(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 December 2019 (05.12.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/231763 A1

(51) International Patent Classification:
A61L 24/00 (2006.01) *C08L 99/00* (2006.01)
A61L 24/04 (2006.01) *A61K 6/087* (2006.01)
A61L 26/00 (2006.01)

(21) International Application Number:
PCT/US2019/033324

(22) International Filing Date:
21 May 2019 (21.05.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/676,987 27 May 2018 (27.05.2018) US

(72) Inventor; and
(71) Applicant: **ANGELETAKIS, Christos** [US/US]; 4 Fabius Ct., Bear, Delaware 19701 (US).

(74) Agent: **MENDELSON, Elliot**; 233 S. Wacker Dr., Suite 8400, Chicago, Illinois 60606 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2019/231763 A1

(54) **Title:** TISSUE ADHESIVES AND SEALANTS USING NATURALLY DERIVED ALDEHYDES

(57) **Abstract:** The present invention a hydrogel or aqueous-based biodegradable crosslinked polymer that is a reaction product of a polymer having aldehyde-reactive functional groups and a naturally derived dialdehyde in the presence of a polyol additive to improve stability as well as other additives such as buffers and fillers to modify the properties of the hydrogel for the specific use. This hydrogel may be useful as a sealant for dental and medical applications and as a tissue adhesive.

TISSUE ADHESIVES AND SEALANTS USING NATURALLY DERIVED ALDEHYDES

This application claims priority to U.S. Application No. 62676987, filed 05/27/2018, which is incorporated herein in its entirety.

FIELD OF THE INVENTION

This invention relates to a hydrogel or aqueous-based biodegradable crosslinked polymer which is a reaction product of a polymer having aldehyde-reactive functional groups and a naturally derived dialdehyde in the presence of a polyol additive to improve stability as well as other additives such as buffers and fillers to modify the properties of the hydrogel for the specific use. This hydrogel may be useful as a sealant for dental and medical applications and as a tissue adhesive.

BACKGROUND OF THE INVENTION

Hydrogels are a common class of biomaterials utilized in a wide range of applications including as tissue adhesives, tissue engineering scaffolds, drug delivery vehicles, or as sealants and space filling agents. There are two methods for achieving gelation of the synthetic materials. One is by an external stimulus applied on a one-part material to result in gel formation. Stimuli such as pH, temperature or addition of water are commonly used. The second method is by mixing two components and gelation is achieved by rapid crosslinking reactions induced by mixing. Hydrogels made by the first method are generally weak and are not effective as adhesives or sealants. The second method is preferred since the kinetics of the crosslinking can be controlled and are not dependent on how the environment where the material is applied.

Tissue adhesives and sealants are known in interventional medicine for many years. Surgical adhesive compositions for tissue are also well known such as described in US5385606. Various biomedical applications of the subject hydrogels include those described in U.S. Pat. Nos. 3438374; 5092841; 5292362; 5583114; 5843156; 6162241; 6290729; 6302898; 6310036; 6329337; 6371975; 6,372,229; 6423333; 6458147; 6475182; and 6547806. These adhesives are usually two-part systems typically comprised of a polymer having aldehyde-reactive functional groups such as a water soluble proteinaceous material (e.g., albumin, particularly bovine or human serum albumin) and glutaraldehyde or its

polymers in appropriate amounts and allowing the combined mixture to react in situ on the tissue surface or surfaces to be bonded. Minimal use of sutures can be achieved in this manner. In a similar fashion these two-part systems can be used as sealants for applications in the lung or other body tissues. A major advantage of these two-part systems is that these materials are absorbable materials which are intended to bond and/or seal tissue as it heals and then be absorbed over a period of time.

Glutaraldehyde, a C5 dialdehyde and its polymers (see US9101536 where a heat-treated glutaraldehyde solution is described), is known to quickly react with proteins to give hydrogels commonly used in biomedical applications. Additionally, use of C6 to C10 alicyclic or aromatic dialdehydes and their polymers is mentioned in US9783650. Reaction of glutaraldehyde with albumin polymers causes mainly a decrease in lysine together with lesser losses of tyrosine, histidine and arginine. This occurs via covalent bonding by forming conjugated Schiff's bases primarily with amino groups of lysine and amino bonds of the other N-terminal amino acids. Insoluble cross-linked aggregates result with a minimal amount of amino acid modification. This reaction has been used for the formulation of tissue adhesives and sealants in medical applications.

However, the ratio of proteinaceous substrate to the dialdehyde that is required to give a hydrogel that cures quickly enough for the application and resists flow results in a concentration of Glutaraldehyde which is toxic. Moreover, a small excess of Glutaraldehyde is generally used to allow the hydrogel to bond to the tissue substrate and function as an adhesive. Glutaraldehyde toxicity is well known. At concentrations of 0.75% or greater, glutaraldehyde produces significant tissue necrosis. Concentrations below this level produce limited local toxicity associated with clinically acceptable side effects for many applications. See, for example, Speer, D. P. et al. J. Biomedical Mat. Res. 1980, 14, 753; Huang-Lee, L. L. H. et al. J. Biomedical Mat. Res. 1990, 24, 1185; Fuerst, W. et al. Ann. Thorac. Surg. 2005, 79, 1522; and Zeiger, E. et al. Mutation Res. 2005, 589, 136. This has been addressed by substituting glutaraldehyde in some of these adhesives with pre-polymerized glutaraldehyde to reduce this toxicity, but its polymerization is reversible resulting in small amounts still to be present.

