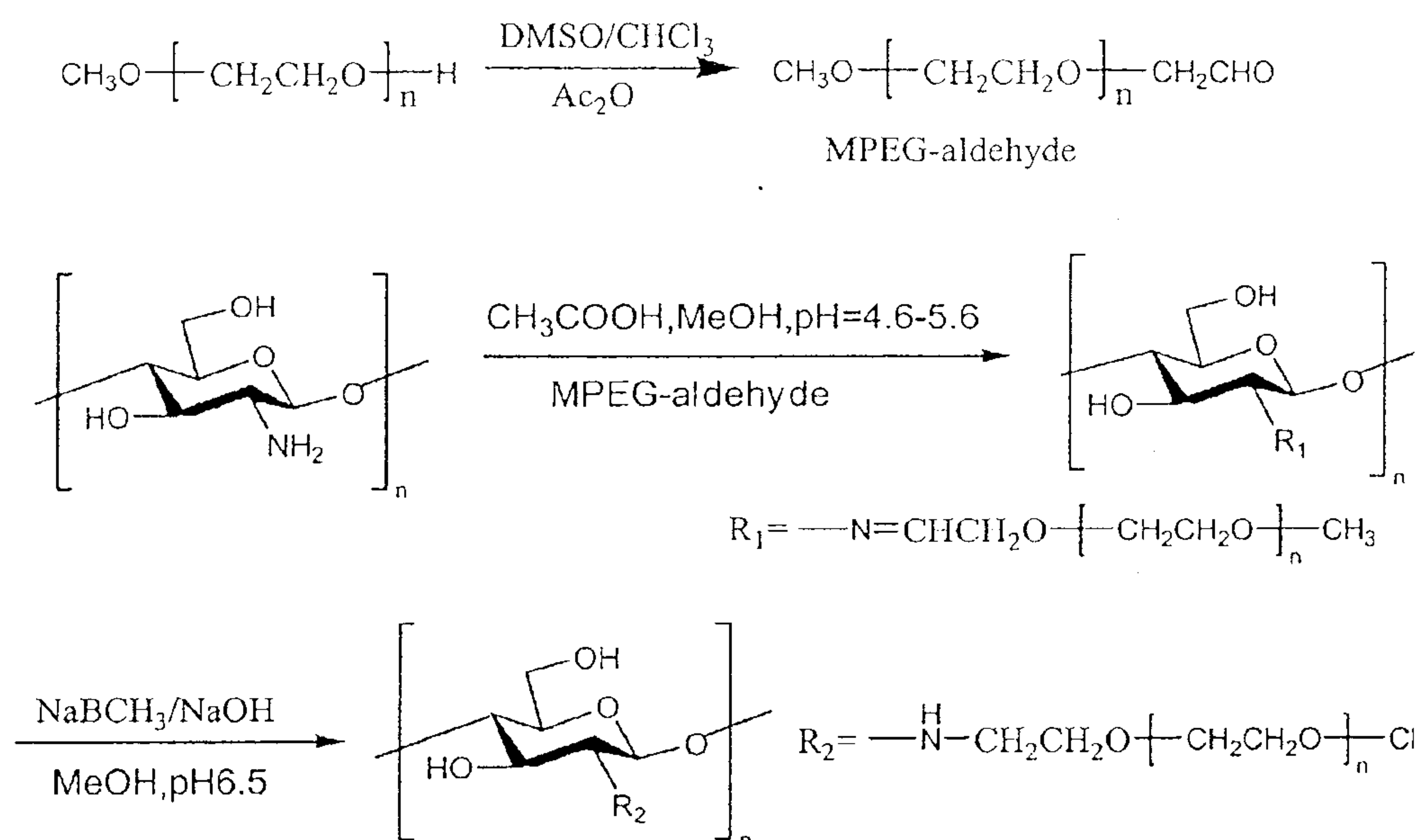
10

5.52 ml of acrylic acid was dissolved in 150 ml of double distilled water and 3g of chitosan (Kraeber® 9012-76-4, molecular weight 200-600 kD) was added to it. The mixture was heated to 50C and vigorously stirred for 3 days. After removal of insoluble fragments by centrifugation, the product was

15 collected and its pH was adjusted to 11 by adding NaOH solution. The mixture was dialyzed extensively to remove impurities.

Example 2

Preparation of PEG-chitosan



5

Monomethyl-PEG-aldehyde was prepared by the oxidation of Monomethyl-PEG (MPEG) with DMSO/acetic anhydride: 10 g of the dried MPEG was dissolved in anhydrous DMSO (30 ml) and chloroform (2 ml).

- 10 Acetic anhydride (5 ml) was introduced into the solution and the mixture is stirred for 9h at room temperature. The product was precipitated in 500 ml ethyl ether and filtered. Then the product was dissolved in chloroform and re-precipitated in ethyl ether twice and dried.

