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(54) **Title**: PROTECTIVE GEL BASED ON CHITOSAN AND OXIDIZED POLYSACCHARIDE

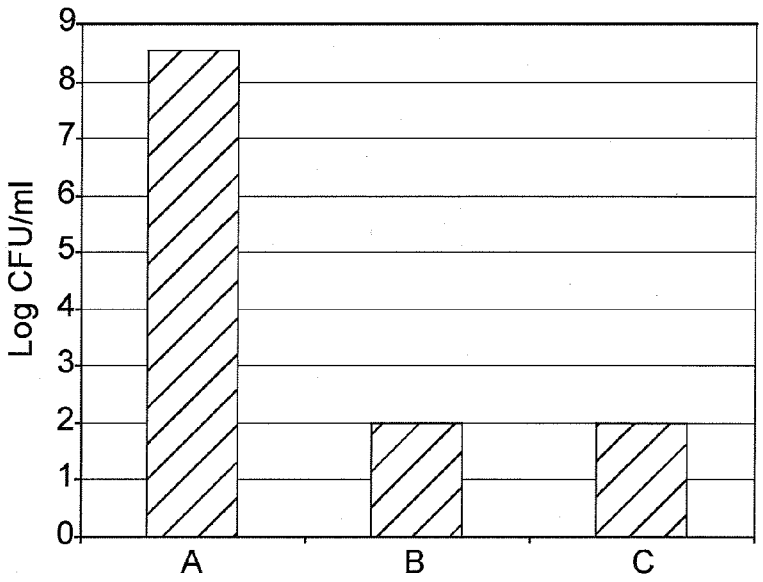

Fig. 3

(57) **Abstract**: Bodily tissue and structures may be protected using a fluid layer containing a mixture of chitosan and oxidized polysaccharide. The mixture forms a protective gel layer via in situ crosslinking. Compared to crosslinking using a low molecular weight aldehyde such as glutaraldehyde or genipin, oxidized polysaccharides appear to provide faster gelation while avoiding the use of potentially less bioacceptable low molecular weight aldehydes.

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PROTECTIVE GEL BASED ON CHITOSAN AND OXIDIZED POLYSACCHARIDE

FIELD OF THE INVENTION

[0001] This invention relates to polysaccharides and to materials for use in or on tissue and structures in the ears, nose, throat, limbs and spinal column.

BACKGROUND

[0002] Certain polysaccharide materials have been used for surgical repair or drug delivery. Documents relating to such materials include U.S. Patent Nos. 6,514,522 (Domb) and 7,053,068 B2 (Prinz), U.S. Patent Application Publication Nos. US 2005/0176620 A1 (Prestwych et al.) and US 2005/0238702 A1 (Ishihara et al.), Canadian Patent Application No. 2 348 842 A1 (Bernkop-Schnürch), Published PCT Application Nos. WO 98/31712 A2 (B.F. Goodrich Co.), WO 01/00246 A2 (Bentley et al.) and WO 03/020771 A1 (Mucobiomer Biotechnologische Forschungs-und Entwicklungs GmbH), Mi et al., *Synthesis and Characterization of a Novel Chitosan-Based Network Prepared Using Naturally-Occurring Crosslinker*, J Polym Sci, Part A: Polym Chem, 38, 2804-2814 (2000), Mi et al., *Synthesis and characterization of biodegradable TPP/genipin co-crosslinked chitosan gel beads*, Polymer, 44, 6521-30 (2003), Roldo et al., *Mucoadhesive thiolated chitosans as platforms for oral controlled drug delivery: synthesis and in vitro evaluation*, European Journal of Pharmaceutics and Biopharmaceutics, 57, 115–121 (2004), Krauland et al., *Viscoelastic Properties of a New in situ Gelling Thiolated Chitosan Conjugate*, Drug Development And Industrial Pharmacy, 31, 885-893 (2005), Bernkop-Schnürch, *Thiomers: A new generation of mucoadhesive polymers*, Advanced Drug Delivery Reviews, 57, 1569-1582 (2005), Bernkop-Schnürch et al., *Thiomers: Preparation and in vitro evaluation of a mucoadhesive nanoparticulate drug delivery system*, International journal of Pharmaceutics, 317, 76-81 (2006) and Weng et al., *Rheological Characterization of in Situ Crosslinkable Hydrogels Formulated from Oxidized Dextran and N-Carboxyethyl Chitosan*, Biomacromolecules, 8, 1109-1115 (2007).

SUMMARY OF THE INVENTION

[0003] The present invention provides, in one aspect, a fluid layer atop a bodily tissue or structure, the layer comprising chitosan and oxidized polysaccharide in amounts sufficient to form a protective gel layer *in situ*. The protective gel layer may assist in returning an injured, inflamed or surgically repaired tissue surface to a normal state, e.g., through one or more healing mechanisms such as modulation of an inflammatory response, phagocytosis, mucosal remodeling, reciliation or other full or partial restoration of normal function.

[0004] The present invention provides in another aspect a method for treating a bodily tissue or structure, which method comprises:

a) applying to such tissue a fluid layer containing a mixture of chitosan and an oxidized polysaccharide, and

b) allowing the mixture to form a protective gel layer *in situ*.

[0005] The disclosed fluid layer desirably is spray applied, and packaged in a multicomponent spray dispenser. The disclosed method and layer are especially useful for treating mucosal tissues in the ears, nose or throat and openings, recesses, passageways or joints in the limbs or spinal column.

BRIEF DESCRIPTION OF THE DRAWING

[0006] **Fig. 1** is a schematic view showing the disclosed method;

[0007] **Fig. 2** is a perspective view of a dispensing instrument which may be used in the disclosed method;

[0008] **Fig. 3** is a graph showing the antimicrobial properties of two *in situ* crosslinked gel layers formed from chitosan and oxidized polysaccharide, and of a trypticase soy broth control;

[0009] **Fig. 4** is a graph showing antimicrobial activity as a function of time for three *in situ* crosslinked gel layers formed from chitosan and oxidized polysaccharide, and for a trypticase soy broth control;

[0010] **Fig. 5** is a graph showing drug release behavior for three *in situ* crosslinked gel layers; and

[0011] **Fig. 6** is a graph showing degradation of an *in situ* crosslinked gel layer.

[0012] Like reference symbols in the various figures of the drawing indicate like elements. The elements in the drawing are not to scale.

DETAILED DESCRIPTION

[0013] The following detailed description describes certain embodiments and is not to be taken in a limiting sense. All weights, amounts and ratios herein are by weight, unless otherwise specifically noted. The terms shown below have the following meanings:

[0014] The term "adhesion" refers to the sticking together of a body structure or prosthetic material to tissue, to the sticking together of tissue to tissue with which it is in intimate contact for extended periods, or to the formation of tissue that connects body structures, prosthetic materials or tissues to one another across a normally open space.

[0015] The term "antimicrobial" refers to an ability to cause greater than a 90% numeric reduction (*viz.*, at least a 1-log order reduction) in a population of one or more of *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pneumonia, Haemophilus influenzae* or *Moraxella catarrhalis*.