A commercially available albumin/glutaraldehyde tissue glue is Bioglue® (Cryolife) commonly used in heart surgery which contains 36 w % bovine serum albumin and 2% w % Glutaraldehyde (final concentrations after mixing) polymerizes rapidly, within a few seconds.

However, for such a glue to be efficacious for certain applications such as bronchoscopic lung volume reduction BLVR), it must have a long working time, about 5 minutes, to allow delivery through a catheter while spillage is avoided at the treatment site. Because of the rapid rate of polymerization of Bioglue® (starts to thicken in less than thirty seconds), the Bioglue® composition is not well suited for such procedures. Moreover, decreasing the ratio of bovine serum albumin to glutaraldehyde to achieve the desired polymerization kinetics results in a glutaraldehyde concentration which is highly toxic. Therefore, there is a need for a less toxic crosslinker. In dentistry, when there is direct pulp exposure, pulp withdrawal and necrosis is expected. Endodontic procedures are then performed such as removal of the tooth pulp. With deciduous teeth a pulp capping agent is used such as formocresol or ferric sulfate. During restorative procedures, when the remaining dentin thickness (RDT), defined as the distance between the cavity floor and pulp tissue, is less than 0.5 mm alkaline and calcium releasing liners are used as they may decrease the long-term inflammatory response possibly by stimulating the formation of reparative dentin. However, the pulp withdraws during the process, with the general outcome of making the tooth more brittle and more subject to fracture. Portland cement or mineral trioxide aggregate (MTA) is an alternative pulp capping material that is more biocompatible, but it has a poor delivery system and a long setting time of approx. 4 hours. There is thus a need for a non-toxic resorbable curable material that can be used as a cavity lining material in restorative dentistry for assisting the regeneration of the dental pulp by acting as a scaffold and that reliably seals the pulp cavity during a dental restorative procedure.

There can be other uses for a resorbing curable material in restorative dentistry. For example, there is a need in dentistry for a wound dressing that degrades over time. Post-surgical sites intraorally are subject to food bolus trauma as well as tooth brushing that can irritate the site and delay healing. A wound dressing that can remain in place after surgery to protect the site is important – if the dressing is additionally resorbing, then an additional benefit is obtained.

Another application for a resorbing curable material is for care of furcation involvement during endodontic surgery. The area at the root furcation can often have iatrogenic involvement extending through the tooth structure and into the underlying bone. Placing a resorbable material that is compatible with the bone is important during the surgical treatment to seal off the area and promote healing. A related application would be anytime

the root structure needs to receive palliative treatment – i.e. fracture, or again iatrogenic puncturing of the root during root canal treatment.

Therefore, hydrogels for dental and medical applications that contain all-natural or plant extract ingredients and thereby have reduced toxicity can avoid the use of glutaraldehyde or its polymers will be an advancement in the art.

SUMMARY OF THE INVENTION

The present invention is based on the discovery that certain natural dialdehydes when mixed with water or water mixed with a hydrophilic solvent, react with polymers having aldehyde-reactive functional groups such as aqueous solutions of proteins to give hydrogels. These hydrogels may contain additives used to modify its properties for the specific application. These hydrogels have utility for use in biomedical applications.

Natural dialdehydes are known minor ingredients occurring in several plant species, most commonly as secoiridoid compounds in plants of the family Oleaceae and derivatives thereof.

The main compounds of interest are the hydroxy tyrosol ester of elenolic acid (oleuropein), the hydroxyl tyrosol ester of decarboxymethyl elenolic acid (oleacein) and the tyrosol ester of decarboxymethyl elenolic acid (oleocanthal). Elenolic acid and decarboxymethyl ester of elenolic acid also naturally occurring are also of interest. All these compounds are dialdehydes in at least one of their tautomeric forms and are reactive with proteins to give hydrogels. Additionally, these ester compounds release tyrosol or hydroxy tyrosol upon resorption, both dietary antioxidants.

Specific proteins that can be used to react with the natural dialdehydes are, but are not limited to: albumins, collagens, elastins, fibrins, and the like. More specifically Bovine Serum Albumin (BSA) is preferred.

In a preferred embodiment of the present invention, the hydrogel composition contains at least one glycol or polyol containing 2 to 1000 carbon atoms in a molar ratio of said glycol or polyol to said dialdehyde to sufficiently displace the water of hydration from binding to said dialdehyde in favor of binding to said glycol or polyol, such that deterioration

of the mono or dialdehyde by internal polymerization is no more than 20 percent over two years at room temperature.

Biologically active agents may be included, e.g., bone growth factors, tissue activators, cartilage growth activators, small molecule active agents. The hydrogel of the present invention is typically employed in methods where a quantity of the hydrogel is delivered to a particular site or location of a subject, patient or host in need thereof. The subject, patient or host are either human or animal. The solutions or suspensions can be dispensed by a controlled delivery system or mixed manually to form the hydrogel of the present invention.

