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(71) Applicants: **PSIVIDA US, INC.** [US/US]; 400 Pleasant Street, Watertown, MA 02472 (US). **PSIMEDICA LIMITED** [GB/GB]; Malvern Hills Science Park, Geraldine Road, Malvern, Worcestershine WR14 3SZ (GB).

(72) Inventors: **BARNETT, Christian**; 3 Lower House Farm Barns, Throckmorton, Pershore Worcestershire WR10 2JX (GB). **ASHTON, Paul**; 76 Page Road, Newton, MA 02460 (US). **CANHAM, Leigh, T.**; 73 Barnards Green Road, Malvern, Worcestershire WR14 3LR (GB).

(74) Agents: **HALSTEAD, David, P.** et al.; Foley Hoag LLP, Seaport West, 155 Seaport Boulevard, Boston, MA 02210-2600 (US).

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(54) Title: BIOERODIBLE SILICON-BASED COMPOSITIONS FOR DELIVERY OF THERAPEUTIC AGENTS

(57) Abstract: The invention comprises a composition comprising a bioerodible porous silicon-based carrier material wherein the carrier material carries at least one large molecule therapeutic agent and at least one amorphous sugar, optionally further comprising a crystallization inhibitor. The composition may be used *in vitro* or *in vivo* to deliver the therapeutic agent, preferably in a controlled fashion over an intended period of time such as over multiple days, weeks or months. The composition may be used for treating or preventing conditions of a patient such as chronic diseases.

Bioerodible Silicon-Based Compositions for Delivery of Therapeutic Agents

RELATED APPLICATIONS

This application claims the benefit of priority to United States Provisional Patent Application serial number 61/798,324, filed March 15, 2013, the contents of which are hereby incorporated by reference herein in their entirety.

BACKGROUND

There has been considerable interest within the pharmaceutical industry in the development of dosage forms which provide controlled release of therapeutic agents over a period of time. Releasing an active substance in this way can help to improve bioavailability and ensure that appropriate concentrations of the agent are provided for a sustained period without the need for repeated dosing. In turn, this also helps to minimize the effects of patient non-compliance which is frequently an issue with other forms of administration.

Patients may be reluctant to comply with their treatment regime, as compliance may be painful and traumatic. For example, today there exist therapeutic agents that can treat, with good clinical success, ophthalmic conditions, such as age-related macular degeneration, diabetic macular edema, diabetic retinopathy, choroidal neovascularization, and other conditions that can lead to blindness or near blindness. Often the afflicted population is an older patient group who must adjust their activities of daily living to cope with the early stages of these diseases. However, as the disease progresses, permanent eye damage occurs and many clinically effective treatments are only preventative, and not restorative. Thus, consistent compliance to the treatment regime is nearly mandatory to prevent loss of sight.

Unfortunately, treatment regimens typically require the patient to hold still while the physician pierces the patient's eye with a hypodermic needle to deliver the therapeutic agent into the eye, typically the vitreous of the eye. This can be traumatic and painful and accordingly a patient may be reluctant to receive the injections. The ability to provide a longer-term benefit for each injection, and thus reduce the pain and trauma suffered by the patient, turns on the required pharmacokinetics of the therapeutic agent and the delivery vehicle that carries and releases the agent.

Some known delivery vehicles have active ingredients that are incorporated into

polymer and sol-gel systems by entrapment during synthesis of the matrix phase. Microencapsulation techniques for biodegradable polymers include such methods as film casting, molding, spray drying, extrusion, melt dispersion, interfacial deposition, phase separation by emulsification and solvent evaporation, air suspension coating, pan coating and in-situ polymerization. Melt dispersion techniques are described, for example, in U.S. Pat. No. 5,807,574 and U.S. Pat. No. 5,665,428.

In an alternative approach, the active ingredient is loaded after formation of the porous matrix is complete. Such carrier systems generally have micron-sized rather than nanometer-sized pores to allow the agents to enter into the pores. U.S. Pat. No. 6,238,705, for example, describes the loading of macroporous polymer compositions by simple soaking in a solution of the active ingredient and U.S. Pat. Nos. 5,665,114 and 6,521,284 disclose the use of pressure to load the pores of implantable prostheses made of polytetrafluoroethene (PTFE). While this approach may be effective for small organic molecules, larger molecules such as proteins tend to aggregate in large pores and do not effectively release *in vivo* in a controlled manner.

With smaller pores, it has proved difficult to incorporate high concentrations of therapeutic agents due to blocking of the narrow pores. Deposition of material towards the opening of the pores tends to prevent a high proportion of the material from occupying the pore system. The problem of achieving high loading of the active ingredient limits the effectiveness of many currently known delivery systems.

Another concern when delivering therapeutic agents through an delivery vehicle is the biocompatibility of the delivery vehicle following release of the drug. Bioerodible or resorbable delivery vehicle materials would be an attractive alternative to delivery vehicles that require removal following release of the drug. The design and preparation of bioerodable delivery vehicles for carrying therapeutic agents has begun to be explored. PCT Publication No. WO2009/009563 describes a drug delivery system comprising a porous silicon material.

Therefore, there remains a continuing need for the development of improved dosage forms for the controlled release of therapeutic agents, which are biocompatible and are capable of delivering large molecules in a sustained fashion.

SUMMARY

The invention comprises a composition comprising a bioerodible porous silicon-based carrier material wherein the carrier material carries at least one large molecule therapeutic agent and at least one amorphous sugar, optionally further comprising a crystallization

inhibitor. In certain embodiments, the composition is prepared using vacuum-assisted flash drying.

The disclosed compositions are for delivering therapeutic agents, particularly large molecules such as proteins, peptides, antibodies, carbohydrates, polymers, vaccines, small interfering RNA (siRNA) or polynucleotides, in a controlled manner. The compositions comprise a porous silicon-based carrier material loaded with the therapeutic agent and an amorphous sugar. In some embodiments, the compositions comprise a porous silicon-based carrier material loaded with the therapeutic agent and a mixture of amorphous sugars. In some embodiments, the compositions comprise a porous silicon-based carrier material loaded with the therapeutic agent, and a mixture of a sugar and a crystallization inhibitor. The compositions may be used *in vitro* or *in vivo* to deliver the therapeutic agent, preferably in a controlled fashion over an intended period of time such as over multiple days, weeks or months. The carrier material is preferably formed from a bioerodible or resorbable material, e.g., a silicon-based material such as elemental silicon or silicon dioxide, such that removal following release of the therapeutic agent is unnecessary. In certain such embodiments, the carrier material and its breakdown products are biocompatible such that the biological side-effects from the bioerosion of the carrier material are minimal or innocuous.

In certain embodiments, the carrier material comprises porous silicon dioxide, such as mesoporous silicon dioxide. The average pore size of the carrier material is typically selected so that it may carry the therapeutic agent, and example pore sizes are from 2-50 nm in diameter, such as from about 15 to about 40 nm in diameter, from about 20 to about 30 nm in diameter, from about 2 to about 15 nm in diameter, or about 5 to about 10 nm in diameter. Silicon-based materials are also disclosed in U.S. 20120177695, which is incorporated herein by reference.

In certain embodiments, the therapeutic agent is a protein with a molecular weight between about 500 amu and about 200,000 amu, and maybe about 800 amu and about 200,000 amu, about 1000 amu and about 200,000 amu, about 1500 amu and about 200,000 amu, about 2,000 amu and about 200,000, about 5,000 amu and about 200,000 amu, about about 10,000 to about 150,000 amu, between about 10,000 and about 50,000 amu, between about 50,000 and about 100,000 amu or between about 100,000 and about 200,000 amu.