- Chitosan (0.5 g, 3 mmol as monosaccharide residue containing 2.5 mmol amino groups, Kraeber 9012-76-4, molecular weight 200-600 kD) was dissolved in 2 % aqueous acetic acid solution (20 ml) and methanol (10 ml). A 15 ml sample of MPEG-aldehyde (8 g, DC: 0.40) in aqueous solution was added into the chitosan solution and stirred for 1h at room temperature. Then the pH of chitosan /MPEG-monoaldehyde solution was adjusted to 6.0-6.5 with aqueous 1 M NaOH solution and stirred for 2h at room temperature. NaCNBH₃ (0.476 g, 7.6 mmol) in 7 ml water was added to the reaction mixture dropwise and the solution was stirred for 18 h at room temperature. The mixture was dialyzed with dialysis membrane (COMW 6000-8000) against aqueous 0.5 M NaOH solution and water alternately. When the pH of outer solution reached 7.5, the inner
- 15
- 20

solution was centrifuged at 5,000 rpm for 20 min. The precipitate was removed. The supernatant was freeze-dried and washed with 100 ml acetone to get rid of unreacted MPEG. After vacuum drying, the final product (white powder) was obtained as water soluble or organic solvent soluble PEG-g-Chitosan. The yield
5 of water soluble derivatives was around 90% based on the weight of starting chitosan and PEG-aldehyde.

Example 3

Preparation of a PEG-chitosan and Hyaluronan Hydrogel

10 Hyaluronan (sodium hyaluronate, Kraeber 9067-32-7) was dissolved in water as a 0.5% solution by weight. PEG-chitosan, prepared as described in Example 2, was dissolved in water as a 5% solution by weight. A sample of each solution (0.5 mL of each) was mixed, then a solution of EDCI (20 μ L of a solution in water at 350 mg/mL) was added and the solution was thoroughly
15 mixed. Immediately a solution of N-hydroxysuccinimide (20 μ L of a solution in water at 125 mg/mL) was added and thoroughly mixed in to form a premix. The premix gelled into a hydrogel in about 7 minutes at ambient temperature (22°C). At 37 °C gelation occurred in about 2 minutes.

20 Example 4

Preparation of a Acrylated Chitosan and Adipic Acid Hydrogel

A sample of acrylated chitosan prepared as described in Example 1 was dissolved in water at a concentration of 2% by weight. A sample of this solution (0.5 mL) was mixed with a solution of adipic acid in water (40 μ L of a 20
25 mg/mL solution), then a solution of EDCI (20 μ L of a 350 mg/mL solution) and the solution thoroughly mixed. Then, a solution of N-hydroxysuccinimide in water (20 μ L of a 125 mg/mL solution) was mixed in. The premix gelled in about 9 minutes at ambient temperature (22°C). At 37 °C gelation occurred in about 3 minutes.

30

Example 5**Preparation of an Acrylated Chitosan and Carboxymethylcellulose Hydrogel**

A sample of acrylated chitosan prepared as described in Example 1 was
 5 dissolved in water at a concentration of 2% by weight. A sample of
 carboxymethylcellulose sodium salt (Polysciences no. 06140, MW 80 kD,
 degree of substitution 0.7) was dissolved in water at a concentration of 5% by
 weight. These two solutions (0.25 mL each) were mixed with a solution of
 EDCI (20 μ L of a 6.5% solution) and the solution thoroughly mixed. Then, a
 10 solution of N-hydroxysuccinimide in water (20 μ L of a 35% solution) was mixed
 in. The solution gelled in about 10 minutes at ambient temperature (22°C).

Example 6**Preparation of Oxidized Dextran**

15 Dextran (5 g) was dissolved in 400 mL of distilled H₂O, then 3.28 g of
 NaIO₄ dissolved in 100mL ddH₂O was added. The mixture was stirred at 25°C
 for 24 hrs. 10 ml of ethylene glycol was added to neutralize the unreacted
 periodate following by stirring at room temperature for an additional hour. The
 final product was dialyzed exhaustively for 3 days against doubly distilled H₂O,
 20 then lyophilized to obtain a sample of pure oxidized dextran.

Example 7**Analyses of Oxidized Dextran**

The degree of oxidation of the oxidized dextran was determined by
 25 quantifying the aldehyde groups formed using t-butyl carbazate titration via
 carbazone formation. A solution of oxidized dextran (10 mg/ml in pH 5.2 acetate
 buffer) was prepared; and a 5-fold excess *tert*-butyl carbazate in the same buffer
 was added and allowed to react for 24 hrs at ambient temperature, then a 5-fold
 excess of NaBH₃CN was added. After 12 hrs, the reaction product was
 30 precipitated three times with acetone and the final precipitate was dialyzed
 thoroughly against water, followed by lyophilization. The degree of oxidation
 (i.e., abundance of aldehyde groups) was assessed using ¹H NMR by integrating
 the peaks: 7.9 ppm (proton attached to *tert*-butyl) and 4.9 ppm (anomeric proton
 of dextran).