The hydrogel is useful as a tissue adhesive or sealant for medical applications including but not limited to, ophthalmic applications; neurosurgery applications, adhesion prevention to prevent undesired tissue to tissue adhesions resulting from trauma or surgery; and as a hemostat sealant. Other applications are bronchoscopic lung volume reduction procedures, in cardiovascular surgery as a tissue adhesive especially for heart surgery. Orthopedic applications are also possible, such a bonding fractured bone. For many of the above applications glutaraldehyde-based adhesives and sealants are used commercially and use of the hydrogel of the current invention that is based on naturally derived dialdehydes has advantages of lower toxicity and better biocompatibility.

The present invention provides a biodegradable, resorbing curable composition for use in an intraoral treatment or procedure, and the use of composition for the manufacture of a medicament for treating an intraoral surface. In one embodiment of the present invention, a resorbing curable cavity lining material is provided that protects the pulp during and after restorative procedures, such as from the harsh chemicals used in acid etching that lead to inflammation of the pulp and from bacteria. After the restoration is placed, this lining material slowly degrades while acting as a scaffold for new cell growth in the remaining space. In accordance with the present invention, the resorbing material, such as a degradable cavity liner, is the product of a two-part curable protein/aldehyde composition.

In addition to the above, this resorbing curable material can have other applications in dentistry such as use as a post oral surgery dressing and adhesive and as a biodegradable adhesive for repair of tooth fractures. The hydrogel may also be useful as a tissue adhesive or

sealant for dental as well as medical applications, including but not limited to, ophthalmic and orthopedic applications.

Specifically, the claimed compositions use exclusively naturally derived components and exhibit substantially reduced inflammation when compared with tissue adhesives and sealants in which regular glutaraldehyde or its polymers are employed. This makes the claimed compositions a significant improvement over the art.

DETAILED DESCRIPTION

The present invention is based on the discovery that the certain natural dialdehydes when diluted to a concentration of about 1M (moles/Liter) in water or water mixed with a hydrophilic solvent, react with polymers having aldehyde-reactive functional groups such as aqueous solutions of proteins to give hydrogels. These hydrogels may contain additives in order to modify its properties for the specific use. These hydrogels have utility for use in biomedical applications.

Natural dialdehydes are known minor ingredients occurring in several plant species, most commonly as secoiridoid compounds in plants of the family Oleaceae and derivatives thereof.

The main secoiridoid compounds of interest are Elenolic acid, decarboxymethyl elenolic acid and their esters. Examples of these esters are the hydroxy tyrosol ester of elenolic acid (oleuropein), the hydroxy tyrosol ester of decarboxymethyl elenolic acid (oleacein), the tyrosol ester of decarboxymethyl elenolic acid (oleocanthal) as well as the tyrosol ester of elenolic acid (ligstroside). All these compounds exist as dialdehydes in one of their tautomeric forms and are reactive with proteins to give hydrogels.

Additionally, these ester compounds release tyrosol or hydroxy tyrosol upon resorption, both dietary antioxidants having desirable systemic effects.

In 1993, Montedoro and co-workers reported the isolation of ligstroside and oleuropein from virgin olive oils (Montedoro, G. et al. J. Agric. Food Chem. 1993, 41, 2228). These phenolic compounds comprise important minor constituents of virgin olive oils that have been implicated in the organoleptic characteristics including bitterness, pungency, and astringency (Andrewes, P. et al. J. Agric. Food Chem. 2003, 57:1415). In addition, these

agents have been suggested to contribute to the oxidative stability of virgin olive oil and as such are associated with health benefits of olive oils, specifically their antioxidant/anticancer activities (Owen, R. W. et al. Food Chem. Toxicology, 2000, 38, 647; Owen, R. W. et al. (2000) Eur. J. Cancer, 2000, 36, 1235-1247; Baldioli, M. et al. J. Am. Oil Chem. Soc. 1996, 73, 1589; Manna, C. et al. J. Agric. Food Chem. 2002, 50, 6521).

These secoiridoid compounds are also present in other plant species. For example, oleacein is obtained from Ligustrum vulgare L. Similar structural features have been reported in the constituents of the Jasminum (Somanadhan, B. et al. Planta Medica 1998, 64, 246; Takenaka, Y. et al. Chem. & Pharm. Bull. 2002, 50, 384) and related plant species (Takenaka, Y. et al. Phytochemistry 2002, 59, 779).

Aqueous solutions of the natural dialdehydes of this invention are mixed with solutions of polymers having aldehyde-reactive functional groups such as proteins to give hydrogels by crosslinking. The two part composition, prior to mixing, includes a protein solution and an aldehyde solution. The volume ratio of the two aqueous solutions can be 10:1 to 1:1 in favor of the aldehyde solution. Optionally, the protein and/or the aldehyde solution can contain additives such as adhesion modifiers, plasticizing agents, foaming agents, fillers and biologically active agents. (e.g., therapeutic compound(s), stabilizing compound(s), antibiotic(s), growth factor(s), etc.), buffers, salts, surfactants, anti-surfactants, lipids, excipients, and/or other suitable compounds. In certain embodiments, compositions of the invention may be sterilized.