The size of a therapeutic agent may alternatively be characterized by the molecular radius, which may be determined, for example, through X-ray crystallographic analysis or by hydrodynamic radius. The therapeutic agent may be a protein, e.g., with a molecular radius selected from 0.5 nm to 20 nm, such as about 0.5 nm to 10 nm, even from about 1 to 8 nm.

Preferably, a suitable pore radius to allow access to particular agents, e.g., proteins, is selected according to a pore-therapeutic agent (agent) differential, defined herein as the difference between the radius of a agent and a radius of a pore. For example, the pore-agent differential for insulin, with a hydrodynamic radius of 1.3 nm and a pore with a minimum radius of 4.8 nm has a pore-protein differential of 3.5 nm. A pore-agent differential may be used to determine minimum suitable average pore size for accommodating a protein of a particular radius. The pore-protein differential may typically be selected from about 3.0 to about 5.0 nm.

Typically the compositions are selected to have an average pore size to accommodate the therapeutic agent. The average pore size of the carrier material may be chosen based on the molecular weight or the molecular radius of the therapeutic agent to be loaded into the pores of the carrier material. For example, a therapeutic agent of molecular weight selected from 100,000 to 200,000 amu may be used with a carrier material of larger average pore size such as from about 15 nm to about 40 nm. In certain embodiments, a therapeutic agent of molecular weight selected from 5,000 to 50,000 amu may be used with a carrier material of smaller average pore size such as from about 2 nm to about 10 nm.

In certain embodiments, the sugars, whether used alone or in combination, are selected from sucrose, fructose, glucose, erythritol, maltitol, lactitol, sorbitol, mannitol, xylitol, D-tagatose, trehalose, trehalose dehydrate, galactose, glycerol, rhamnose, cyclodextrin, raffinose, ribulose, ribose, threose, arabinose, xylose, lyxose, allose, altrose, mannose, idose, lactose, maltose, invert sugar, isotrehalose, neotrehalose, palatinose or isomaltulose, erythrose, deoxyribose, gulose, idose, talose, erythrulose, xylulose, psicose, turanose, cellobiose, glucosamine, mannosamine, fucose, glucuronic acid, gluconic acid, glucono-lactone, abequose, galactosamine, xylo-oligosaccharides, gentio-oligoscaccharides, galacto-oligosaccharides, sorbose, nigero-oligosaccharides, fructooligosaccharides, maltotetraol, maltotriol, maltodextrin, malto-oligosaccharides, lactulose, melibiose, or any combinations thereof. In preferred embodiments, the sugar is selected from trehalose, trehalose dihydrate, sucrose, mannitol, sorbitol, xylitol or glycerol, or a combination thereof.

In certain embodiments, the compositions are prepared by forming the porous carrier material first and then loading the pores with the therapeutic agent, and the amorphous or solution form of the sugar, or a plurality of sugars, or a combination of a sugar and a crystallization inhibitor. In preferred embodiments, the therapeutic agent is loaded before the amorphous or solution form of the sugar or the crystallization inhibitor.

The invention includes methods for loading a therapeutic agent into the pore of a porous silicon-based carrier material, comprising contacting a porous silicon-based carrier material with a therapeutic agent. One exemplary method for loading a therapeutic agent into the pore of a porous silicon-based carrier material comprises selecting a porous silicon-based carrier having pore sizes dimensionally adapted to allow a single protein to load into the pore such that opposite sides of the protein engage opposite sides of the pore. One method for loading a therapeutic agent into the pore of a porous silicon-based carrier material comprises selecting a porous silicon-based carrier having pore sizes dimensionally adapted to admit only a single agent into the width of a single pore at one time (i.e., longitudinal series along the length of a pore are not excluded), e.g., two agents could not be accommodated if positioned side-by-side (laterally) within a pore. Methods for loading an agent into the pore of a silicon-based material and for selecting appropriate carrier materials for an agent of interest are also disclosed in U.S. 20120177695, which is incorporated herein by reference.

The compositions may be disposed on the skin or on the surface of the eye. Alternatively, the compositions may be disposed within the body of a mammal, such as within the eye of a patient, or within any other tissue or organ of the patient's body. In particular applications, the compositions are disposed subcutaneously, subconjunctivally or in the vitreous of the eye. The compositions may be used for treating or preventing conditions of a patient such as chronic diseases. In certain embodiments, the compositions are for treating or preventing diseases of the eye such as glaucoma, macular degeneration, diabetic macular edema and age-related macular degeneration. The therapeutic agent may be released in a controlled manner over a period of weeks or months, for example, to treat or prevent diseases of the eye such as macular degeneration.

The invention comprises stabilized formulations comprising amorphous sugars and methods of stabilizing therapeutic agents in a porous carrier material as described herein. In certain embodiments, the invention comprises stabilized biomolecules, such as antibodies, in the pores of the carrier material such that the half-life or the shelf life of the biomolecule is superior to the half-life or shelf life of the biomolecule outside of the carrier material. In certain embodiments, the proteins of the stabilized formulations are stable to drying under reduced pressure at room temperature ambient conditions. In certain embodiments, the porous carrier material comprising a therapeutic agent and an amorphous sugar is coated with a polymer. In preferred embodiments, the porous carrier material comprising a therapeutic agent and an amorphous sugar is coated with a controlled release polymer.

In certain embodiments of compositions as described herein, the amorphous forms of sugars of the compositions described herein, when in contact with the porous carrier materials described herein, retain their amorphous character at 25 °C / 60% relative humidity after 90 days than under similar conditions without the porous carrier materials. In certain embodiments, the amorphous sugars stabilize biomolecules, e.g., antibodies, at the temperature of 25 °C for at least 15 days, at least 1 month, at least 6 months, at least 1 year, at least 1.5 years, at least 2 years, at least 2.5 years, at least 3 years or at least 4 years.

In some embodiments, the stabilized formulations of the invention are stable when exposed to non-aqueous solvent such as dichloromethane, or any solvent not capable of solubilizing the sugar.

DETAILED DESCRIPTION

Figure 1 shows the stabilisation of the amorphous sugars in mesoporous oxidized anodized silicon (e.g., as prepared by Examples 1-3) for 90 days at 25 °C and 60% relative humidity.

Figure 2 shows the stability of bevacizumab after vacuum drying on mesoporous oxidized anodized silicon versus commercial freeze drying.

Figure 3 shows the dissolution of myoglobin co-formulated with sucrose in coated 60 Å mesoporous oxidized anodized silicon particles.

Overview

Sustained and controlled delivery of therapeutic agents to patients, particularly patients with chronic conditions such as glaucoma or cancer, is becoming increasingly important in modern medical therapy. Many therapies are most effective when administered at frequent intervals to maintain a near constant presence of the active agent within the body. While frequent administration may be recommended, the inconvenience and associated difficulty of patient compliance may effectively prevent treatment in this manner. As a result, sustained release compositions that release therapeutic agents in a controlled manner are very attractive in fields such as cancer therapy and treatment of other chronic diseases.

Compositions that release therapeutic agents *in vivo* or *in vitro* may be formed from a variety of biocompatible or at least substantially biocompatible materials. One type of composition employs a silicon-based carrier material. Silicon-based carrier materials may include, for example, elemental silicon, and oxidized silicon in forms such as silicon dioxide (silica), or silicates. Some silicon-based compositions have demonstrated high

biocompatibility and beneficial degradation in biological systems, eliminating the need to remove the carrier material following release of the therapeutic agent.