[0016] The terms "attached" and "adhered" when used in reference to a bacterial biofilm and a surface mean that the biofilm is established on and at least partially coats or covers the surface, and has some resistance to removal from the surface. As the nature of this relationship is complex and poorly understood, no particular mechanism of attachment or adherence is intended by such usage.

[0017] The term "bacterial biofilm" means a community of bacteria attached to a surface, with the organisms in the community being contained within an extracellular polysaccharide (EPS) matrix produced by the bacteria.

[0018] The term "biocompatible" when used in reference to a substance means that the substance presents no significant deleterious or untoward effects upon the body.

[0019] The term "biodegradable" when used in reference to a substance means that the substance will degrade or erode *in vivo* to form smaller chemical or physical species. Such degradation process may be enzymatic, chemical or physical.

[0020] The term "bioresorbable" when used in reference to a substance means that the substance is capable of being absorbed by the body.

[0021] The term "cohesive" when used in reference to a liquid or gel means that the liquid or gel when placed on a level surface will tend to (but need not in all cases) stick to itself and form a unitary mass.

[0022] The term "comminuted" when used in reference to a particulate material means that the particles have been fractured and reduced in size by cutting, grinding, pulverizing, triturating or other particle fracturing process employing externally-applied force.

[0023] The term "conformal" when used in reference to a composition applied to tissue or other body structure means that the composition can form a substantially continuous layer over an area to which the composition has been applied.

[0024] The terms "detaching", "removing" and "disrupting" when used in reference to a bacterial biofilm attached or adhered to a surface mean that at least a significant amount of the biofilm initially present on the surface no longer is attached or adhered to the surface. No particular mechanism of detachment, removal or disruption is intended by such usage.

[0025] The term "fluid" when used in reference to a substance means that the substance is a liquid having a loss modulus (G′′) greater than its storage modulus (G′) and a loss tangent (tan δ) greater than 1.

[0026] The term "gel" when used in reference to a substance means that the substance is deformable (*viz.*, is not a solid), G′′ is less than G′ and tan δ is less than 1.

[0027] The term "gelation" when used with respect to formation of a gel layer means the time at which G′′ equals G′ and tan δ equals 1.

[0028] The term "hemostat" means a device or material which stops blood flow.

[0029] The term "hydrogel" when used in reference to a gel means that the gel is hydrophilic and contains water.

[0030] The term "hydrated" when used in reference to a device or substance means that the device or substance contains uniformly distributed chemically-bound water. A "fully hydrated" device or substance is incapable of taking up additional water of hydration. A "partially hydrated" device or substance is capable of taking up additional water of hydration.

[0031] The term "inner ear" means the semicircular canals and cochlea.

[0032] The term "middle ear" means the region defined by the tympanic membrane, interior structures such as the ossicular chain, the surrounding lining and bordering structures such as the mastoid.

[0033] The term "mucoadhesive" when used in reference to a device or substance means that the device or substance will adhere to the mucus covering epithelia.

[0034] The term "nasal or sinus cavities" refers to the various tissues defining the normally air-filled passages and chambers within the nose and sinus including but not limited to the nostrils or nares, the nasal concha or turbinates, the frontal, ethmoid, sphenoid and maxillary sinuses, the sinus ostia and the nasopharnyx.

[0035] The term "polysaccharide" includes derivatives of polysaccharides and modified polysaccharides, as well as derivatives of individual polysaccharide species and modified individual polysaccharide species. For example, the term "carboxymethylcellulose" includes carboxymethylcellulose derivatives and modified carboxymethylcelluloses, the term "chitosan" includes chitosan derivatives and modified chitosans, and the term "starch" includes starch derivatives and modified starches.

[0036] The term "protective" when used in reference to a layer of a composition atop tissue or other body structure means that the layer may assist in returning an injured, inflamed or surgically repaired tissue surface to a normal state, e.g., through one or more healing mechanisms such as modulation of an inflammatory response, phagocytosis, mucosal remodeling, reciliation or other full or partial restoration of normal function.

[0037] The term "residence time" when used in reference to a protective gel layer atop tissue or other body structure means the time period during which the gel layer or portion thereof remains in place *in vivo* under gross observation.

[0038] The term "solvating" means to form a solution or dispersion containing a solvent or other carrier within which a solute is dissolved or suspended.

[0039] The term "substantially collagen-free" means containing a sufficiently low amount of collagen so as not to pose a potential risk of transmission of or infection with bovine spongiform encephalopathy (BSE) or variant Creutzfeldt-Jakob disease (vCJD).

[0040] The term "thin" when used in reference to a protective layer atop tissue or other body structure means having an average thickness less than about two millimeters.

[0041] Referring to **Fig. 1**, the disclosed method may be performed for example in the nasal or sinus cavities **100** of a patient, including the maxillary sinuses **110a**, **110b** and frontal sinuses **112a**, **112b**, which may be accessed through nares **114a**, **114b**. It should be noted that external features of the patient, including nares **114a**, **114b**, are shown in dashed lines. When the patient suffers for example from chronic rhinosinusitis, one or more treatment sites such as treatment site **116** associated with a surface of maxillary sinus **110a** may be medically or if need be surgically addressed. Treatment site **116** includes ciliated epithelium of maxillary sinus **110a** and may include an associated layer of bacteria inhabiting an associated biofilm (not shown in **Fig. 1**). The treatment site need not be natural tissue and may instead be an artificial structure (not shown in **Fig. 1**) such as a sinus packing or stent which may also be covered at least in part with a layer of bacterial biofilm. If present, the biofilm may be removed using a solvating system (for example, the solvating system described in U.S. Patent Application Publication No. US 2007/0264310 A1) which may be applied to treatment site **116** using an introducer **120** with an articulatable delivery tube **122** containing an irrigation duct (hidden in **Fig. 1**) through which the solvating system may flow to a nozzle **124** at the distal end of introducer **122** and thence to the treatment site. The solvating system and residues of the biofilm may be removed from the treatment site via an aspiration duct (hidden in **Fig. 1**). The disclosed composition comprising chitosan and oxidized polysaccharide may likewise be applied at the treatment site using the same or a different irrigation duct in introducer **120**. Those skilled in the art will appreciate that the disclosed composition (and if used, the solvating system) may be applied to the treatment site using other methods or devices. Exemplary other methods include power spray or other spray application, lavage, misting, mopping, wicking, dripping and trephination and exemplary other devices include spray nozzles (e.g., single component or multiple component spraying nozzles) and syringes (e.g., single barrel or multiple barrel glass or plastic syringes and bulb syringes). The treatment method may also be performed in other parts of the body. The treatment method has particular utility in non-vascular applications, including treatment of tissues (e.g., mucosal tissues) or structures in or near the ears, throat, limbs or spinal column.