Example 8**Preparation of an Oxidized Dextran / Acrylated Chitosan Gel**

A 1 mL sample of a 1-3% aqueous oxidized dextran in water solution
 5 was mixed with 1 mL of a 1-3% aqueous acrylated chitosan solution. The
 mixture was gently stirred for 10 seconds. Gelation occurred within 30 seconds
 to 10 minutes at temperatures ranging from 5 °C to 37 °C.

Example 9**10 Preparation of Oxidized Hyaluronan**

Sodium hyaluronate (1.0 gram) was dissolved in 80 ml of water in a flask
 shaded by aluminum foil, and sodium periodate (various amounts) dissolved in
 20 ml water was added dropwise to obtain oxidized hyaluronan (oHA) with
 different oxidation degrees. The reaction mixture was incubated at ambient
 15 temperature and 10 ml of ethylene glycol was added to neutralize the unreacted
 periodate following by stirring at room temperature for an additional hour. The
 solution containing the oxidized hyaluronan was dialyzed exhaustively for 3
 days against water, then lyophilized to obtain pure product (yield: 50-67%).

20 Example 10**Analyses of Oxidized Hyaluronan**

The degree of oxidation of oxidized hyaluronan was determined by
 quantifying aldehyde groups formed with t-butyl carbazate titration via
 carbazone formation. A solution of the oxidized hyaluronan (10 mg/ml in pH 5.2
 25 acetate buffer) and a 5-fold excess tertbutyl carbazate in the same buffer were
 allowed to react for 24 hrs at ambient temperature, followed by the addition of a
 5-fold excess of NaBH₃CN. After 12 hrs, the reaction product was precipitated
 three times with acetone and the final precipitate was dialyzed thoroughly
 against water, followed by lyophilization. The degree of oxidation (i.e.,
 30 abundance of aldehyde groups) was assessed using ¹H NMR by integrating the
 peaks: 1.32 ppm (tert-butyl) and 1.9 ppm (CH₃ of hyaluronic acid).

Example 11**Preparation of a PEG-chitosan / Hyaluronan Hydrogels**

A solution of PEG-chitosan (2.5 wt%) and hyaluronan (0.5%) in water at pH in the range of about 3.5 to about 5.5 was made up by first making solutions of the two polymers independently at the stated pH, then mixing the solutions. The mixture rapidly formed a viscous solution.

5 A solution of PEG-chitosan (1.3% w/v, 25 g), hyaluronan (2% w/v, 2.3 g), and 0.1 M HCl (pH 1.45, 0.45 g) was made up. The viscosity increased markedly within about 30 seconds. The pH of the resulting mixture was within the 3.5 to 5.5 range.

10 **Example 12**

Preparation of a Gelatin / Oxidized Hyaluronan Hydrogel

A 20% w/v solution of gelatin in water (1 ml) was mixed with a 20% solution of partially oxidized Hyaluronan (1 ml) (20.3% oxidized). The solution was warmed to about 40-45 °C, above the melting point of the gelatin, and was
15 mixed. At about 37 °C gelation occurs in about 15 minutes. Optionally, a buffer system of 0.1 M borax at pH 9.4 may be used.

Example 13

Application of a Premix to Canine Pericardium *in Vitro*

20 A premix comprising an alkylated chitosan, a polybasic carboxylic acid, a dehydrating reagent and a carboxyl activating reagent in water was made up using two syringes joined by a Luer connector. The somewhat viscous but flowable composition was then applied to a square (about 5 x 5 cm) of damp canine pericardium in which an incision had previously been made. The clear
25 viscous liquid was allowed to stand in place for several minutes. A hydrogel formed and adhered to the tissue such that the pericardium square could be picked up, manipulated and stretched, showing that a clear, elastic hydrogel had sealed the incision. The hydrogel was probed with blunt forceps tips but did not rupture or detach from the surrounding tissue.

30

Example 14

PEG-Chitosan / Hyaluronan Hydrogel Adhesion to Murine Muscle

A premix formed as in Example 13 was disposed on the muscle tissue of a dead mouse. A hydrogel was completely formed after about 10 minutes. A

moderate amount of force applied with a pair of forceps did not either detach the hydrogel from the muscle, or rupture the hydrogel. A high degree of hydrogel elasticity was observed.

5 **Example 15**

**PEG-Chitosan / Hyaluronan Hydrogel Sealing of Full-Thickness
Dermal Wounds in Mouse *in Vivo***

 A wound completely penetrating the thickness of the dermal layer of a living mouse was provided. A premix prepared as in Example 13 was disposed
10 in the wound. After one week, the hydrogel was observed to be in place and detachment had not occurred. Evidence of healing along the original wound bed was observed.

Example 16

15 **Evaluation of PEG-Chitosan / Hyaluronan Hydrogel Adhesion and
Sealing in Canine Dura Mater in Vitro**

 A premix formed as in Example 13 was applied to a sample of canine dura mater bathed in saline at 37 °C. The sealant formed a hard and durable substance within about 90 to 120 seconds of injection. The sample of dura mater
20 was picked up with forceps and gentle pressure was applied to the edges. There was no tearing of the tissue seam.