Tests show that high porosity silicon-based materials, e.g., 80% porosity, are resorbed faster than medium porosity silicon-based material, e.g., 50% porosity, which in turn is resorbed faster than bulk silicon-based material, which shows little to no sign of bioerosion or resorption in biological systems. Furthermore, it is understood that the average pore size of the carrier material will affect the rate of resorption. By adjusting the average pore size of a carrier material as well as the porosity of the material, the rate of bioerosion may be tuned and selected.

Silicon-based carrier materials are often prepared using high temperatures and organic solvents or acidic media to form the porous material and load the therapeutic agent within the pores. These conditions may be suitable for certain molecules such as salts, elements, and certain highly stable small organic molecules. However, for loading large organic molecules such as proteins or antibodies, caustic and/or severe conditions during the preparation or loading of the template could lead to denaturing and deactivation, if not complete degradation of the active agent. Loading large molecules such as antibodies into the carrier material under mild conditions is a feature of the methods described herein that is particularly advantageous for large organic molecules such as proteins.

The particle size of the silicon-based carrier material may also affect the rate in which the pores of the carrier material may be loaded with the therapeutic agent. Smaller particles, e.g., particles in which the largest diameter is 20 microns or less, may load more rapidly than particles in which the largest diameter is greater than 20 microns. This is particularly apparent when the pore diameters are similar in dimensions to the molecular diameters or size of the therapeutic agents. The rapid loading of smaller particles may be attributed to the shorter average pore depth that the therapeutic agent must penetrate in smaller particles.

Definitions

As used herein the specification, "a" or "an" may mean one or more. As used herein in the claim(s), when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein “another” may mean at least a second or more.

The terms "antibody" and "antibodies" broadly encompass naturally occurring forms of antibodies and recombinant antibodies, such as single-chain antibodies, camelized antibodies, chimeric, and humanized antibodies and multi-specific antibodies as well as

fragments and derivatives of all of the foregoing, preferably fragments and derivatives having at least an antigenic binding site. Antibody derivatives may comprise a protein or chemical moiety conjugated to the antibody. The term "antibody" is used in the broadest sense and covers fully assembled antibodies, and recombinant peptides comprising them.

"Antibody fragments" comprise a portion of an intact antibody, preferably the antigen-binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies (Zapata *et al.* (1995) Protein Eng. 8(10):1057-1062); single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')2 fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

Bioerode or bioerosion, as used herein, refers to the gradual disintegration or breakdown of a structure or enclosure over a period of time in a biological system, e.g., by one or more physical or chemical degradative processes, for example, enzymatic action, hydrolysis, ion exchange, or dissolution by solubilization, emulsion formation, or micelle formation.

The term “preventing” is art-recognized, and when used in relation to a condition, such as a local recurrence (e.g., pain), a disease such as cancer, a syndrome complex such as heart failure or any other medical condition, is well understood in the art, and includes administration of a composition which reduces the frequency of, or delays the onset of, symptoms of a medical condition in a subject relative to a subject which does not receive the composition. Thus, prevention of cancer includes, for example, reducing the number of detectable cancerous growths in a population of patients receiving a prophylactic treatment relative to an untreated control population, and/or delaying the appearance of detectable cancerous growths in a treated population versus an untreated control population, e.g., by a statistically and/or clinically significant amount. Prevention of an infection includes, for example, reducing the number of diagnoses of the infection in a treated population versus an untreated control population, and/or delaying the onset of symptoms of the infection in a treated population versus an untreated control population. Prevention of pain includes, for example, reducing the magnitude of, or alternatively delaying, pain sensations experienced by subjects in a treated population versus an untreated control population.

The term "prophylactic or therapeutic" treatment is art-recognized and includes administration to the host of one or more of the subject compositions. If it is administered prior to clinical manifestation of the unwanted condition (e.g., disease or other unwanted state of the host animal) then the treatment is prophylactic, (i.e., it protects the host against developing the unwanted condition), whereas if it is administered after manifestation of the unwanted condition, the treatment is therapeutic, (i.e., it is intended to diminish, ameliorate, or stabilize the existing unwanted condition or side effects thereof).

Resorption or resorbing as used herein refers to the erosion of a material when introduced into or onto a physiological organ, tissue, or fluid of a living human or animal.

A "therapeutically effective amount" of a compound with respect to the subject method of treatment refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, e.g., at a reasonable benefit/risk ratio applicable to any medical treatment.

As used herein, the term "treating" or "treatment" includes reversing, reducing, or arresting the symptoms, clinical signs, and underlying pathology of a condition in manner to improve or stabilize a subject's condition.

Unless otherwise indicated, the term large therapeutic molecule refers to molecules with molecular weights equal to or greater than 2000 amu, or even greater than 3000 amu.

Unless otherwise indicated, the term "small molecule" refers to an organic molecule having a molecular weight less than about 2000 amu, preferably less than about 1500 amu, more preferably less than about 1000 amu, or most preferably less than about 750 amu. Preferably, a small molecule contains one or more heteroatoms.

Unless otherwise indicated, the term "sugar" refers to monosaccharides, disaccharides, oligosaccharides or sugar alcohols. Examples for the term "sugar" are, but not limited to, sucrose, fructose, glucose, erythritol, maltitol, lactitol, sorbitol, mannitol, xylitol, D-tagatose, trehalose, trehalose dehydrate ,galactose, glycerol, rhamnose, cyclodextrin, raffinose, ribulose, ribose, threose, arabinose, xylose, lyxose, allose, altrose, mannose, idose, lactose, maltose, invert sugar, isotrehalose, neotrehalose, palatinose or isomaltulose, erythrose, deoxyribose, gulose, idose, talose, erythrulose, xylulose, psicose, turanose, cellobiose, glucosamine, mannosamine, fucose, glucuronic acid, gluconic acid, glucono-lactone, abequose, galactosamine, xylo-oligosaccharides, gentio-oligoscaccharides, galacto-oligosaccharides, sorbose, nigero-oligosaccharides, fructooligosaccharides, maltotetraol,

maltotriol, maltodextrin, malto-oligosaccharides, lactulose, melibiose, or any combinations thereof.

Silicon-Based Carrier Materials

The devices and methods described herein provide, among other things, compositions comprising a porous silicon-based carrier material wherein at least one therapeutic agent and an amorphous sugar are disposed in a pore of the carrier material. The described methods use such compositions for treatment or prevention of diseases, particularly chronic diseases. Furthermore, the described methods of preparing compositions provide compositions which are characterized by sustained and controlled release of therapeutic agents, particularly large molecules such as proteins or antibodies.

The composition typically comprises a silicon-based carrier material such as elemental silicon, silicon dioxide (silica), silicon monoxide, silicates (compounds containing a silicon-bearing anion, e.g., SiF_6^{2-}, $Si_2O_7^{6-}$, or SiO_4^{4-}), or any combination of such materials. In certain embodiments, the carrier material comprises a complete or partial framework of elemental silicon and that framework is substantially or fully covered by a silicon dioxide surface layer. In other embodiments, the carrier material is entirely or substantially entirely silica.

In certain embodiments, the carrier material comprises silica, such as greater than about 50% silica, greater than about 60 wt% silica, greater than about 70 wt% silica, greater than about 80 wt% silica, greater than about 90 wt% silica, greater than about 95 wt% silica, greater than 99 wt% silica, or even greater than 99.9 wt% silica. Porous silica may be purchased from suppliers such as Davisil, Salicycle, and Macherey-Nagel.