[0042] **Fig. 2** shows an exemplary instrument **200** which may be used in the disclosed treatment method. Instrument **200** includes a handle **202** and an introducer **222** whose

distal end **224** (referenced generally) includes a spray nozzle, irrigation and aspiration ducts (not separately numbered in **Fig. 2**). Instrument **200** can optionally further include a first actuator assembly **226** (referenced generally) and a second actuator assembly **228** (referenced generally). A control wheel **230** in first actuator assembly **226** may be operable by a user to effectuate bending of the introducer **222**, and a control wheel **232** in second actuator assembly **228** may be operable by a user to effectuate movement or rotation relative to introducer **222** of liquid sprayed from distal end **224** of introducer **222**. Handle **202** serves generally as a housing for various other components of instrument **200** and as a support for introducer **222**. Handle **202** may have a pistol grip-like shape, defining a grip portion **234** and a nose **236**. Grip portion **234** is sized and shaped for grasping by a user's hand, whereas nose **236** is adapted for connection to introducer **222**. Trigger **238** and an associated sensor and valve (not shown in **Fig. 2**) may be used to control the flow of the disclosed rehydrated gel (and if used, the disclosed solvating system) through irrigation tubing **240** and thence through the spray nozzle in distal end **224** and onto the desired treatment site. Trigger **238** may be provided with a multidirectional range of motion and associated with one or more additional sensors and valves (not shown in **Fig. 2**) to control removal from a treatment site of the solvating system, biofilm residue and other debris through the aspiration duct in distal end **224** and thence to aspiration tubing **242**. Trigger **238** may also be used to control the flow of the disclosed rehydrated gel through a separate lumen in irrigation tubing **240** and thence through the spray nozzle in distal end **224** and onto the desired treatment site.

[0043] The applied composition comprising chitosan and oxidized polysaccharide may fill the treatment site (e.g., a nasal or sinus cavity, or an opening, recess, passageway or joint in a portion of the limbs or spinal column), in which case the disclosed layer of such composition may be very thick and not exposed to air or other nearby gases, and with differing thicknesses throughout the layer. The disclosed composition may also be applied as a thin film or other conformal coating in which case the disclosed layer may be relatively thin and exposed to air or other nearby gases, and with a substantially uniform thickness throughout the layer. After gelation the protective gel layer may be viscous, elastic or viscoelastic. The protective gel layer desirably adheres to mucosal or other natural tissues (e.g., cartilage or bone) at the treatment site and resists detachment or other

disruption until natural degradation or resorption of the gel layer takes place, e.g., after a residence time in vivo of from one day to a few (e.g., 2, 3 or 4) days, weeks or months. Meanwhile bacterial recolonization or reinfection may be significantly reduced or prevented, and improved healing and reciliation may take place. The protective gel layer may provide various therapeutic advantages including but not limited to bacterial adhesion repellence, anti-infective properties, local immune modulation, tissue protection, reduction or elimination of pain or bleeding, reduction in inflammation, optimization of environment for ciliary regrowth, reduction in adhesions to critical anatomy, and the like. These advantages may arise due to a variety of mechanisms including a) killing bacteria, b) inhibiting bacterial colonization, c) inhibiting the adherence of bacteria to tissue, d) reducing tissue morbidity or abscess formation, e) reducing or preventing disease recurrence (for example, specifically reducing the chronic inflammation related to bacterial toxin and EPS), f) coating and protecting tissue during healing, such as by maintenance of a moist wound which promotes platelet aggregation, or by closure of a dry wound without excessive scabrous formation, g) hemostasis, h) optimizing the environment for reciliation of the mucosa, i) speeding the growth or regrowth of cilia and j) delivering therapeutic agent(s) to the treatment site. Desirably the protective gel layer will adhere to a portion of the mucosa while leaving the cilia in unadhered portions free to undergo natural rhythmic cilia motion (*viz.*, cilia beating), will if desired also deliver antimicrobial agents or additional therapeutic agents, and desirably will discourage or prevent bacteria from adhering to the treatment site.

[0044] A wide variety of chitosans (including salts and other chitosan derivatives) may be employed in the disclosed fluid layer and method. Exemplary unmodified chitosans and their salts (including citrate, nitrate, lactate, phosphate, chloride and glutamate salts) may be obtained from a variety of commercial sources including KitoZyme S.A., Fluka Chemie AG, the NovaMatrix unit of FMC BioPolymer AS and Sigma-Aldrich Co. Chitosan may also be synthesized by deacetylation of chitin (poly-N-acetyl-D-glucosamine) to eliminate acetyl groups on the nitrogen atom by hydrolysis. The resulting polymer has a plurality of repeating units (e.g., about 30 to about 3000 repeating units, about 60 to about 600 repeating units, or such other amount as may be desired for the chosen end use) some or all of which contain deacetylated amino groups (e.g., about 30 to

about 100% or about 60 to about 95% of the total repeating units), with the remaining repeating units (if any) containing acetylated amino groups. The polymer is cationic and may be regarded as being composed from glucosamine monomers. The chitosan may have a variety of number average molecular weights, e.g., about 5 to about 2000 kDa, about 10 to about 500 kDa, or about 10 to about 100 kDa. The chitosan may for example be an ultralow molecular weight material having a number average molecular weight less than about 50 kDa, a low molecular weight material having a number average molecular weight of about 50 to about 200 kDa, a medium molecular weight material having a number average molecular weight of about 200 to about 500 kDa or a high molecular weight material having a number average molecular weight greater than about 500 kDa. Chitosan derivatives may also be employed, for example derivatives in which one or more hydroxyl or amino groups have been modified for the purpose of altering the solubility or mucoadhesion characteristics of the derivative. Exemplary derivatives include thiolated chitosans, and non-thiolated chitosan derivatives such as acetylated, alkylated or sulfonated chitosans (for example O-alkyl ethers, O-acyl esters, cationized trimethyl chitosans and chitosans modified with polyethylene glycol). Chitosan derivatives may be obtained from a variety of sources. For example, thiolated chitosans may be obtained from ThioMatrix Forschungs Beratungs GmbH and Mucobiomer Biotechnologische Forschungs-und Entwicklungs GmbH or prepared by reaction of chitosan with a suitable thiolated reactant, e.g., as described in the above-mentioned Published PCT Application No. WO 03/020771 A1 or in the above-mentioned Roldo et al., Krauland et al., Bernkop-Schnürch and Bernkop-Schnürch et al. papers.

[0045] The chitosan desirably is obtained in dry particulate form, for example, as free-flowing granules whose average particle diameter is less than about 1 mm, less than about 100 μm, about 1 to about 80 μm, or less than 1 μm. The chitosan preferably is packaged and shipped to a user in such dry particulate form so as to reduce degradation of the chitosan during prolonged storage. The chitosan fluid may be formed for example by dissolving the chitosan in water or another suitable solvent just prior to use. Recommended chitosan amounts will depend on the chitosan molecular weight, and may for example be about 1 to about 20 %, about 1 to about 10 % or about 1 to about 5 % of the resulting solution. Copending U.S. Provisional Application Serial No. 61/047,580,

filed April 24, 2008, describes a preferred technique for rehydrating a chitosan, by dispersing free-flowing chitosan particles in a biocompatible water-miscible polar dispersant, and combining the dispersion with sufficient aqueous solvent for the particles to convert them to a cohesive hydrogel. The chitosan may be comminuted but desirably is non-comminuted.