The cross-linkers of the present invention are naturally derived di- or polyaldehydes. As will be appreciated by one skilled in the art, the aldehydes described herein can exist as hydrates in aqueous solution, e.g., existing as hemi-acetals in aqueous solution. In certain embodiments, such hydrates can revert to the corresponding aldehyde for cross-linking. In some embodiments of this invention, alcohols or polyols are added to the aldehyde phase to form hemiacetals and thereby improve its stability and thereby prevent decomposition. These hemiacetals can revert to the corresponding aldehyde for crosslinking. Use of such additives has been described in the case of glutaraldehyde solutions (see i.e. US4436754) Alcohols such as isopropanol, butanol and the like or diols such as triethylene glycol or higher ethylene glycols such as polyethylene glycol 200 or polyethylene glycol 400 can be used.

In a preferred embodiment of the present invention, the hydrogel composition contains at least one glycol or polyol containing 2 to 1000 carbon atoms in a molar ratio of said glycol or polyol to said dialdehyde to sufficiently displace the water of hydration from binding to said dialdehyde in favor of binding to said glycol or polyol, such that deterioration of the mono or dialdehyde by internal polymerization is no more than 20 percent over two years at room temperature.

The proteinaceous component of the substrate is made up of one or more distinct proteins. The proteins of this component may be either synthetic or naturally occurring proteins, where the proteins may be obtained/prepared using any convenient protocol, e.g., purification from naturally occurring sources, recombinant production, synthetic production, and the like, where in certain embodiments the proteins are obtained from naturally occurring, e.g., bovine or human, sources. Specific proteins of interest include, but are not limited to: albumins, collagens, elastins, fibrins, and the like.

Albumin refers generally to any protein with water solubility, which is moderately soluble in concentrated salt solutions, and which experiences heat coagulation (protein denaturation). Substances containing albumin, such as egg white, are called albuminoids. The most well-known type of albumin is serum albumin in the blood, but there is also the storage protein ovalbumin in egg white, and other storage albumins in the seeds of some plants.

Serum albumin is the most abundant blood plasma protein and is produced in the liver and forms a large proportion of all plasma protein. Human serum albumin, a water-soluble protein of 585 amino acids with a molecular weight of 66 kD, is the most abundant protein in plasma (3.5-5.0 g/100 mL in blood plasma), but also exists in lower concentrations in extra vascular fluids such as the fluid occurring in dentinal tubules. It has a large number of charged amino acids (about 100 negative charges and 100 positive charges) with an isoelectric point of 5.0 and a net negative charge of −15 at a plasma pH of 7.4 and attracts both anions and cations. In certain embodiments, the albumin protein of the invention is a mammalian serum albumin, human serum albumin, porcine serum albumin and/or bovine serum albumin (BSA) or a recombinant protein. BSA is preferred as is also commonly available.

Adhesion modifiers may be present in some embodiments to improve the adhesive strength of the sealant or adhesive to the biological surface. In some embodiments, the

adhesion modifiers are polymeric compounds having charged functionalities, e.g., amines, etc. Among the numerous adhesion modifiers that may be used, polyethyleneimine (PEI) is preferred. PEI is a long chain branched, alkyl polymer containing primary, secondary and tertiary amines. The presence of these highly ionic groups results in significant attachment through ionic interactions with the underlying surface. In addition, the presence of PEI in the substrate significantly enhances the presence of amine terminals suitable to produce crosslinks with the crosslinking agent. Additional adhesion modifiers of interest include, but are not limited to: gelatin, carboxymethylcellulose, butylhydroxytoluene, etc. Chitosan and acetylated chitin to improve sealing of injuries for use as a sealant for blood control.

Plasticizing Agents may be present in some embodiments in the substrate. These can improve mixing, wetting of a biological surface, or modify the elastic modulus of the hydrogel and improve its strength. Numerous plasticizing agents exist, including polyvinyl pyrrolidone (NVP), dextran and polyacrylic acid and its salts. Addition of PVP or dextran shortens polymerization time such that lower concentrations of albumin and/or dialdehyde can be used. This effect may be used to improve the deliverability and/or biocompatibility of the hydrogel of the present invention with appropriate polymerization characteristics. NVP is preferred since can also increase the strength properties of the hydrogel. Other plasticizers are fatty acids, e.g., oleic acid, palmitic acid, etc., phospholipids, and phosphatidic acid. Some of these plasticizers have low water solubility, so it is necessary to increase their miscibility with water by premixing the appropriate plasticizer with an alcohol to reduce the surface tension associated with the solution. Many alcohols may be used for this purpose. In one embodiment of this invention, oleic acid is mixed with ethanol to form a 50% (w/w) solution and this solution then is used to plasticize the proteinaceous substrate during the formulation process. Whereas the type and concentration of the plasticizing agent is dependent upon the application, in certain embodiments the final concentration of the plasticizing agent is from about 0.01 to 20% (w/w). Other plasticizing agents of interest include, but are not limited to: polyethylene glycol, glycerin, butylhydroxytoluene, etc.

In certain embodiments, the substrate may include a foaming agent which, upon combination with the crosslinker composition, results in a foaming composition, e.g., compositions that includes gaseous air bubbles interspersed about. Any convenient foaming agent may be present, where the foaming agent may be an agent that, upon contact with the crosslinking composition, produces a gas that provides bubble generation and, hence, the

desired foaming characteristics of the composition. For example, a salt such as sodium bicarbonate in an amount ranging from about 2 to about 5% w/w may be present in the substrate. Upon combination of the substrate with an acidic crosslinker composition, e.g., having a pH of about 5, a foaming composition is produced.