In certain embodiments, the carrier material comprises elemental silicon, greater than 60 wt% silicon, greater than 70 wt% silicon, greater than 80 wt % silicon, greater than 90 wt % silicon, or even greater than 95% silicon. Silicon may be purchased from suppliers such as Vesta Ceramics.

Purity of the silicon-based material can be quantitatively assessed using techniques such as Energy Dispersive X-ray Analysis, X-ray fluorescence, Inductively Coupled Optical Emission Spectroscopy or Glow Discharge Mass Spectroscopy.

The carrier material may comprise other components such as metals, salts, minerals or polymers. The carrier material may have a coating (such as a polymer coating) disposed on at least a portion of the surface, e.g., to improve biocompatibility of the carrier material and/or affect release kinetics.

The silicon-based carrier material may comprise elemental silicon or compounds thereof, e.g., silicon dioxide or silicates, in an amorphous form. In some embodiments, the silicon-based carrier material comprises fumed silica. In certain embodiments, the elemental silicon or compounds thereof is present in a crystalline form. In other embodiments, the carrier material comprises amorphous silica and/or amorphous silicon. In certain embodiments, the silicon-based material is greater than about 60 wt% amorphous, greater than about 70 wt% amorphous, greater than about 80 wt% amorphous, greater than about 90 wt% amorphous, greater than about 92 wt% amorphous, greater than about 95 wt% amorphous, greater than about 99 wt% amorphous, or even greater than 99.9 wt% amorphous.

X-ray diffraction analysis can be used to identify crystalline phases of silicon-based material. Powder diffraction can be taken, for example, on a Scintag PAD-X diffractometer, e.g., equipped with a liquid nitrogen cooled germanium solid state detector using Cu K-alpha radiation.

The silicon-based material may have a porosity of about 40% to about 95% such as about 60% to about 80%. Porosity, as used herein, is a measure of the void spaces in a material, and is a fraction of the volume of voids over the total volume of the material. In certain embodiments, the carrier material has a porosity of at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or even at least about 90%. In particular embodiments, the porosity is greater than about 40%, such as greater than about 50%, greater than about 60%, or even greater than about 70%.

The carrier material of the compositions may have a surface area to weight ratio selected from about 20 m^2/g to about 2000 m^2/g, such as from about 20 m^2/g to about 1000 m^2/g, or even from about 100 m^2/g to about 300 m^2/g. In certain embodiments, the surface area is greater than about 200 m^2/g, greater than about 250 m^2/g or greater than about 300 m^2/g.

In certain embodiments, the therapeutic agent is distributed to a pore depth from the surface of the carrier material of at least about 10 microns, at least about 20 microns, at least about 30 microns, at least about 40 microns, at least about 50 microns, at least about 60 microns, at least about 70 microns, at least about 80 microns, at least about 90 microns, at least about 100 microns, at least about 110 microns, at least about 120 microns, at least about 130 microns, at least about 140 microns or at least about 150 microns. In certain

embodiments, the therapeutic agent is distributed in the pores of the carrier material substantially uniformly.

The therapeutic agent may be loaded into the carrier material to a depth which is measured as a ratio to the total width of the carrier material. In certain embodiments, the therapeutic agent is distributed to a depth of at least about 10% into the carrier material, to at least about 20% into the carrier material, at least about 30% into the carrier material, at least about 40% into the carrier material, at least about 50% into the carrier material, or at least about 60% into the carrier material.

The amorphous sugar may be loaded into the carrier material to a depth which is measured as a ratio to the total width of the carrier material. In certain embodiments, the amorphous sugar is distributed to a depth of at least about 1% to at least about 9%, to at least 10% into the carrier material, to at least about 20% into the carrier material, at least about 30% into the carrier material, at least about 40% into the carrier material, at least about 50% into the carrier material, or at least about 60% into the carrier material. In some embodiments, the amorphous sugar may seal the pores.

The amorphous sugar may be loaded into the carrier material to a weight that is measured as a ratio to the combined weight of the carrier material and therapeutic agent. In certain embodiments, the amorphous sugar is loaded to a weight at least about 1% to at least about 80%, at least about 1% to at least about 70%, at least about 1% to at least about 60%, at least about 1% to at least about 50%, at least about 1% to at least about 40%, at least about 1% to at least about 30%, at least about 1% to at least about 20%, to at least about 1% to at least about 15%, about 1% to at least about 10%, about 1% to at least about 5%, about 1% to at least about 4%, at least about 1% to at least about 3%, or at least about 1% to at least about 2%. In certain embodiments, the amorphous sugar is loaded to a weight at least about 5% to at least about 10%, at least about 10% to at least about 20%, at least about 10% to at least about 30%, at least about 30% to at least about 40%, at least about 40% to at least about 50%, at least about 50% to at least about 60%, at least about 60% to at least about 70%, or at least about 70% to at least about 80%. In certain embodiments, the amorphous sugar may be loaded to a weight of about 30%. Quantification of gross loading may be achieved by a number of analytic methods, for example, gravimetric, EDX (energy-dispersive analysis by x-rays), Fourier transform infra-red (FTIR) or Raman spectroscopy of the pharmaceutical composition or by UV spectrophotometry, titrimetric analysis, HPLC or mass spectroscopy of the eluted therapeutic agent in solution. Quantification of the uniformity of loading may be

obtained by compositional techniques that are capable of spatial resolution such as cross-sectional EDX, Auger depth profiling, micro-Raman and micro-FTIR.

Porous silicon-based materials of the invention may be categorized by the average diameter of the pore size. Microporous silicon-based material has an average pore size less than 2 nm, mesoporous silicon-based material has an average pore size of between 2-50 nm and macroporous silicon-based material has a pore size of greater than 50 nm. In certain embodiments, greater than 50% of the pores of the silicon-based material have a pore size from 2-50 nm, greater than 60% of the pores of the silicon-based material have a pore size from 2-50 nm, greater than 70% of the pores of the silicon-based material have a pore size from 2-50 nm, greater than 80% of the pores of the silicon-based material have a pore size from 2-50 nm, or even greater than 90% of the pores of the silicon-based material have a pore size from 2-50 nm.

In certain embodiments, the carrier material comprises porous silicon dioxide, such as mesoporous silicon dioxide. In certain embodiments, the average pore size of the carrier material is selected from 2-50 nm, such as from about 15 to about 40 nm, such as about 20 to about 30 nm. In certain embodiments, the average pore size is selected from about 2 to about 15 nm, such as about 5 to about 10 nm. In certain embodiments, the average pore size is about 30 nm.

The pore size may be preselected to the dimensional characteristics of the therapeutic agent to control the release rate of the therapeutic agent in a biological system. Typically, pore sizes that are too small preclude loading of the therapeutic agent, while oversized pores do not interact with the therapeutic agent sufficiently strongly to control the rate of release. For example, the average pore diameter for a carrier material may be selected from larger pores, e.g., 15 nm to 40 nm, for high molecular weight molecules, e.g., 200,000-500,000 amu, and smaller pores, e.g., 2 nm to 10 nm, for molecules of a lower molecular weight, e.g., 10,000-50,0000 amu. For instance, average pore sizes of about 6 nm in diameter may be suitable for molecules of molecular weight around 14,000 to 15,000 amu such as about 14,700 amu. Average pore sizes of about 10 nm in diameter may be selected for molecules of molecular weight around 45,000 to 50,000 amu such as about 48,000 amu. Average pore sizes of about 25-30 nm in diameter may be selected for molecules of molecular weight around 150,000 nm.