[0046] A wide variety of oxidized polysaccharides may be employed in the disclosed fluid layer and method. Exemplary polysaccharides include agars, alginates, carrageenans, celluloses, chitins, chitosan (thus enabling chitosan to be crosslinked using its oxidized counterpart), chondroitin sulfates, dextrans, galactomannans, glycogens, hyaluronic acids, starches and other biocompatible polysaccharides capable of being oxidized. Oxidized polysaccharides such as oxidized cellulose, chitin, chitosan, chondroitin sulfate, dextran, glycogen, hyaluronic acid and starch are preferred. The polysaccharide desirably is oxidized to an extent sufficient to provide aldehyde groups capable of promoting rapid crosslinking of the chitosan when the chitosan and oxidized polysaccharide are combined in aqueous solution. Representative oxidizing agents or techniques include the use of a) sodium periodate, b) hypochlorite ion in the presence of di-tert-alkylnitroxyl catalysts, c) metal-catalyzed oxidation, using for example ruthenium, d) anhydrous oxidation using for example nitrogen dioxide in for example a halocarbon, e) enzymatic or chemo-enzymatic oxidation of starch, guar and other polysaccharides, and other oxidation agents and techniques that will be known to persons having ordinary skill in the art. Depending on the selected oxidizing agent or technique, a variety of degrees of oxidation, degrees of polymerization and oxidation sites may be employed. For example, oxidation may be directed at a primary hydroxyl group (for example, the 6-hydroxyl group in the anhydroglucose units of glucans), resulting in carboxyl-polysaccharides with preserved ring structures. Oxidation may also be directed at a vicinal diol function present in a monosaccharide ring (for example, the C2-C3 site in anhydroglucose units), resulting in cleavage of the monosaccharide units and the production of dialdehyde or dicarboxyl functional groups. The dialdehyde content of such an oxidized polysaccharide may range from a degree of oxidation of, for example, 2 % to virtually 100 %, e.g., more than 30 % or more than 50 % of the available oxidation sites. The oxidized polysaccharide may also contain other functional groups, for example hydroxyalkyl groups, cationic groups,

carboxyl groups and other acid groups. As a generalization, reduced amounts of oxidized polysaccharide may be employed in the disclosed fluid layer and method as the degree of polysaccharide oxidation is increased.

[0047] The oxidized polysaccharide desirably is dissolved in water or another suitable solvent prior to use. Recommended oxidized polysaccharide amounts typically will depend on the oxidized polysaccharide molecular weight, and may for example be about 1 to about 20 %, about 1 to about 10 % or about 1 to about 5 % of the resulting solution. The oxidized polysaccharide solution normally is kept separate from the chitosan solution until just prior to use.

[0048] Compared to crosslinking using a low molecular weight aldehyde such as glutaraldehyde or genipin, oxidized polysaccharides appear to provide faster gelation while avoiding the use of potentially less bioacceptable low molecular weight aldehydes. In addition to their ability to react with amine groups in the chitosan, aldehyde groups in the oxidized polysaccharide may also enhance mucoadhesion. The oxidized polysaccharides may provide additional benefits including improved or better controlled biodegradability, bioresorbability, drug delivery or haemostatic properties. The presence of phosphate ions appears to accelerate the crosslinking reaction. Phosphate may be provided by using phosphate buffered saline (PBS) as a solvent for one or both of the chitosan and oxidized polysaccharide.

[0049] Sufficient chitosan and oxidized polysaccharide desirably are employed so that a protective gel layer will form in less than 30 minutes after the chitosan and oxidized polysaccharide are mixed, and more preferably in less than 20 minutes, less than 10 minutes, less than 5 minutes or essentially immediately after mixing. The resulting fluid mixture may for example contain chitosan and oxidized polysaccharide in a combined amount representing about 1 to about 20 %, about 1 to about 10% or about 1 to about 5% of the composition. The chitosan and oxidized polysaccharide may for example be combined in a ratio of about 10:1 to about 1:20, about 5:1 to about 1:10, or about 3:1 to about 1:5. These ratios depend on the degree of oxidation of the oxidized polysaccharide(s), with lower oxidized polysaccharide amounts generally being used when more highly-oxidized polysaccharides are employed. For some applications the chitosan amount will preferably be as high as may be feasible in order to provide good

antimicrobial properties, and in such cases it will be preferable to use a low amount of highly oxidized polysaccharide so as to obtain rapid gel formation.

[0050] The disclosed compositions desirably are substantially collagen-free. Preferably the compositions are sufficiently free of collagen (e.g., containing no collagen at all) so as to be saleable worldwide for use without restriction in humans.

[0051] The disclosed compositions may optionally include a variety of other ingredients. These other ingredients may be disposed before mixing in the first part, second part or both parts of a two-part composition. Exemplary other ingredients include water and other solvents (e.g., alcohols), acids, bases, buffering agents, antimicrobial agents, therapeutic agents and other adjuvants. An acid, base or buffering agent may for example maintain the composition at an appropriate pH for contacting human tissue, e.g., a pH greater than 5, a near-neutral pH, or a pH less than 8.5. Exemplary buffering agents include barbitone sodium, glycinamide, glycine, potassium chloride, potassium phosphate, potassium hydrogen phthalate, sodium acetate, sodium citrate, sodium phosphate and their conjugate acids.

[0052] The disclosed compositions desirably are inherently antimicrobial without requiring addition of a separate antimicrobial agent. Antimicrobial activity may be influenced by the proportion of chitosan in the composition (with higher chitosan proportions tending to provide greater antimicrobial activity) and by the number of available chitosan amine hydrogen atoms. Accordingly, use of chitosan derivatives containing low numbers of available amino hydrogen atoms (such as the N-carboxyethyl derivatives desired in the above-mentioned Weng et al. paper) may be contraindicated. In any event, a separate antimicrobial agent may be employed if desired. A useful list of such antimicrobial agents may be found, for example, in the above-mentioned U.S. Patent Application Publication No. US 2007/0264310 A1.

[0053] Exemplary therapeutic agents which may be employed in the disclosed compositions include any material suitable for use at the intended treatment site including analgesics, anti-cholinergics, anti-fungal agents, antihistamines, steroidal or non-steroidal anti-inflammatory agents, anti-parasitic agents, antiviral agents, biostatic compositions, chemotherapeutic/antineoplastic agents, cytokines, decongestants, hemostatic agents (e.g., thrombin), immunosuppressors, mucolytics, nucleic acids, peptides, proteins, steroids,

vasoconstrictors, vitamins, mixtures thereof, and other therapeutic materials that will be known to those skilled in the art. A useful list of such therapeutic agents may be found, for example, in the above-mentioned U.S. Patent Application Publication No. US 2007/0264310 A1.

[0054] Other adjuvants that may be included in the disclosed compositions include dyes, pigments or other colorants (e.g., FD & C Red No. 3, FD & C Red No. 20, FD & C Yellow No. 6, FD & C Blue No.2, D & C Green No. 5, D & C Orange No. 4, D & C Red No. 8, caramel, titanium dioxide, fruit or vegetable colorants such as beet powder or beta-carotene, turmeric, paprika and other materials that will be known to those skilled in the art); indicators; flavoring or sweetening agents including but not limited to anise oil, cherry, cinnamon oil, citrus oil (e.g., lemon, lime or orange oil), cocoa, eucalyptus, herbal aromatics (e.g., clove oil, sage oil or cassia oil), lactose, maltose, menthol, peppermint oil, saccharine, sodium cyclamate, spearmint oil, sorbitol, sucrose, vanillin, wintergreen oil, xylitol and mixtures thereof; antioxidants; antifoam agents; and rheology modifiers including thickeners and thixotropes. The disclosed compositions desirably do not contain ingredients which might potentially harm mucosal tissues or structures, e.g., tissues in the nasal or sinus cavities.