CLAIMS:

1. A hydrogel for use in sealing a biological tissue in a subject, the hydrogel comprising an alkylated chitosan having amino groups and a polybasic carboxylic acid having carboxylic acid groups, at least some of the amino groups and at least some of the carboxylic acid groups being joined together by amide bonds.
2. The hydrogel of claim 1, further comprising an oxidized polysaccharide having aldehyde groups.
3. The hydrogel of claim 2, wherein the oxidized polysaccharide comprises an oxidized dextran, an oxidized starch, or an oxidized hyaluronan.
4. The hydrogel of claim 1 or 2, wherein the polybasic carboxylic acid comprises an acidic polysaccharide, a hyaluronan, a carboxymethylcellulose, or an alkane α,ω -dicarboxylic acid.
5. The hydrogel of any one of claims 1, 2 or 4, further comprising a carboxyl activating reagent.
6. The hydrogel of claim 5, wherein the carboxyl activating reagent is an N-hydroxy compound.
7. The hydrogel of any one of claims 1, 2, 4, 5, or 6, further comprising a dehydrating reagent.
8. The hydrogel of claim 7, wherein the dehydrating reagent is a carbodiimide.
9. The hydrogel of any one of claims 1 to 8, wherein the alkylated chitosan comprises poly(oxyalkylene) chitosan.

10. The hydrogel of any one of claims 1 to 8, wherein the alkylated chitosan comprises acrylated chitosan.
11. The hydrogel of any one of claims 1 to 10, further comprising a dye material or a radio-opaque material.
12. The hydrogel of any one of claims 1 to 11, further comprising a therapeutic or protective agent.
13. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises an antimicrobial agent.
14. The hydrogel of claim 13, wherein the antimicrobial agent is an antibacterial agent.
15. The hydrogel of claim 14, wherein the antibacterial agent is an aminoglycoside antibiotic, a cephalosporin antibiotic, a carbacephem antibiotic, a glycopeptide antibiotic, a macrolide antibiotic, a penicillin antibiotic, a polypeptide antibiotic, a quinolone antibiotic, a tetracycline antibiotic or a sulfonamide antibiotic.
16. The hydrogel of claim 15, wherein the aminoglycoside antibiotic is gentamicin or streptomycin.
17. The hydrogel of claim 15, wherein the cephalosporin antibiotic is cephalexin or cephtriaxone.
18. The hydrogel of claim 15, wherein the carbacephem antibiotic is loracarbef.
19. The hydrogel of claim 15, wherein the glycopeptide antibiotic is vancomycin.

20. The hydrogel of claim 15, wherein the macrolide antibiotic is erythromycin.
21. The hydrogel of claim 15, wherein the penicillin antibiotic is amoxicillin or ampicillin.
22. The hydrogel of claim 15, wherein the polypeptide antibiotic is bacitracin or polymyxin B.
23. The hydrogel of claim 15, wherein the quinolone antibiotic is ciprofloxacin.
24. The hydrogel of claim 15, wherein the tetracycline antibiotic is oxytetracycline.
25. The hydrogel of claim 13, wherein the antimicrobial agent is an antifungal agent.
26. The hydrogel of claim 25, wherein the antifungal agent is ketoconazole, miconazole or amphotericin B.
27. The hydrogel of claim 13, wherein the antimicrobial agent is an antiviral agent.
28. The hydrogel of claim 27, wherein the antiviral agent is acyclovir or AZT.
29. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises a peptide, a protein, a growth factor, or a bone morphogenic factor.
30. The hydrogel of claim 29, wherein the peptide or protein is an antibody.
31. The hydrogel of claim 29, wherein the growth factor is an interleukin, transforming growth factor- β , fibroblast growth factor or vascular endothelial growth factor.
32. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises a bone powder or a bone substitute.

33. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises a nucleic acid or a nucleic acid analog.
34. The hydrogel of claim 33, wherein the nucleic acid analog is Cytovene®, Epivir®, Gemzar®, Hivid®, Rebetron®, Videx®, Zerit®, or Zovirax®.
35. The hydrogel of claim 33, wherein the nucleic acid analog comprises an antisense nucleic acid or nucleic acid analog, or a small interfering nucleic acid or nucleic acid analog, or a recombinant plasmid.
36. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises an anti-inflammatory agent.
37. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises an anti-cancer agent.
38. The hydrogel of claim 37, wherein the anti-cancer agent is a nucleotide or nucleoside analog, an antineoplastic agent, an adjunct antineoplastic agent, an alkylating agent, a nitrogen mustard, a nitrosourea, an antibiotic, an antimetabolite, a hormonal agonist or antagonist, an androgen, an antiandrogen, an antiestrogen, a combination of estrogen and nitrogen mustard, a gonadotropin releasing hormone (GNRH) analogue, a progestin, an immunomodulator, a photosensitizing agent, or a skin or mucous membrane agent.
39. The hydrogel of claim 38, wherein the nucleotide or nucleoside analog is 2-chlorodeoxyadenosine.
40. The hydrogel of claim 12, wherein the therapeutic or protective agent comprises a radioactive material.