The pore size may be preselected to be adapted to the molecular radii of the therapeutic agent to control the release rate of the therapeutic agent in a biological system. For instance, average pore sizes of about 25 nm to about 40 nm in diameter may be suitable

for molecules with a largest molecular radius from about 6 nm to about 8 nm. Molecular radii may be calculated by any suitable method such as by using the physical dimensions of the molecule based on the X-ray crystallography data or using the hydrodynamic radius which represents the solution state size of the molecule. As the solution state calculation is dependant upon the nature of the solution in which the calculation is made, it may be preferable for some measurements to use the physical dimensions of the molecule based on the X-ray crystallography data. As used herein the largest molecular radius reflects half of the largest dimension of the therapeutic agent.

In certain embodiments, the average pore diameter is selected to limit the aggregation of molecules, e.g., proteins, within a pore. It would be advantageous to prevent biomolecules such as proteins from aggregating in a carrier material as this is believed to impede the controlled release of molecules into a biological system. Therefore, a pore that, due to the relationship between its size and the size of a biomolecule, allows, for example, only one biomolecule to enter the pore at any one time, will be preferable to a pore that allows multiple biomolecules to enter the pore together and aggregate within the pore. In certain embodiments, multiple biomolecules may be loaded into a pore, but due to the depth of the pore, the proteins distributed throughout this depth of the pore will aggregate to a lesser extent.

In certain embodiments, the therapeutic agent is selected from any agent useful in the treatment or prevention of diseases. In certain embodiments, the agent is selected from small molecule therapeutic agents, i.e., compounds with molecular weights less than 1000 amu. In preferred embodiments, the therapeutic agents are selected from large molecules with molecular weight equal to or greater than 1000 amu. In certain embodiments, the therapeutic agent of the invention is a biomolecule. Biomolecules, as used herein, refer to any molecule that is produced by a living organism, including large polymeric molecules such as proteins, polysaccharides, and nucleic acids as well as small molecules such as primary metabolites, secondary metabolites, and natural products or synthetic variations thereof. In particular, proteins such as antibodies, ligands, and enzymes may be used as therapeutic agents of the invention. In particular embodiments, the biomolecules of the invention have molecular weights ranging from about 10,000 amu to about 500,000 amu. In certain embodiments, the therapeutic agent is selected from one or more monoclonal antibodies, such as ranibizumab (Lucentis) and bevacizumab (Avastin).

In certain embodiments, the therapeutic agent has a molecular weight between 10,000 and 50,000 amu, between 50,000 and 100,000 amu or between 100,000 and 150,000 amu. In

certain embodiments, the therapeutic agent is a protein with a molecular weight between 5,000 amu and 200,000 amu, such as about 10,000 to about 150,000 amu.

The size of a therapeutic agent may alternatively be characterized by the molecular radius, which may be determined, for example, through X-ray crystallographic analysis or by hydrodynamic radius. The therapeutic agent may be a protein, e.g., with a molecular radius selected from 0.5 nm to 20 nm such as about 0.5 nm to 10 nm, even from about 1 to 8 nm.

A therapeutic agent with molecular radius from 1 to 2.5 nm may be advantageously used with a carrier material with a minimum pore radius of from 4.5 to 5.8 nm. A therapeutic agent with a molecular radius of 7 nm may be advantageously used with a carrier material with a minimum pore radius of from 11 to 13 nm, such as about 12 nm. For example, insulin with a hydrodynamic radius of 1.3 nm may be used with a carrier material that has an average minimum pore radius of 4.8 nm.

The protein-pore differential may be used to choose a suitable carrier material to accommodate the therapeutic agent. This calculation subtracts the molecular radius from the pore radius. Typically, the radius of the therapeutic agent would be the hydrodynamic radius or largest radius determined through x-ray crystallographic analysis. The pore radius would typically be the average pore radius of the carrier material. For example, the pore-protein differential for insulin, with a hydrodynamic radius of 1.3 nm and a pore with a minimum radius of 4.8 nm has a protein-pore differential of 3.5 nm. In certain embodiments, the protein-pore differential is selected from 3 to 6 nm, such as from 3.2 to 4.5 nm. The protein-pore differential may be about 3.2 nm, about 3.3 nm, about 3.4 nm, about 3.5 nm, about 3.6 nm, about 3.7 nm, about 3.8 nm, about 3.9 nm, about 4.0 nm, about 4.1 nm, about 4.2 nm , about 4.3 nm, about 4.4 nm or about 4.5 nm.

In certain embodiments, the therapeutic agent is an antibody and the average pore size of the carrier material is selected from about 20 nm to about 40 nm such as from about 25 nm to 35 nm such as about 30 nm. In certain embodiments, the therapeutic agent is an antibody selected from bevacizumab or ranibizumab and the average pore size of the carrier material is selected from about 20 nm to about 40 nm such as from about 25 nm to 35 nm such as about 30 nm. In certain embodiments, the therapeutic agent is bevacizumab and the average pore size of the carrier material is about 30 nm.

In certain embodiments, the walls of the carrier material that separate the pores have an average width of less than 5 nm, such as about 4.8 nm, about 4.6 nm, about 4.4 nm, about 4.2 nm, about 4.0 nm, about 3.8 nm, about 3.6 nm, about 3.4 nm, about 3.2 nm, about 3.0 nm, about 2.8 nm, or even about 2.6 nm. In certain embodiments, the walls of the carrier material

that separate the pores have an average width of less than about 3 nm, such as about 2.8 nm, about 2.6 nm, about 2.4 nm, about 2.2 nm, about 2.0 nm, about 1.8 nm, about 1.6 nm, about 1.4 nm, about 1.2 nm, about 1.0 nm, or even about 0.8 nm.

Dimensionality and morphology of the carrier material can be measured, for example, by Transmission Electron Microscopy (TEM) using a 2000 JEOL electron microscope operating, for example, at 200 keV. Samples for TEM can be prepared by dispensing a large number of porous carrier material particles onto a holey carbon film on a metal grid, via a dilute slurry.

In certain embodiments, the pores of the carrier material define space having a volume of about 0.1 mL/g to about 5 mL/g of the carrier material. In certain embodiments, the pore volume is about 0.2 mL/g to about 3 mL/g, such as about 0.4 mL/g to about 2.5 mL/g, such as about 1.0 mL/g to about 2.5 mL/g.

In certain embodiments, the load level of the carrier material is up to 80% by weight based on the combined weight of the carrier material and the therapeutic agent. The load level is calculated by dividing the weight of the loaded therapeutic agent by the combined weight of the loaded therapeutic agent and carrier material and multiplying by 100. In certain embodiments, the load level of the carrier material is greater than 1%, such as greater than 3%, such as greater than 5%, such as greater than 10%, such as greater than 15%, greater than 20%, greater than 25%, greater than 30%, greater than 35%, greater than 40%, greater than 45%, such as greater than 50%, such as greater than 60%, or greater than 70%. The load level may be between about 5% and about 10%. In certain embodiments, the load level of the carrier material is between about 10% and about 20%, between about 20% and about 30%, between about 30% and about 40%, between about 40% and about 50%, between about 50% and about 60%, between about 60% and about 70% or between about 70% and about 80% by weight.