Biocompatible fillers as particles or fibers can also be included in the substrate to form a stable suspension. Examples of such particles are nanosized hydroxyapatite, mineral trioxide aggregate (MTA) and nanosilicas. These particles can also be therapeutic agents in solid form such as antibiotics that can slowly dissolve resulting in a therapeutic effect. Reinforcing fillers may be included, such as chopped fibrous silk, polyester, PTFE, NYLON, carbon fibers, polypropylene, polyurethane, glass, etc. Fibers can be modified, e.g., as described above for the other components, as desired, e.g., to increase wettability, mixability, etc. Reinforcing fillers may be present from about 0 to 40%, such as from about 10 to about 30%. Non-reinforcing fillers may also be included, e.g., clay, mica, hydroxyapatite, calcium sulfate, bone chips, etc. Where desired, these fillers may also be modified, e.g., as described above. Non-reinforcing fillers may be present from about 0 to 40%, such as from about 10 to about 30%.

Biologically active agents may be included, e.g., bone growth factors, tissue activators, cartilage growth activators, small molecule active agents such as N-acetyl cysteine (NAC), etc. NAC is favored as will act as a Reactive Oxygen Species (ROS) scavenger, particularly useful when placed on tissues such as dental pulp that may be exposed to acrylic monomers (see Krifka S. et al, Biomaterials, 2012 33, 2012, 740). An amount of NAC of 0.1% to 0.7% (w/w) in the subject hydrogel can be used. This concentration may also be effective for enhancing osteoblastic differentiation in the case of orthopedic applications as described in US8101198.

Upon mixing the polymer having aldehyde-reactive functional groups and the naturally derived dialdehyde to produce the subject hydrogel, buffering of this resultant hydrogel is important for several reasons, e.g., to optimize the bonding strength of the composition to the attaching surface, to optimize the conditions necessary for internal crosslinking to occur, etc. For example, optimum crosslinking for proteins using dialdehyde crosslinkers occurs at pH range from about 6 to about 8. Buffers capable of maintaining this range are useful in this invention, if they do not interfere with the carbonyl terminal of the crosslinker or modify the amine terminus of the amino acids. For example, phosphate buffer

up to 1M in strength is suitable for use as a buffer in the present invention, and preferably about 0.2M in strength. While phosphate buffering of the solutions is ideal for the stability of the protein substrate in applications where increased adhesion is required, an acidic buffer may be used as well, such as citrate buffer at 0.1- 1M concentration and having a pH range of about 4.5 to about 6. The buffer may be present in either component, or present in both components, as desired.

The hydrogel of the present invention is typically employed in methods where a quantity of the hydrogel is delivered to a particular site or location of a subject, patient or host in need thereof. The subject, patient or host are either human or animal. This hydrogel is locally delivered to a particular region, site or location of the host, where the site or location may vary Representative sites or locations include, but are not limited to: vessels, organs, and the like. Depending on the application, the composition may be delivered to the site of interest manually or with a delivery device, e.g., the delivery device employed to deliver the composition in stenting applications, described in greater detail below.

The solutions or suspensions can be dispensed by a controlled delivery system, such as one that includes a reusable delivery device, applicator tips, and applicator tip extenders. Once dispensed, the solutions are mixed in a predefined ratio in the applicator tip where cross-linking begins. In one embodiment of this invention, the delivery system can be like the ones used for tissue adhesives, two-part dental cements or dental impression materials such as Mixpac ™ (Sulzer Co. Grabs Switzerland). The aldehyde molecules covalently bond (cross-link) the protein molecules to each other and, upon application, to the tissue proteins i.e. dentin and/or pulp, creating a flexible mechanical seal. The protein/aldehyde composition begins to polymerize within seconds, for example about 15 to about 30 seconds, and reaches its bonding strength within about 2 minutes, and generally in less than 1 minute. In another embodiment plasticizer additives can be used to extend the working time in order to deliver the uncured hydrogel through a tube, as this is convenient for delivery inside the lung. Good adhesion is obtained to wet surfaces, thus providing usefulness for use in the body.

After placement and cure, the resultant hydrogel composition begins to slowly biodegrade at a rate dependent on the environment. Factors affecting the rate of degradation include the rate of fluid turnover and the availability of proteolytic enzymes. As the hydrogel degrades, it acts as a scaffold for new cell growth in the remaining space. If the liner contains

particles, these can assist in reinforcing the structure of the scaffold. As a scaffold, the liner is effective for the transport of nutrients, oxygen and waste.

As summarized above, the subject hydrogel compositions are prepared by combining a polymer having aldehyde-reactive functional groups and a naturally derived dialdehyde in appropriate amounts and under conditions sufficient for the hydrogel composition to be produced. The, substrate and crosslinker are typically combined in a ratio (v/v) ranging from about 1:5 to about 10:1; so that a resultant hydrogel composition is produced in which the total protein concentration typically ranges from about 10 to about 60%, such as from about 20 to about 50%, including from about 30 to about 40% and the total crosslinker compositions typically ranges from about 0.1 to about 20%, such as from about 0.5 to about 15%, including from about 1 to about 10%.