41. The hydrogel of claim 40, wherein the radioactive material is a radionuclide.
42. The hydrogel of claim 41, wherein the radionuclide is technetium-99m, iodine-123, iodine-125, iodine-131, or gallium-67.
43. The hydrogel of any one of claims 12 to 42, wherein the therapeutic or protective agent is further contained within a microsphere or a nanosphere.
44. The hydrogel of any one of claims 1 to 43, wherein the alkylated chitosan has a degree of substitution of a chitosan backbone with alkyl groups in the range of from about 0.25 to about 0.5.
45. The hydrogel of claim 44, wherein the degree of substitution is from about 0.25 to about 0.45.
46. The hydrogel of claim 44, wherein the degree of substitution is about 0.5.
47. The hydrogel of any one of claims 1 to 46, wherein the alkylated chitosan concentration is about 3% to about 7% by weight.
48. The hydrogel of any one of claims 1 to 46, wherein the alkylated chitosan concentration is about 5% by weight.
49. The hydrogel of any one of claims 2 to 48, wherein the oxidized polysaccharide concentration is about 0.1% to about 5% by weight.
50. The hydrogel of any one of claims 2 to 48, wherein the oxidized polysaccharide concentration is about 0.3% to about 0.7% by weight.

51. The hydrogel of any one of claims 2 to 48, wherein the oxidized polysaccharide concentration is about 0.5% by weight.
52. The hydrogel of any one of claims 1 to 51, wherein the polybasic carboxylic acid concentration is about 0.1% to about 5% by weight.
53. The hydrogel of any one of claims 1 to 51, wherein the polybasic carboxylic acid concentration is about 0.3% to about 0.7% by weight.
54. The hydrogel of any one of claims 1 to 51, wherein the polybasic carboxylic acid concentration is about 0.5% by weight.
55. The hydrogel of any one of claims 1 to 54, wherein the hydrogel is adapted to adhere to biological tissue of the subject to adhesively seal the tissue.
56. The hydrogel of any one of claims 1 to 55, wherein the subject is a human.
57. A composition comprising the hydrogel of any one of claims 1 to 56, wherein the hydrogel, when applied to a living biological tissue, adheres to the living biological tissue.
58. A wound closure device comprising the hydrogel of any one of claims 1 to 56.
59. The device of claim 58, further comprising a tissue clip.
60. The device of claim 58, further comprising a suture clip.
61. Use of the hydrogel of any one of claims 1 to 56 for sealing a biological tissue of a subject.

- 62. The use of claim 61, wherein the biological tissue comprises a meninx of a nervous system organ.
- 63. The use of claim 62, wherein the meninx comprises dura mater.
- 64. The use of claim 62, wherein the nervous system organ comprises brain or spinal cord.
- 65. The use of claim 61, wherein the biological tissue comprises blood vessels or musculature.
- 66. The use of claim 61, wherein the biological tissue comprises the annulus of an intervertebral disk.
- 67. The use of claim 61, wherein the biological tissue comprises cartilage or bone.
- 68. The use of claim 61, wherein the biological tissue comprises neural tissue or dermal tissue.
- 69. The use of claim 68, wherein the hydrogel is capable of sealing a laceration, cut, abrasion, or disruption of the dermal tissue.
- 70. The use of any one of claims 61 to 69, wherein the sealing comprises adhesively sealing.
- 71. The use of claim 61, wherein the hydrogel is capable of adhesively sealing the biological tissue against leakage of a biological fluid.
- 72. The use of claim 71, wherein the biological fluid is cerebrospinal fluid.

73. The use of claim 61, wherein the hydrogel is for providing reinforcement of a sutured biological tissue.

74. The use of any one of claims 61 to 73, wherein the biological tissue is disrupted by an injury or by a surgical procedure.