In certain embodiments, the load level of the carrier material is up to 40% weight based on the weight of the composition. In certain embodiments, the load level of the carrier material is greater than 1%, such as greater than 3%, such as greater than 5%, such as greater than 10%, such as greater than 15%, greater than 20%, greater than 25%, greater than 30%, or greater than 35%. The load level may be between about 5% and about 10%. In certain embodiments, the load level of the carrier material is between about 10% and about 20%, between about 20% and about 30%, between about 30% and about 40% by weight. The load level is calculated by dividing the weight of the loaded therapeutic agent divided by the weight of the composition and multiplying by 100. The composition may comprise the carrier

material, the therapeutic agent, the amorphous sugar and optionally other components such as a crystallization inhibitor. In some embodiments, the composition comprises:
a therapeutic agent (such as a protein) in the range of 1% to 40% by weight,
an amorphous sugar in the range of 1% to 50% by weight, and
a carrier material in the range of 10% to 30% by weight.

The load volume of the carrier materials described herein may be evaluated in terms of the volume of the pores in the porous material being occupied by the therapeutic agent. The percentage of the maximum loading capacity that is occupied by the therapeutic agent (that is, the percentage of the total volume of the pores in the porous carrier material that is occupied by the therapeutic agent) for carrier materials according to the invention may be from about 30% to about 100%, such as from about 50% to about 90%. For any given carrier material, this value may be determined by dividing the volume of the therapeutic agent taken up during loading by the void volume of the carrier material prior to loading and multiplied by one hundred.

In certain embodiments, the carrier materials of the invention are particles that, measured at the largest diameter, have an average size of about 1 to about 500 microns, such as about 5 to about 100 microns. In certain embodiments, a single carrier material particle measured at its largest diameter is about 1 to about 500 microns, such as about 5 to about 500 microns.

In order to increase the rate of loading of the particles of the invention, it may be advantageous to use relatively small particles. As smaller particles have pores with less depth for the therapeutic agent to penetrate, the amount of time needed to load the particles may be reduced. This may be particularly advantageous when the pore diameters are similar in dimensions to the molecular diameters or size of the therapeutic agents. Smaller particles may be from 1-20 microns, such as about 10-20 microns, e.g., about 15-20 microns, measured at the largest dimension.

In some aspects, greater than 60%, greater than 70%, greater than 80% or greater than 90% of the particles have a particle size of from 1-20 microns, preferably 5-15 microns, measured at the largest dimension. The particles may have an average particle size between 1 and 20 microns such as between 5-15 microns or about 15 microns, about 16 microns, about 17 microns, about 18 microns, about 19 microns.

Particle size distribution, including the mean particle diameter can be measured, for example, using a Malvern Particle Size Analyzer, Model Mastersizer, from Malvern Instruments, UK. A helium-neon gas laser beam may be projected through an optical cell

containing a suspension of the carrier material. Light rays striking the carrier material are scattered through angles which are inversely proportional to the particle size. The photodetector array measures the light intensity at several predetermined angles and electrical signals proportional to the measured light flux values are then processed by a microcomputer system against a scatter pattern predicted from the refractive indices of the sample carrier material and aqueous dispersant.

Larger carrier material particles or implants are also envisioned for controlled delivery of therapeutic agents. The particles/implants of the invention may have an average size of about 1 mm to about 5 cm measured at the largest dimension. In certain embodiments, the particles/implants have an average size of about 5 mm to about 3 cm measured at the largest dimension. Particles greater than 1 mm, as measured at the largest dimension, may be useful for intramuscular subcutaneous, intravitreal or subdermal drug delivery.

In certain embodiments, the amorphous sugars described herein present in the pores are used to stabilize sensitive therapeutic compounds, such as biomolecules, e.g., antibodies. In certain embodiments, biomolecules that are partially or wholly unstable at elevated temperatures, such as room temperature or above, can be made stable at room temperature for prolonged periods of time. For example, the biomolecule formulated with amorphous sugars within the carrier material is stable to drying under reduced pressure at room temperature.

In certain embodiments, the porous carrier materials described herein are used to stabilize sensitive therapeutic compounds, such as biomolecules, e.g., antibodies. In certain embodiments, biomolecules that are partially or wholly unstable at elevated temperatures, such as room temperature or above, can be made stable at room temperature for prolonged periods of time. The biomolecules may be loaded into a carrier material such that an aqueous suspension of the biomolecule loaded into the carrier material is more stable than a corresponding aqueous solution of the biomolecule (i.e., an identical aqueous solution with and without the addition of the porous carrier material). For example, the biomolecule within the carrier material may have a half-life at room temperature (e.g., about 23 °C) that is greater than a half-life of the biomolecule without the carrier material under the same conditions. In certain embodiments, a biomolecule in the pores of the carrier material has a half-life that is at least twice as long as the biomolecule outside of the carrier material under the same conditions, more preferably, at least five times, at least 10 times, at least than 15 times, at least 20 times, at least 30 times, at least 40 times, at least 50 times, at least 60 times, or at least 100 times as long as the biomolecule outside of the carrier material. For example, an antibody within the pores of the carrier material may have a half-life that is at least 10 times

as long as the antibody outside of the carrier material, more preferably, at least 20 times as long.

Similarly, biomolecules formulated with amorphous sugars may have a longer shelf life within the pores of the carrier material than in a corresponding aqueous solution, preferably at least twice as long, at least five times as long, at least 10 times as long, at least 20 times as long, at least 30 times as long, at least 40 times as long, at least 50 times as long, at least 60 times as long or at least 100 times as long. For example, an antibody within the pores of the carrier material may have a longer shelf life than an antibody outside of the carrier material, preferably at 10 times as long, at least 20 times as long.

In certain embodiments, porous compositions comprising the carrier material and, a biomolecule, such as an antibody, and amorphous sugars exhibit stability at the temperature of 25 °C for at least 15 days, or even about 1 month. Additionally or alternatively, in certain embodiments, the antibody-loaded carrier materials are stable at 25 °C for at least 6 months, at least 1 year, at least 1.5 years, at least 2 years, at least 2.5 years, at least 3 years or at least 4 years. Stability may be assessed, for example, by high performance size exclusion chromatography (HPSEC) or by comparing the biological activity of the stored biomolecule-loaded compositions against a sample of freshly prepared biomolecule-loaded compositions or against the activity of the compositions as measured prior to storage. Activity of antibodies, for example, can be assessed by various immunological assays including, for example, enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay. Preferably, at the end of the storage period, the activity of the stored compositions is at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, at least 99.5%, at least 99.8%, or even at least 99.9% of the activity of the corresponding freshly prepared compositions. Accordingly, the invention contemplates methods of treatment wherein biomolecule-loaded compositions are stored at 25 °C for at least 6 months, at least 1 year, at least 1.5 years, at least 2 years, at least 2.5 years, at least 3 years or at least 4 years prior to administering the compositions to a patient.

The invention further comprises methods of stabilizing biomolecules. Methods of the invention comprise loading biomolecules into the pores of the carrier material through any suitable method to form the compositions of the invention.

Methods of Preparation

The invention also provides methods of preparing silicon-based carrier materials. In certain embodiments, porous silicon-based carrier material may be prepared synthetically.

For example, porous silica may be synthesized by reacting tetraethyl orthosilicate with a template made of micellar rods. In certain embodiments, the result is a collection of spheres or rods that are filled with a regular arrangement of pores. The template can then be removed, for example, by washing with a solvent adjusted to the proper pH. In certain embodiments, the porous silicon-based carrier material may be prepared using a sol-gel method or a spray drying method. In certain embodiments, the porous silicon based carrier material may be prepared by flame hydrolysis of silicon tetrachloride in an oxy-hydrogen flame. In certain embodiments, the preparation of the carrier material involves one or more techniques suitable for preparing porous silicon-based material.