The hydrogel is useful as a tissue adhesive or sealant for medical applications including but not limited to, ophthalmic applications such as sealing wounds resulting from trauma such as corneal lacerations, or from surgical procedures such as vitrectomy procedures, cataract surgery, LASIK surgery, glaucoma surgery, and corneal transplants; neurosurgery applications, such as sealing the dura; as a plug to seal a fistula or the punctum; adhesion prevention to prevent undesired tissue to tissue adhesions resulting from trauma or surgery; and as a hemostat sealant. Other applications are bronchoscopic lung volume reduction procedures, in cardiovascular surgery as a tissue adhesive especially for heart surgery. Orthopedic applications are also possible, such a bonding fractured bone. For many of the above applications glutaraldehyde-based adhesives and sealants are used commercially and use of the hydrogel of the current invention that is based on naturally derived dialdehydes has advantages of lower toxicity and better biocompatibility.

The present invention also provides a biodegradable, resorbing curable composition for use in an intraoral treatment or procedure, and the use of composition for the manufacture of a medicament for treating an intraoral surface. In one embodiment of the present invention, a resorbing curable cavity lining material is provided that protects the pulp during and after restorative procedures, such as from the harsh chemicals used in acid etching that lead to inflammation of the pulp and from bacteria. After the restoration is placed, this lining material slowly degrades while acting as a scaffold for new cell growth in the remaining space. In accordance with the present invention, the resorbing material, such as a degradable cavity liner, is the product of a two-part curable protein/aldehyde composition.

In an exemplary embodiment of the present invention, a method for the protection and regeneration of dental pulp in a dental restorative procedure comprises preparing a tooth area to provide a cavity floor within the tooth, wherein a remaining dentin thickness between the cavity floor and the dental pulp is less than about 0.5 mm, and applying to the cavity floor a solution of a proteinaceous material and an aldehyde and allowing a polymerization reaction to occur to thereby form a biodegradable cavity liner comprising the proteinaceous material in cross-linked form of gel consistency. With respect to use, the solution of the proteinaceous material and the aldehyde is used for the manufacture of a medicament, wherein after the medicament is applied to the cavity floor, the polymerization reaction is allowed to occur to thereby form the biodegradable cavity liner to protect and regenerate dental pulp.

The method may further comprise etching the remaining tooth area with an etchant, such as an aqueous phosphoric acid or polyacrylic acid solution, after applying the liner solution in the deepest part of the cavity and allowing it to cure, whereby the biodegradable cavity liner prevents the etchant from contacting the dental pulp and applying a restorative composition to the tooth area after etching to restore the tooth.

In addition to the above, this resorbing curable material can have other applications in dentistry such as use as a post oral surgery dressing and adhesive and as a biodegradable adhesive for repair of tooth fractures. Thus, in its broadest form, the present invention provides a method for treating an intraoral surface, comprising applying to the intraoral surface a solution of a proteinaceous material and an aldehyde and allowing a polymerization reaction to occur to thereby form a biodegradable material comprising the proteinaceous material in cross-linked form. With respect to use, the solution of the proteinaceous material and the aldehyde is used for the manufacture of a medicament for treating an intraoral surface, wherein after the medicament is applied to the intraoral surface, the polymerization reaction is allowed to occur to thereby form the biodegradable material.

EXAMPLE

Two solutions are prepared. Solution A contains 30 parts polyethylene glycol 200, 30 parts Oleocanthal (derived from olive fruit and its content assayed by 1H NMR and additionally the aldehyde content is assayed using the hydroxylamine titration method (A. R. Barnes,et al, Pharmaceutica Acta Helvetiae, 1994, 69, 21)) and 40 parts water. Solution B contains 40 parts BSA and 60 parts water. Solutions A and B are loaded in a 5 mL Mixpac

1:4 double barreled syringe and a mixing tip is mounted. Upon extrusion of about 0.5 mL of the combined solutions, a hydrogel begins to form after 15 seconds and its formation is complete after two minutes as judged by poking with a plastic dental stick.

The subject biocompatible hydrogel compositions are typically employed in methods where a quantity of the hydrogel composition is simultaneously mixed and delivered through an in-line mixing/dispensing tip directly to the site. Alternatively, the hydrogel composition is premixed and then applied to the site in need of the biomaterial. By using either of the two methods, delivery occurs to a particular site or location on a subject, patient or host in need thereof. The subjects may be animals or humans.

1. A cross-linked polymer characterized by comprising a reaction product of a first solution containing a secoiridoid dialdehyde and a second solution containing a polymer having aldehyde reactive functional groups, wherein said cross-linked polymer reaction product is biodegradable.

2. The biodegradable cross-linked polymer of Claim 1, where the first solution and the second solution are combined in a ratio from about 5:1 to about 1:10 to produce the biodegradable cross-linked polymer reaction product.

3. The biodegradable cross-linked polymer of Claim 1, wherein said first solution further comprises at least one glycol or polyol containing 2 to 1000 carbon atoms, where said glycol or polyol is present in a sufficient molar ratio between said glycol or polyol and said dialdehyde to sufficiently displace the water of hydration from binding to said dialdehyde in favor of binding to said glycol or polyol, such that deterioration of the dialdehyde in said first solution by internal polymerization is no more than 20 percent over two years at room temperature.