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Figure 1

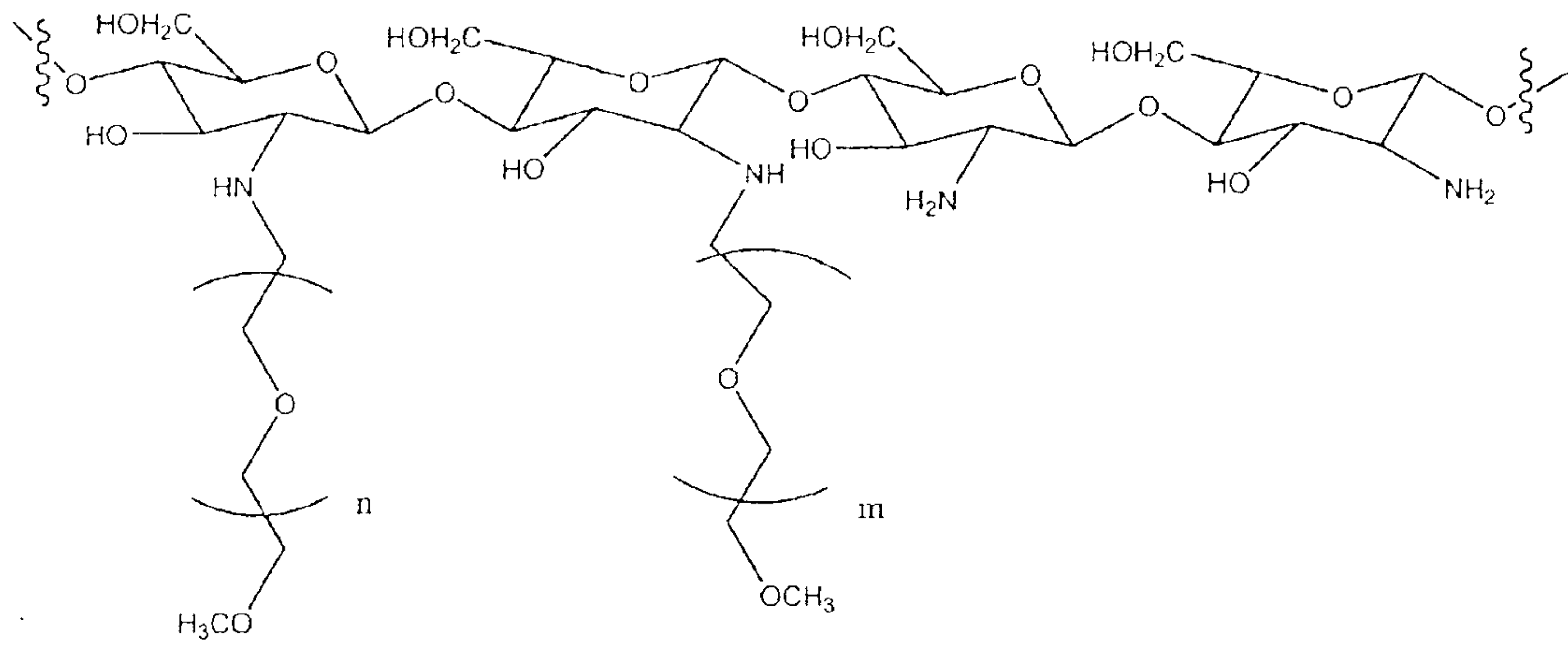