Pores may be introduced to the silicon-based carrier material through techniques such as anodization, stain etching, or electrochemical etching. In an exemplary embodiment, anodization employs a platinum cathode and silicon wafer anode immersed in Hydrogen Fluoride (HF) electrolyte. Corrosion of the anode producing pores in the material is produced by running electrical current through the cell. In particular embodiments, the running of constant DC is usually implemented to ensure steady tip-concentration of HF resulting in a more homogeneous porosity layer.

In certain embodiments, pores are introduced to the silicon-based carrier material through stain-etching with hydrofluoric acid, nitric acid and water. In certain embodiments, a combination of one or more stain-etching reagents are used, such as hydrofluoric acid and nitric acid. In certain embodiments, a solution of hydrofluoric acid and nitric acid are used to form pores in the silicon-based material.

The porosity of the material can be determined by weight measurement. BET analysis may be used to determine any one or more of the pore volume, pore size, pore size distribution and surface area of the carrier material. BET theory, named after the combined surname initials of authors of the theory, applies to the physical adsorption of gas molecules on a solid surface and serves as the basis for an important analysis technique for the measurement of the specific surface area of a material (J. Am. Chem. Soc.v. 60, p 309 (1938)). The BET analysis may be performed, for example, with a Micromeritics ASAP 2000 instrument available from Micromeritics Instrument Corporation, Norcross, Georgia. In an exemplary procedure, the sample of carrier material may be outgassed under vacuum at temperatures, for example, greater than 200 °C for a period of time such as about 2 hours or more before the measurements are taken. In certain embodiments, the pore size distribution curve is derived from the analysis of the adsorption branch of the isotherm output. The pore volume may be collected at the P/P0 = 0.985 single point.

One or more drying techniques may be used in the preparation of porous silicon-based materials of the invention. For example, to prevent cracking of the porous silicon-based material, the material may be dried by supercritical drying, freeze drying, pentane drying, slow evaporation, spray drying or vacuum-assisted flash drying. Supercritical drying involves superheating the liquid pore above the critical point to avoid interfacial tension. Freeze drying involves freezing and subliming any solvents under vacuum. Pentane drying uses pentane as the drying liquid instead of water and as a result may reduce capillary stress due to the lower surface tension. Slow evaporating is a technique which can be implemented following the water or ethanol rinsing and may be effective at decreasing the trap density of solvent within the material. Spray drying is a technique whereby a solution of protein and sugar is spray dried so that the water is evaporated sufficiently quickly to allow the sugar to go from a solution to a solid without reordering into a crystal. Vacuum-assisted flash drying is a technique whereby the porous matrix assists the rapid drying of the formulation under reduced pressure whilst stabilising the amorphous sugar. Vacuum-assisted flash drying may be performed at room temperature, which is desirable for physically stabilized amorphous systems such as biomolecules and sugars.

The surface of the porous silicon-based material may be modified to exhibit properties such as improved stability, cell adhesion or biocompatibility. Optionally, the material may be exposed to oxidizing conditions such as through thermal oxidation. In an exemplary embodiment, the process of thermal oxidation involves heating the silicon-based material to a temperature above 1000 °C to promote full oxidation of the silicon-based material. Alternatively, the surface of the carrier material may be oxidized so that the carrier material comprises a framework of elemental silicon partially, substantially or fully covered by an oxizided surface such as a silicon dioxide surface.

The surface of the porous silicon-based material or a portion thereof may be derivatized. In an exemplary embodiment, the surface of a porous silicon-based material may be derivatized with organic groups such as alkanes or alkenes. In a particular embodiment, the surface of the carrier material may be derivatized by hydrosilation of silicon. In particular embodiments, the derivatized carrier materials may function as biomaterials, incorporating into living tissue.

Any one or more of electrostatic interactions, capillary action and hydrophobic interactions may enable loading of the therapeutic agent into the pores of the carrier material. In certain embodiments, the carrier material and therapeutic molecules are placed in a solution and the large molecules, e.g., proteins or other antibodies, are drawn from the

solution into the pores of the carrier material, reminiscent of a molecular sieve's ability to draw water from an organic liquid. Hydrophobic drugs may be better suited for loading into carrier materials that are predominantly formed from silicon (e.g., greater than 50% of the material is silicon) while hydrophilic drugs may be better suited for loading into a carrier material that is characterized as mostly silica (e.g., greater than 50% of the carrier material is silica). In certain embodiments, the loading of large molecules into the pores of the carrier material is driven by external factors such as sonication or heat. The carrier material may have an electrostatic charge and/or the therapeutic agent may have an electrostatic charge. Preferably, the carrier material has the opposite electrostatic charge as the therapeutic agent such that adsorption of the therapeutic agent into the pores of the carrier material is facilitated by the attractive electrostatic forces. In certain embodiments, the therapeutic agent or the carrier material itself does not have an electrostatic charge under neutral conditions, but is polarizable or ionizable. For example, in such embodiments, the carrier material and/or the therapeutic agent may be ionized to facilitate the adsorption of the therapeutic agent in the pores of the carrier material. For example, in the body, at physiological pH, silicon dioxide exhibits a negatively charged surface, which promotes electrostatic adsorption of positively charged peptides. Similarly, molecules with carboxylic acids, phosphoric, and/or sulfonic acids are ionized with increasing pH to negatively charged carboxylate, phosphate, and/or sulfonate salts, while nitrogenated molecules (e.g., bearing amine, guanidine, or other basic substituents) are protonated with decreasing pH to ammonium, guanidinium, or other positively charged salts.

The carrier material may comprise a coating or surface modification to attract the therapeutic agent into the pores. In certain embodiments, the carrier material is coated or modified in whole or in part with a material comprising moieties that are charged in order to attract a protein or antibody into the pores of the carrier material. In other embodiments, the moieties may be appended directly to the carrier material. For example, amine groups may be covalently appended onto the surface of the carrier material such that when protonated at physiological pH, the surface of the carrier material carries a positive charge, thereby, for example, attracting a protein or antibody with a negatively charged surface. In other embodiments, the carrier material may be modified with carboxylic acid moieties such that when deprotonated at physiological pH, the carrier material carries a negative charge, thereby attracting proteins or antibodies with positively charged surfaces into the pores.

In certain embodiments, the therapeutic agent may be incorporated into the carrier material following complete formation of the carrier material. Alternatively, the therapeutic agent may be incorporated into the carrier material at one or more stages of preparation of the carrier material. For example, the therapeutic agent may be introduced to the carrier material prior to a drying stage of the carrier material, or after the drying of the carrier material or at both stages. In certain embodiments, the therapeutic agent may be introduced to the carrier material following a thermal oxidation step of the carrier material.

More than one therapeutic agent may be incorporated into a carrier material. In certain such embodiments, each therapeutic agent may be individually selected from small organic molecules and large molecules such as proteins and antibodies. For example, an ocular carrier material may be impregnated with two therapeutic agents for the treatment of glaucoma, or one therapeutic agent for the treatment of macular degeneration and another agent for the treatment of glaucoma.

In certain aspects, e.g., when both small molecule therapeutic agents and larger molecular therapeutic agents such as proteins are incorporated into a carrier material, the therapeutic agents may be incorporated into the carrier material at different stages of the preparation of the composition. For example, a small molecule therapy may be introduced into the carrier material prior to an oxidation or drying step and a large molecule therapeutic agent may be incorporated following an oxidation or drying step. Similarly, multiple different therapeutic agents of the same or different types may be introduced into a finished carrier material in different orders or essentially simultaneously.