[0055] In those instances where it is desirable to remove water from tissue, e.g., to remove fluid from polyps or edematous tissue, a hyperosmolar agent may be employed in the disclosed compositions. Exemplary hyperosmolar agents include furosemide, sodium chloride gel and other salt preparations that draw water from tissue or substances which directly or indirectly change the osmolar content of the mucous layer. Where sustained release or delayed release of a therapeutic agent is desirable, a release agent modifier may also be included.

[0056] The disclosed composition typically will be subjected to sterilization and placed in suitable sealed packaging (for example, a multicomponent syringe, a vial or vials, or a multi-chamber pouch made of suitable materials) prior to shipment to an end user. Additional property customization may be carried out by using a sterilization procedure such as gamma radiation or electron beam (E-Beam) processing to cause controlled chain scission. Cold ionizing radiation sterilization (e.g., cold E-Beam sterilization) may be employed to limit the degree of chain scission, as discussed in

copending PCT Application No. (Attorney Docket Nos. P0035142.00 and 151-P-35142WO01), filed even date herewith. Whether or not sterilized, the first part containing the chitosan normally will be kept separate from the second part containing the oxidized polysaccharide until just prior to use.

[0057] The disclosed compositions may desirably be used as a part of a multi-step treatment regimen which disrupts a bacterial biofilm and discourages its return. For example, a series of steps that may be broadly classified as Cleansing/Disrupting, Killing, Aerating, Protecting/Coating, and Healing may be carried out. The Cleansing/Disrupting step may be carried out by administering a solvating system as discussed above in connection with **Fig. 1** and **Fig. 2**. The Killing step may be carried out by applying a suitable antimicrobial agent to the treatment site. This may for example be accomplished by including an antimicrobial agent in the solvating system, as a separately-applied composition, or in both the solvating system and in a separately-applied composition. An antimicrobial agent may also be applied or administered post operatively. The Aerating step may be carried out by providing air passageways or improving air passageways to the treated tissues by opening occluded or partially occluded passages, e.g., the sinuses or sinus ostia for nasal applications. This may for example be accomplished by surgically removing obstructive tissue structures or by manually displacing such structures. The Protecting/Coating step may be carried out by coating at least part of the thus-treated tissue with the disclosed composition containing chitosan and oxidized polysaccharide as described above. The Healing step may be carried out by allowing the cleansed, protected and sealed tissue surface to undergo a return to a normal state, e.g., through one or more healing mechanisms such as modulation of an inflammatory response, phagocytosis, mucosal remodeling, reciliation or full or partial restoration of normal function. The multi-step treatment regimen may include or be followed by a Clearing step in which the disclosed composition containing chitosan and oxidized polysaccharide is sufficiently biodegradable or bioresorbable to disappear from the treatment site in a desired time period, e.g., more than 1 day, more than 3 days, or about 4 to 7 days, and desirably without shedding large solid chunks. The disclosed method may advantageously be accomplished without requiring surgery, for example by applying and removing the optional solvating system and by applying the disclosed composition containing chitosan and oxidized

polysaccharide through normal aspiration/suction techniques or by simple flushing of affected tissue. A comparable series of steps may be performed in a multi-step treatment regimen in a portion of the middle or inner ear. Further details regarding such a regimen may be found in U.S. Patent Application Publication No. US 2007/0264310 A1.

[0058] The invention is further illustrated in the following non-limiting examples.

Example 1

Gel Formulations

[0059] Chitosan solutions were prepared by dissolving varying amounts of chitosan glutamate (PROTASAN™ UP G 113 or PROTASAN UP G 213 from the NovaMatrix unit of FMC BioPolymer AS) overnight in PBS. An oxidized starch (OXST) solution was prepared by dissolving P9265 polymeric dialdehyde (from Sigma-Aldrich) in PBS while heating at 80° C for 1-2 hours. Oxidized methylcellulose (MC) and oxidized hydroxypropylmethylcellulose (HPMC) solutions were prepared by reacting MC or HPMC with sodium periodate, lyophilizing the resulting products, and dissolving the lyophilized products in PBS. The resulting chitosan solutions and oxidized polysaccharide solutions were mixed in various ratios and concentrations. Rheological measurements determined the gelling time and storage modulus (G′) for the resulting hydrogels. The results are shown below in Table 1.

Table 1

Gelation Time and Storage Modulus G′

Run No.	Chitosan	Oxidized Polysaccharide	Chitosan:Oxidized Polysaccharide Ratio	Total Conc. (%)	Gel time (min)	G′ (Pa)
1	G 113	OXST	2:1	5	< 2[1]	5,200
2	G 113	OXST	1:1	5	< 2[1]	4,000
3	G 113	OXST	1:2	5	< 2[1]	
4	G 113	OXST	2:1	3.75	< 2[1]	800
5	G 113	OXST	1:1	3.33	3.2	400
6	G 113	OXST	1:2	3	6.4	200
7	G 213	OXST	2:1	2.5	< 2[1]	750
8	G 213	OXST	1:1	2.5	< 2[1]	450
9	G 113	Oxidized MC	1:2	7.5	< 1 day	
10	G 113	Oxidized MC	1:10	5	< 2[1]	
11	G 113	Oxidized MC	1:5	5	< 2[1]	100
12	G 113	Oxidized MC	1:1	5	< 1 day	
13	G 113	Oxidized MC	5:1	5	no gelling	
14	G 113	Oxidized HPMC	1:4	12.5	< 60	
15	G 113	Oxidized HPMC	1:2	7.5	< 1 day	
16	G 113	Oxidized HPMC	1:1	5	no gelling	

[1] Gelled before the rheometer measurement started

[0060] Each of the chitosan/oxidized polysaccharide formulations shown in Table 1 had a viscosity below 500 cP, and should be injectable at a mucosal tissue treatment site

using for example a device like that shown in **Fig. 2** or a variety of other devices that will be known to persons having ordinary skill in the art. The formulations shown in Table 1 should also be sprayable onto a mucosal treatment site. Due to the rapidity with which gelation occurred for some formulations, spray application would in many instances be a preferred mode of application. The Run No. 2 formulation (G 113 Chitosan/OXST 1:1 at a total concentration of 5 %) was spray-applied using a gas-assisted applicator (FibriJet™ SA-6030 regulator, from Micromedics, Inc., controlling a FibriJet SA-3652 spray set equipped with a pair of 3 cc syringes). The OXST solution was dyed using toluidine blue to make the applied fluid layer easier to see. A thin, rapidly formed strong gel was obtained.