Figure 2

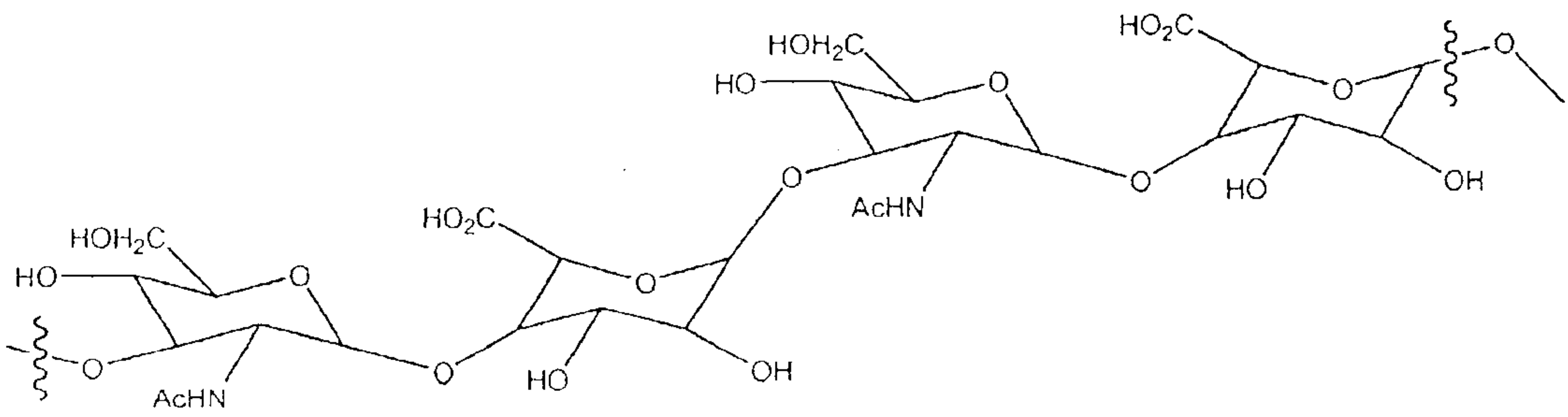


Figure 3

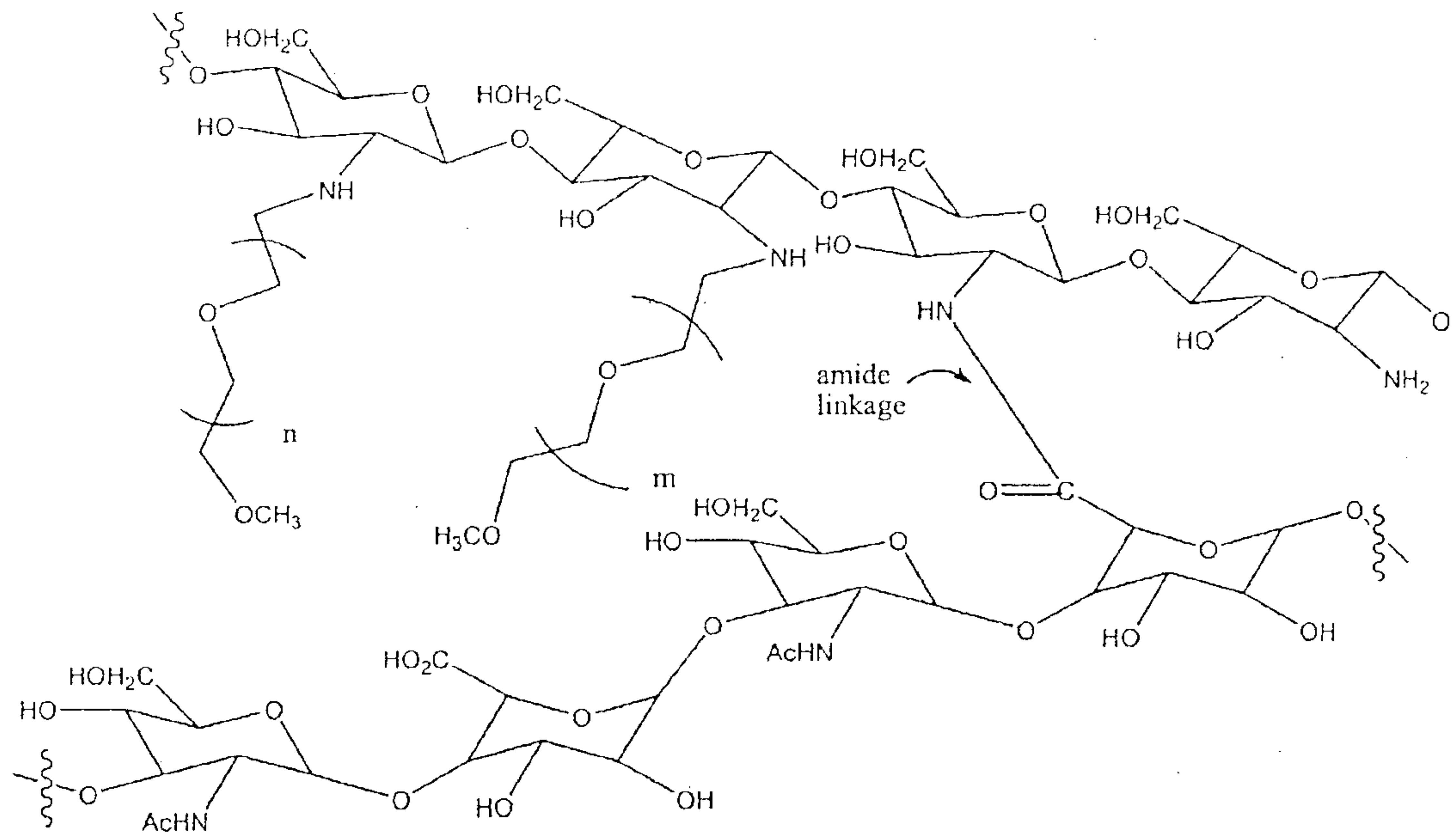


Figure 4