4. The biodegradable cross-linked polymer of Claim 3 where the glycol is selected from the group comprising triethylene glycol, ethylene glycol, diethylene glycol, tetraethylene glycol or mixtures thereof.

5. The biodegradable cross-linked polymer of Claim 3 where the glycol is tetraethylene glycol.

6. The biodegradable cross-linked polymer of Claim 3 where the polyol is selected from the group comprising polyethylene glycol 200, polyethylene glycol 400, polyethylene glycol 8000 or mixtures thereof.

7. The biodegradable cross-linked polymer of Claim 3 where the polyol is polyethylene glycol 200.

8. The biodegradable cross-linked polymer of Claim 3 where the polyol is polyethylene glycol 400.

9. The biodegradable cross-linked polymer of Claim 3, where the polymer having aldehyde-reactive functional group is a synthetic protein.

10. The biodegradable cross-linked polymer of Claim 3, where the polymer having aldehyde-reactive functional group is a naturally occurring protein selected from the group consisting of albumin, collagen, elastin, and fibrin.

11. The biodegradable cross-linked polymer of Claim 10, where the polymer having aldehyde-reactive functional groups is albumin.

12. The biodegradable cross-linked polymer of Claim 11, where the albumin is bovine serum albumin.

13. The biodegradable cross-linked polymer of Claim 3, where the secoiridoid dialdehyde is isolated from the leaves or fruit of an Olive tree.

14. The biodegradable cross-linked polymer of Claim 3, where the secoiridoid dialdehyde is oleocanthal.

15. The biodegradable cross-linked polymer of Claim 3, where the secoiridoid dialdehyde is oleacein.

16. The biodegradable cross-linked polymer of Claim 3, where the secoiridoid dialdehyde is elenolic acid.

17. The biodegradable cross-linked polymer of Claim 3, where the secoiridoid dialdehyde is decarboxymethyl elenolic acid.

18. The biodegradable cross-linked polymer of Claim 3, further comprising an adhesion modifier.

19. The biodegradable cross-linked polymer of Claim 18, where the adhesion modifier is selected from the group consisting of polyethyleneimine, gelatin, carboxymethylcellulose, butylhydroxytoluene, chitosan, and acetylated chitin.

20. The biodegradable cross-linked polymer of Claim 3, further comprising a plasticizing agent.

21. The biodegradable cross-linked polymer of Claim 20, where the plasticizing agent selected from the group consisting of fatty acids, polyethylene glycol, glycerin,

butylhydroxytoluene, polyvinyl pyrrolidone (NVP), dextran, polyacrylic acid, and its salts.

22. The biodegradable cross-linked polymer of Claim 20, where the plasticizing agent is present in a final concentration of about 0.01% to about 20% (w/w).

23. The biodegradable cross-linked polymer of Claim 3, further comprising a foaming agent.

24. The biodegradable cross-linked polymer of Claim 23, where the foaming agent is sodium bicarbonate.

25. The biodegradable cross-linked polymer of Claim 3, further comprising neutral salt, a wetting agent, a preservative, a dye, or a thickening agent.

26. The biodegradable cross-linked polymer of Claim 3, further comprising a biocompatible filler.

27. The biodegradable cross-linked polymer of Claim 26, where the biocompatible filler is a particle or fiber.

28. The biodegradable cross-linked polymer of Claim 27, where the particle or fiber is selected from the group consisting of nano-sized hydroxyapatite, mineral trioxide aggregate (MTA), nanosilica, therapeutic agent, chopped fibrous silk, polyester, PTFE, NYLON, carbon fibers, polypropylene, polyurethane, glass, clay, mica, hydroxyapatite, calcium sulfate, calcium phosphate, and bone chips.

29. The biodegradable cross-linked polymer of Claim 27, where the particle or fiber is present in amounts up to about 40%.

30. The biodegradable cross-linked polymer of Claim 27, where the particle or fiber is in amounts from about 10% to about 30%.

31. The biodegradable cross-linked polymer of Claim 3, further comprising a biologically active agent.

32. The biodegradable cross-linked polymer of Claim 31, where the biologically active agent is selected from the group consisting of a bone growth factor, a tissue activator, a cartilage growth activator, and a small molecule active agent.

33. The biodegradable cross-linked polymer of Claim 32, where the small molecule active agent is an antibiotic.

34. The biodegradable cross-linked polymer of Claim 32, where the small molecule active agent is N-acetyl cysteine.

35. The biodegradable cross-linked polymer of Claim 3, further comprising a pH buffer.

36. The biodegradable cross-linked polymer of Claim 35, where the pH is buffered to a pH of from about 6 to about 8.

37. The biodegradable cross-linked polymer of Claim 35, where the pH is buffered to a pH of from about 4.5 to about 6.

38. A method for applying a coating a site on tissue of a living organism comprising the steps of:

i) applying a hydrogel-forming solution to the site, said hydrogel-forming solution comprising a secoiridoid dialdehyde and a polymer having aldehyde reactive functional groups, where said hydrogel-forming solution is applied under conditions where the secoiridoid dialdehyde and the polymer having aldehyde reactive functional groups can polymerize to form a biodegradable cross-linked polymer, and

ii) allowing the secoiridoid dialdehyde and the polymer having aldehyde reactive functional groups to polymerize so as to form a biodegradable cross-linked polymer.