Example 2

Antimicrobial Properties

[0061] The Run No. 7 and 8 formulations from Table 1 (G 213 Chitosan/OXST 2:1 and 1:1 at a total concentration of 2.5 %, respectively shown as bars **B** and **C** in **Fig. 3**) were evaluated to determine their antimicrobial activity versus *S. Aureus*, using a plate procedure whose detection limit was log 2. The gel formulations were placed in duplicate under sterile conditions directly into a 24-well polystyrene tissue culture plate. Each well was incubated with 1 mL (25,000 colony forming units) of a bacterial suspension of *S. Aureus* (ATCC 25923). Positive controls were incubated with 1 ml of trypticase soy broth (TSB). After 6 hours incubation at 37 °C, the media was transferred in new tubes and serial ten-fold dilutions were performed. Ten µL aliquots from the appropriate dilution were plated in triplicate on trypticase soy agar plates using the dilution track method (Jett B.D. et al., Biotechniques, 23, 648-650 (1997). The plates were incubated at 37 °C for 24 hours and Colony Forming Units (CFU) were counted. As shown in **Fig. 3**, both formulations exhibited complete (greater than 6 log reduction) killing of the bacteria vs. the TSB control (see bar **A** in **Fig. 3**).

[0062] The Run Nos. 2, 10 and 11 formulations from Table 1 (G 113 Chitosan/OXST 1:1, G 113 Chitosan/Oxidized MC 1:10 and G 113 Chitosan/Oxidized MC 1:5 at a total concentration of 5 %, respectively shown as curves **B, C** and **D** in **Fig. 4**) were evaluated as described above to determine their antimicrobial activity as a function of time versus *S.*

Aureus, using a 140,000 CFU/mL bacterial loading, with measurements being recorded at 1, 3 and 6 hours against a TSB control (see curve **A** in **Fig. 4**). As shown in **Fig. 4**, complete killing was observed after 6 hours for gels made from all three chitosan/oxidized polysaccharide formulations, with significant killing being observed after 3 hours. The G 213 Chitosan/OXST formulation appeared to provide faster killing than the G 113 Chitosan/Oxidized MC formulations.

Example 3

Drug Delivery

[0063] The Run No. 1, 2 and 3 formulations from Table 1 (G 113 Chitosan/OXST 2:1, 1:1 and 1:2 at a total concentration of 5 %, respectively shown as curves **A, B** and **C** in **Fig. 5**) were used to prepare drug-loaded hydrogels in PBS buffer by mixing the chitosan and oxidized starch solutions with dexamethasone phosphate as the drug to be delivered. As shown in **Fig. 5**, relatively fast but controlled release for up to 3 days was obtained for dexamethasone phosphate, possibly aided by an interaction between the anionic drug and the cationic chitosan polymer.

Example 4

Degradation

[0064] The degradation behavior of various chitosan/oxidized polysaccharide gel formulations was determined by placing the gels in various buffer systems, including PBS at pH 7.4 with and without lysozyme (1 mg/mL), PBS with lipase, 2-(N-morpholino)ethanesulfonic acid (MES) at pH 6.0, and trishydroxymethylaminomethane (TRIS) at pH 7.4. Weight loss was determined at various time points up to 28 days. Weight loss occurred using all buffer systems, with about 30-60% of the original (dry) sample weight remaining after 28 days. Gels with higher oxidized starch content exhibited greater weight loss. The weight loss results for chitosan/oxidized polysaccharide in PBS at pH 7.4 and 37° C (without lysozyme) are shown in **Fig. 6**.

[0065] The oxidized starch-based gels appeared to degrade into a 'shell-like' material, whereas the oxidized cellulose-based gels appeared to remain as gels during degradation. The oxidized cellulose-based gels may be haemostatic.

[0066] The results in Examples 1-4 show that chitosan and oxidized polysaccharides may be combined to prepare injectable or sprayable formulations which quickly form strong protective gel layers *in situ* with inherent antimicrobial properties. The formulations were in each instance sprayable, antibacterial, biodegradable or bioresorbable and capable of serving as a scaffold for drug delivery.

[0067] Although specific embodiments have been illustrated and described herein for purposes of description of the preferred embodiments, it will be appreciated by those of ordinary skill in the art that a wide variety of alternate or equivalent implementations calculated to achieve the same purposes may be substituted for the specific embodiments shown and described without departing from the scope of the present invention. This application is intended to cover any adaptations or variations of the preferred embodiments discussed herein. Therefore, it is manifestly intended that this invention be limited only by the claims and the equivalents thereof.

We claim:

1. A fluid layer atop a bodily tissue or structure, the layer comprising chitosan and oxidized polysaccharide in amounts sufficient to form a protective gel layer *in situ*.

2. A fluid layer according to claim 1 wherein the tissue comprises nasal tissue.

3. A fluid layer according to claim 1 wherein the tissue comprises cartilage or bone.

4. A fluid layer according to claim 1 wherein the chitosan is an unmodified chitosan or a chitosan salt.

5. A fluid layer according to claim 1 wherein the chitosan is a chitosan derivative.

6. A fluid layer according to claim 1 wherein the oxidized polysaccharide comprises starch.

7. A fluid layer according to claim 1 wherein the oxidized polysaccharide comprises cellulose.

8. A fluid layer according to claim 1 wherein the oxidized polysaccharide comprises chitin, chitosan, chondroitin sulfate, dextran, glycogen or hyaluronic acid.

9. A fluid layer according to claim 1 wherein the chitosan and oxidized polysaccharide are combined in a ratio of about 10:1 to about 1:20.

10. A fluid layer according to claim 1 wherein the chitosan and oxidized polysaccharide are combined in a ratio of about 3:1 to about 1:5.

11. A fluid layer according to claim 1 further comprising phosphate ions.

12. A method for treating bodily tissue or structure, which method comprises:
    a) applying to such tissue a fluid layer containing a mixture of chitosan and an oxidized polysaccharide, and
    b) allowing the mixture to form a protective gel layer in situ.

13. A method according to claim 12 comprising applying the fluid layer to a nasal or sinus cavity.

14. A method according to claim 12 comprising applying the fluid layer to a middle or inner ear.

15. A method according to claim 12 comprising applying the fluid layer to an opening, recess, passageway or joint in a limb.

16. A method according to claim 12 comprising applying the fluid layer to an opening, recess, passageway or joint in a spinal column.

17. A method according to claim 12 comprising applying the fluid layer from a multiple-barrel syringe.

18. A method according to claim 12 comprising applying the fluid layer by spraying.

19. A method according to claim 12 wherein the chitosan is an unmodified chitosan or a chitosan salt.

20. A method according to claim 12 wherein the chitosan is a chitosan derivative.

21. A method according to claim 12 wherein the oxidized polysaccharide comprises starch.

22. A method according to claim 12 wherein the oxidized polysaccharide comprises cellulose.

23. A method according to claim 12 wherein the oxidized polysaccharide comprises chitin, chitosan chondroitin sulfate, dextran, glycogen or hyaluronic acid.

24. A method according to claim 12 wherein the chitosan and oxidized polysaccharide are combined in a ratio of about 10:1 to about 1:20.

25. A method according to claim 12 wherein the chitosan and oxidized polysaccharide are combined in a ratio of about 3:1 to about 1:5.