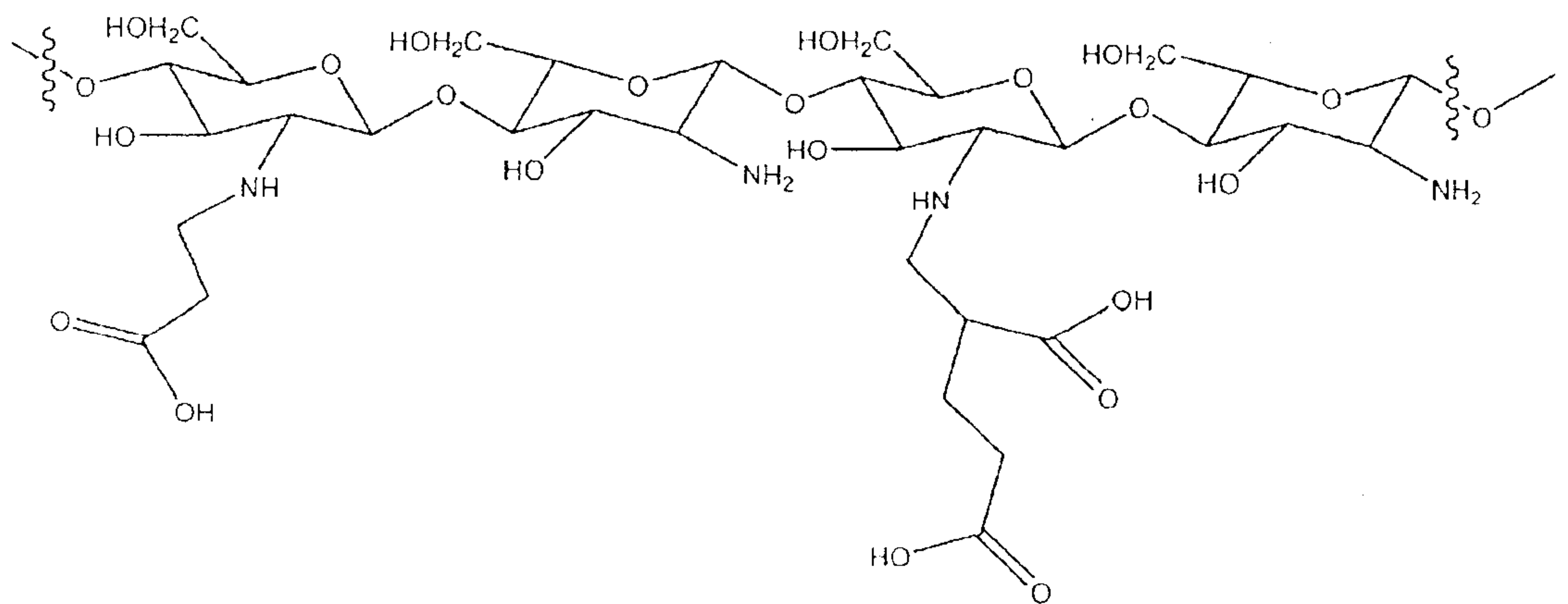


Figure 5

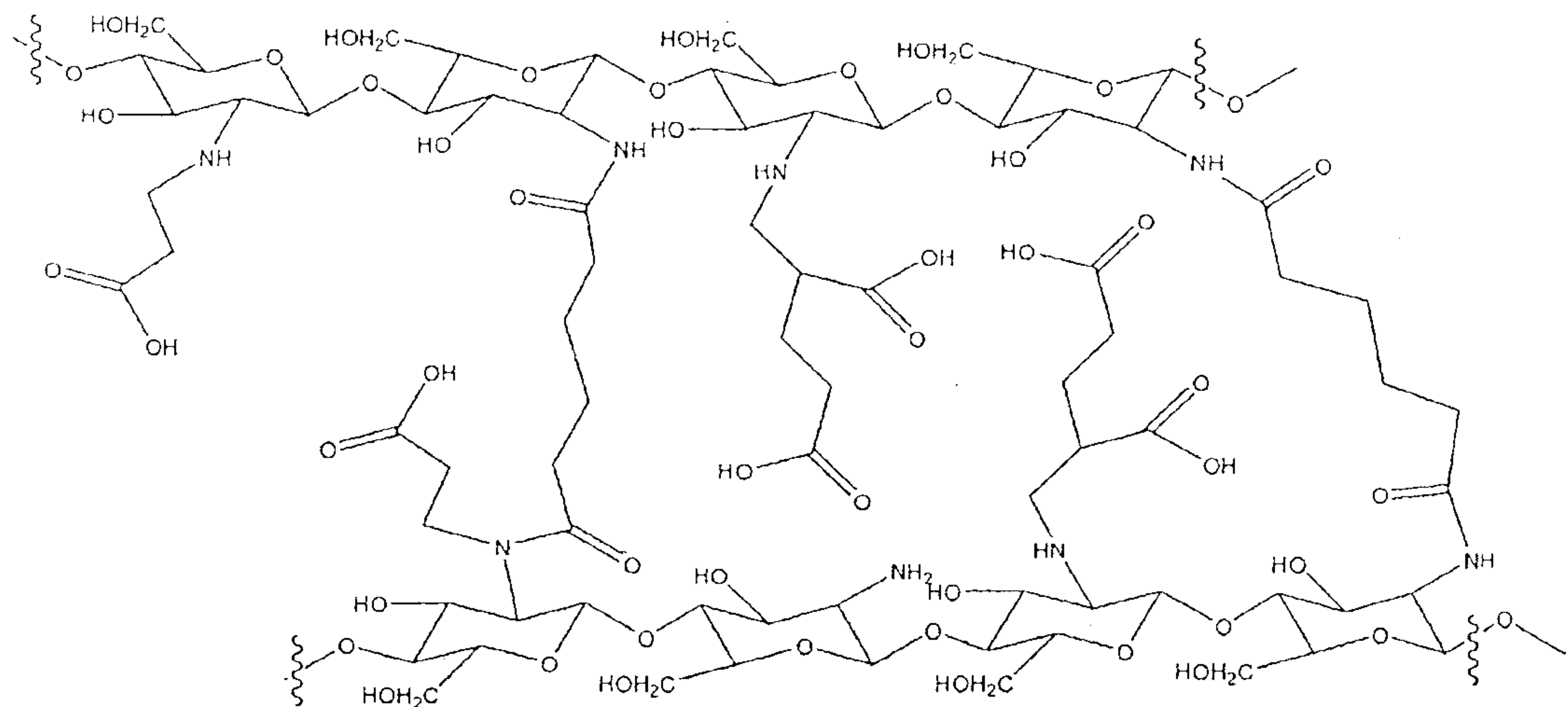


Figure 6