39. The method of Claim 38, where the hydrogel-forming solution is applied with a controlled delivery system, said controlled delivery system having a first solution and a second solution, said first solution comprising a secoiridoid dialdehyde and said second solution comprising a polymer having aldehyde reactive functional groups, said controlled delivery system further having an applicator tip, said applicator tip mixing said first and said second solutions in a predefined ratio when said hydrogel-forming solution is applied.

40. The method of Claim 38, where said first solution additionally comprises at least one glycol or polyol containing 2 to 1000 carbon atoms, where said glycol or polyol is present in a sufficient molar ratio between said glycol or polyol and said secoiridoid dialdehyde to sufficiently displace the water of hydration from binding to said secoiridoid dialdehyde in favor of binding to said glycol or polyol, such that deterioration of the secoiridoid dialdehyde in said first solution by internal polymerization is no more than 20 percent over two years at room temperature.

41. The method of claim 40, where the polymerization to form a biodegradable cross-linked polymer occurs at a pH of about 6 to about 8.

42. The method of Claim 41, where the coating acts as an adhesive.

43. The method of claim 40, where the polymerization to form a biodegradable cross-linked polymer occurs at a pH of about 4.5 to about 6.

44. The method of claim 40, where the hydrogel-forming solution begins polymerize to form a biodegradable cross-linked polymer within about 15 to about 30 seconds following mixing of said first solution and said second solution.

45. The method of claim 44, where the hydrogel-forming solution achieves a bonding strength in about 2 minutes.

46. The method of claim 40, where the hydrogel-forming solution is used in medical application selected from the group consisting of dental procedure, ophthalmic procedure, neurosurgery procedure, sealing a fistula, tissue adhesive, adhesion prevention, hemostat sealant, bronchoscopic lung volume reduction, cardiovascular surgery, and bonding fractured bone.

47. The method of claim 46, where the ophthalmic application is selected from the group consisting of corneal laceration, vitrectomy procedure, cataract surgery, LASIK surgery, glaucoma surgery, and corneal transplant.

48. The method according to Claim 38 wherein the anatomical site is an intraoral surface.

49. A method of protecting and regenerating a dental pulp in a dental restorative procedure, the method comprising:

i) preparing a tooth area to create a cavity having a cavity floor within a tooth wherein a remaining dentin thickness between the cavity floor and the dental pulp is less than about 0.5 mm,

ii) applying a hydrogel-forming solution to the cavity floor, wherein the hydrogel-forming solution comprises a secoiridoid dialdehyde and a polymer having aldehyde reactive functional groups,

iii) allowing a polymerization reaction to occur to form a cross-linked biodegradable material derived from the hydrogel-forming solution, and

iv) filling the cavity with a restorative material.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/033324

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61L24/00 A61L24/04 A61L26/00 C08L99/00 A61K6/087
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61L C09J C08L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 489 135 A1 (NIZO FOOD RES [NL]) 22 December 2004 (2004-12-22) column 1, paragraph 0001 column 3, paragraphs 0010-0012, 0015 column 4, paragraph 0022 column 5, paragraphs 0023, 0024, 0026 examples ----- -/--	1-49

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

[X] See patent family annex.

* Special categories of cited documents :

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

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 September 2019	25/09/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Dudás, Eszter

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2019/033324

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ANTUNES A P M ET AL: "Utilisation of oleuropein as a crosslinking agent in collagenic films", INTERNET CITATION, 1 May 2006 (2006-05-01), XP002717349, Retrieved from the Internet: URL:http://www.aaqtic.org.ar/congresos/istanbul2006/ [retrieved on 1077-05-01] Abstract, Introduction; page 1 page 2, paragraph 4 page 4, paragraph 2.3	1,2, 38-49
A	US 2014/227665 A1 (ANGELETAKIS CHRISTOS [US]) 14 August 2014 (2014-08-14) page 1, paragraphs 0002, 0010 page 2, paragraphs 0014, 0019 page 3, paragraphs 0020, 0021, 0023, 0024	1-49
A	US 6 329 337 B1 (MORITA YASUNOBU [JP] ET AL) 11 December 2001 (2001-12-11) cited in the application column 1, line 60 - column 2, line 22 column 3, line 51 - column 4, line 27	1-49
A	US 2016/022862 A1 (ALSBERG EBEN [US]) 28 January 2016 (2016-01-28) page 1, paragraphs 0004, 0007	1-49

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2019/033324

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 1489135 A1	22-12-2004	EP 1489135 A1	22-12-2004
		WO 2004113431 A1	29-12-2004
US 2014227665 A1	14-08-2014	EP 2956110 A1	23-12-2015
		US 2014227665 A1	14-08-2014
		WO 2014126945 A1	21-08-2014
US 6329337 B1	11-12-2001	EP 1043034 A1	11-10-2000
		JP 2000288079 A	17-10-2000
		US 6329337 B1	11-12-2001
US 2016022862 A1	28-01-2016	US 2016022862 A1	28-01-2016
		WO 2014138628 A1	12-09-2014