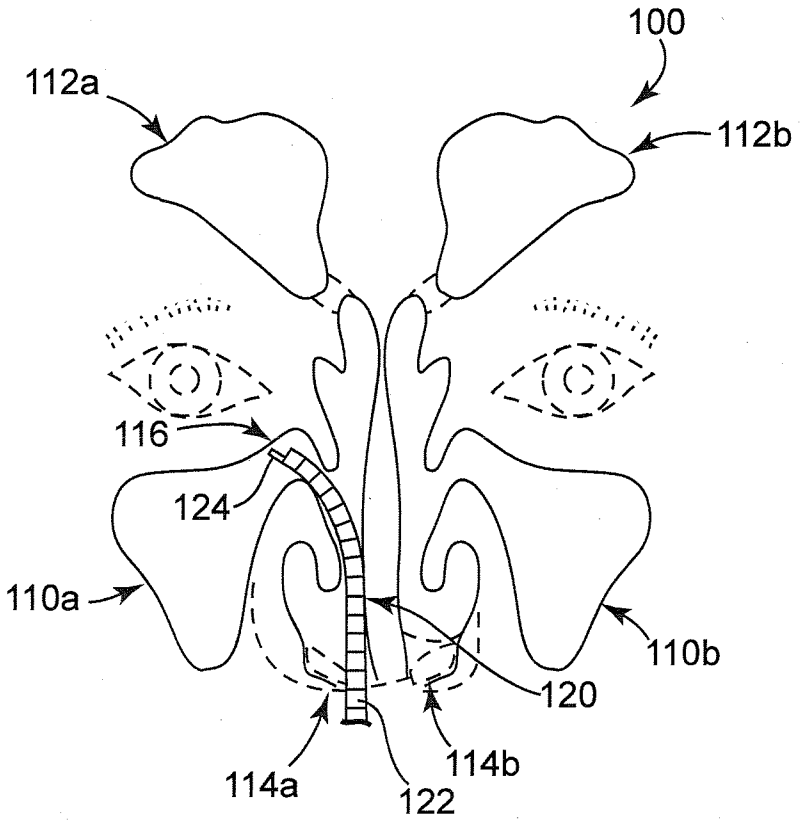


Fig. 1

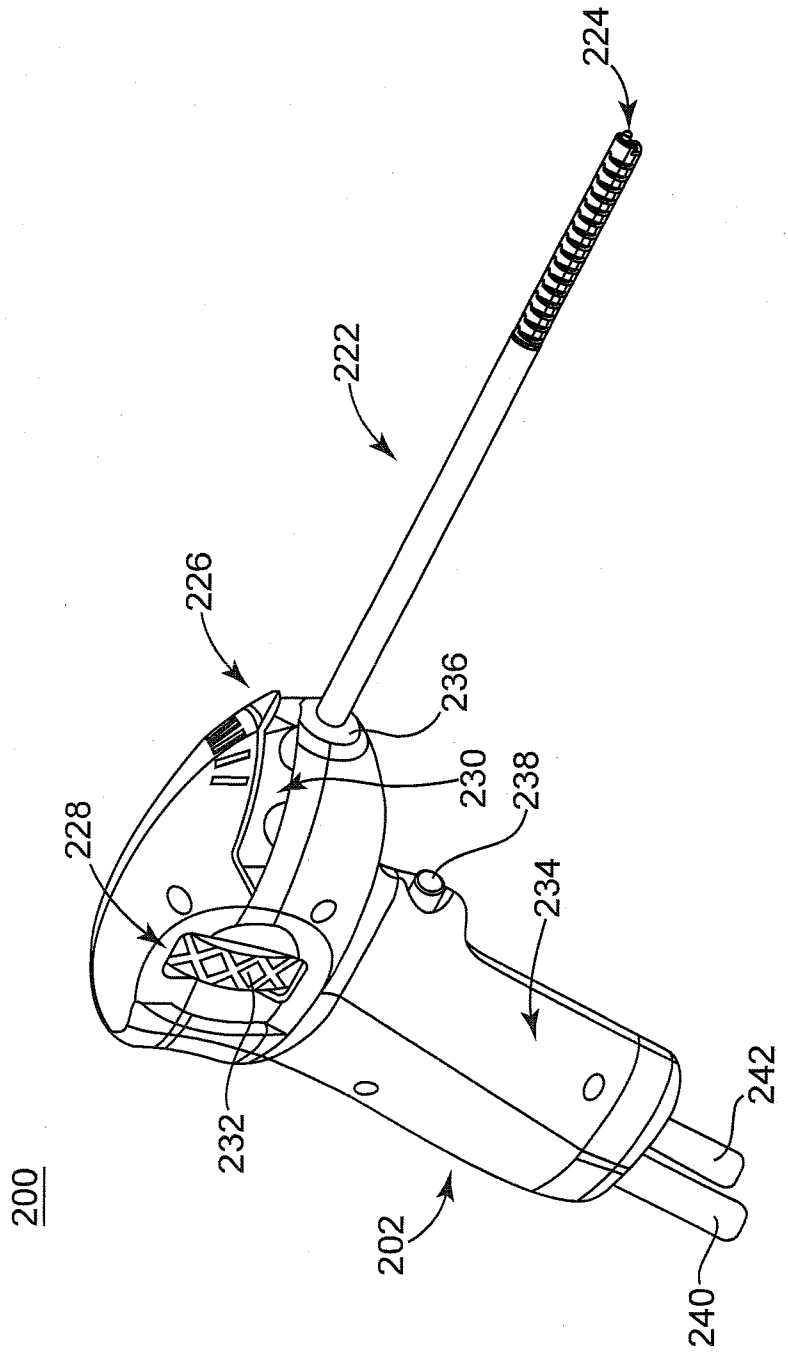
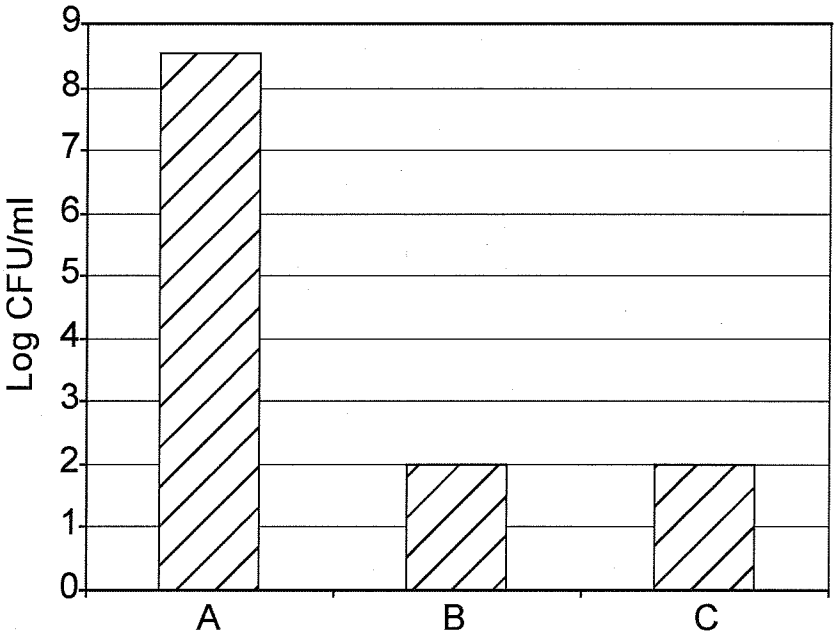


Fig. 2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7
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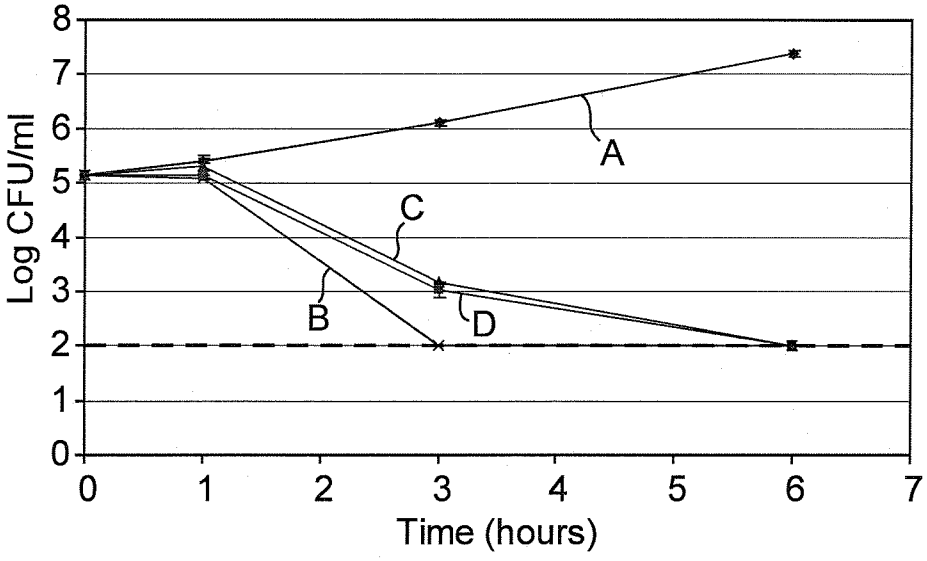
5
4
3
2
1
0
A
B
C

Fig. 3


Log CFU/ml
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6
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4
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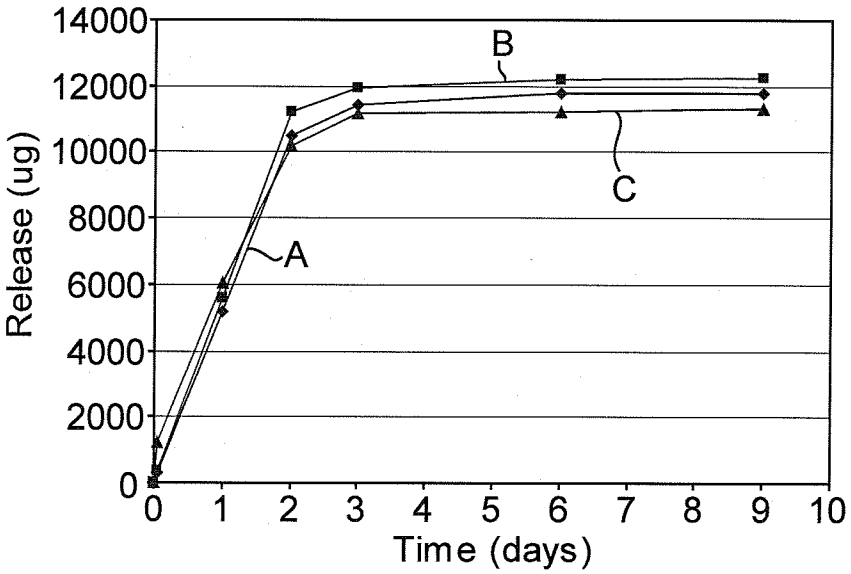
B
C
D
0
1
2
3
4
5
6
7
Time (hours)

Fig. 4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12000
10000
8000
6000
4000
2000
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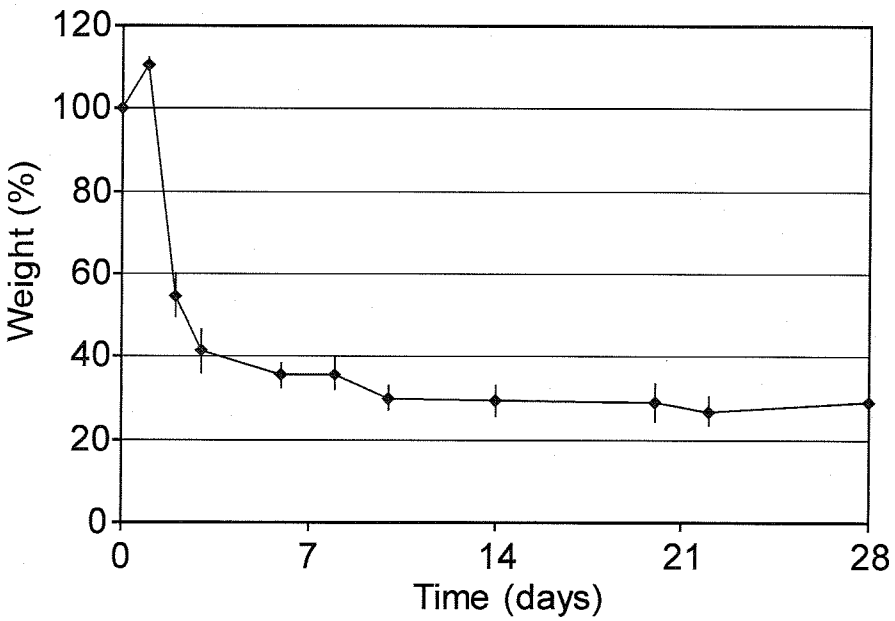
Release (ug)
0 1 2 3 4 5 6 7 8 9 10
Time (days)
A
B
C

Fig. 5


120
100
80
60
40
20
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Weight (%)
0 7 14 21 28
Time (days)

Fig. 6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/041591

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K9/70 A61K31/717 A61K31/718 A61K31/722

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)
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 practical, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2008/075657 A1 (ABRAHAMS JOHN M [US] ET AL) 27 March 2008 (2008-03-27) claims	1-25
X	WO 2004/026200 A (JOHNSON & JOHNSON MEDICAL LTD [GB]; CULLEN BREDA MARY [GB]; SILCOCK DE) 1 April 2004 (2004-04-01) example 3	1-25
P,X	WO 2008/067655 A (Q6 BIOMATERIALS INC [CA]; RAFAT MEHRDAD [CA]; LI FENGFU [CA]; LATORRE) 12 June 2008 (2008-06-12) example 11/12	1-25

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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"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
13 July 2009	17/07/2009

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	Zimmer, Barbara

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/041591

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2008075657 A1	27-03-2008	WO 2009017753 A2	05-02-2009
WO 2004026200 A	01-04-2004	AU 2003264890 A1	08-04-2004
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WO 2008067655 A	12-06-2008	NONE	