When a carrier material comprises a single material, or combination of multiple materials with multiple pore sizes, the larger therapeutic agent is preferably added to the carrier material prior to adding the smaller therapeutic agent to avoid filling the larger pores with the smaller therapeutic agent and interfering with adsorption of the larger therapeutic agent. For example, if a carrier material comprises a single material, or combination of multiple materials, that has some well-defined pores that are about 6 nm in diameter (i.e., suitable for molecules of molecular weight around 14,000 to 15,000 amu) and some well-defined pores that are about 10 nm in diameter (i.e., suitable for molecules of molecular weight around 45,000 to 50,000 amu), the latter therapeutic agent (i.e., the one with molecules of molecular weight around 45,000 to 50,000 amu) are preferably added to the carrier material prior to adding the smaller therapeutic agent (i.e., the one with molecules of molecular weight around 14,000 to 15,000 amu). Alternatively and additionally, in the embodiment wherein the two different porous materials together comprise the device, each

carrier material may be separately loaded with a different therapeutic agent and then the carrier materials may be combined to yield the device.

The therapeutic agent may be introduced into the carrier material in admixture or solution with one or more pharmaceutically acceptable excipients. The therapeutic agent may be formulated for administration in any suitable manner, typically in the form of a composition, suitably for subcutaneous, intramuscular, intraperitoneal or epidermal introduction or for implantation into an organ (such as the liver, lung or kidney). Therapeutic agents according to the invention may be formulated for parenteral administration in the form of an injection, e.g., intraocularly, intravenously, intravascularly, subcutaneously, intramuscularly or infusion, or for oral administration.

The carrier material may be in any suitable form prior to loading with the therapeutic agent such as in the form of a dry powder or particulate or formulated in an aqueous slurry, e.g., with a buffer solution or other pharmaceutically acceptable liquid. The therapeutic agent may be in any suitable form prior to loading into the carrier material such as in a solution, slurry, or solid such as a lyophilisate. The carrier material and/or the therapeutic agent may be formulated with other components such as excipients, preservatives, stabilizers, e.g., sugars, or therapeutic agents, e.g., antibiotic agents.

The therapeutic agent may be formulated (and packaged and/or distributed) as a solution with a concentration of >50mg/mL, such as >60mg/mL, such as >75 mg/mL. In an exemplary embodiment, the therapeutic agent is becacizumab and the becacizumab may be formulated with a concentration of >50mg/mL, such as >60mg/mL, such as >75 mg/mL in, for example, a phosphate buffer solution. The therapeutic agent may be formulated (and packaged and/or distributed) with a surfactant and/or a stabilizer, e.g., sugars, wherein the therapeutic agent has a maximum concentration of 50 mg/mL. A protein fragment, such as an antibody fragment, may be formulated (and packaged and/or distributed) as a solution with a concentration of >10 mg/mL, >15 mg/mL or >20 mg/mL.

The therapeutic agent may be formulated (and packaged and/or distributed) with stabilizers, excipients, surfactants or preservatives. In some embodiments, the stabilizers, excipients, surfactants or preservatives are sugars. In particular embodiments, the sugars are selected from thehalose, sucrose, mannitol, sorbitol, xylitol or glycerol. In other embodiments, the therapeutic agent is formulated (and packaged and/or distributed) essentially free of any one or more of stabilizers, excipients, surfactants and preservatives, e.g., contains less than 1 mg/mL or preferably less than 0.1 mg/mL of a stabilizer, excipients,

surfactant or preservative. The formulation of the therapeutic agent may contain less than 1 mg/mL of surfactants such as less than 0.1 mg/mL of surfactants.

In certain embodiments, the composition may comprise a coating surrounding the particles (e.g., the carrier material/agent/sugar complex) to regulate release of the therapeutic agent. For example, the particles may be coated with a polymeric coating (e.g., by spray-drying) an excipient such as cocoa butter to obtain a desired release profile of the therapeutic agent from the delivery vehicle. A polymeric coating may be biodegradable or non-biodegradable, permeable or non-permeable to release of the agent. One of skill in the art will recognize that it is preferred for the polymer to be permeable, biodegradable, or both in order for the agent to be released from the particles.

In certain embodiments, the particles of the composition may be coated with a range of polymers/solvents such as polyurethane, polysilicone, poly(ethylene-co-vinyl acetate), polyvinyl alcohol, polyanhydride, polylactic acid (PLA), poly(lactic-co-glycolic acid) (PLGA), polyorthoester, polyalkylcyanoacrylate, polycaprolactone, derivatized cellulose based polymers and derivatives and copolymers thereof, such as polymethacrylate-based copolymers, to obtain a desired release profile of the therapeutic agent from the carrier material.

Methods of Use

In certain embodiments, the compositions are used to prevent or treat a condition of a patient. The various embodiments provided herein are generally provided to deliver a therapeutically affective amount of a therapeutic agent locally, i.e., to the site of the pain, disease, etc., in a patient. In certain embodiments, the compositions of the invention may be delivered to any site on the surface or within the body of a patient. For example, compositions of the invention may used on the surface of the skin or eye or may be implanted under the skin, within a muscle, within an organ, adjacent to a bone, within the eye or at any other location where controlled release of a therapeutic agent would be beneficial. The compositions may be administered intravitreally, subcutaneously, subconjunctivally, intraperitoneally, intramuscularly or subretinally. In certain embodiments, the compositions of the invention is delivered to the surface of the eye or within the eye such as within the sclera of the eye or within the vitreous of the eye.

In certain embodiments, the compositions of the invention are used to treat intraocular diseases, such as back of the eye diseases. Exemplary intraocular diseases include glaucoma, age-related macular degeneration such as wet age-related macular degeneration, diabetic

macular edema, geographic atrophy, choroidal neovascularization, uveitis, diabetic retinopathy, retinovascular disease and other types of retinal degenerations.

In certain embodiments, the compositions of the invention are used to treat diseases on the surface of the eye. Exemplary diseases include viral keratitis and chronic allergic conjunctivitis.

In certain embodiments, the method for treating an ocular condition comprises disposing the composition on the surface of the eye or within the eye such as within the vitreous or aqueous of the eye. In certain embodiments, the composition is injected or surgically inserted within the eye of the patient. In certain embodiments, the composition is injected within the eye of the patient, e.g., into the vitreous of the eye. In certain embodiments, the composition is injected as a composition. In certain embodiments, a composition comprises multiple carrier material particles. The composition may comprise particles with an average size between about 1 micron to about 500 microns. In certain embodiments, the composition comprises particles with an average particle size between 5 microns and 300 microns such as between about 5 microns and 100 microns.

In certain aspects, compositions of the invention may be used to administer any therapeutic agent in a sustained fashion to a patient in need thereof. The compositions of the invention are not limited to ocular and intraocular use and may be used in any part of the body. For example, compositions of the invention may be used to administer therapeutic agents subdermally similar to the Norplant contraceptive device. In other embodiments, compositions of the invention are used to administer biomolecules over a sustained period of time for the treatment of chronic diseases such as arthritis. For example, compositions of the invention may be used to deliver therapeutic agents such as etanercept or adalimumab to patients in need of this therapy. The compositions of the invention may be located any place in the body such as within a muscle. The composition may comprise multiple small particles such as multiple particles 500 microns or less. The compositions may comprise larger particles such as greater than 500 microns or one or more particles greater than 1 mm in size such as greater than 10 mm.

The therapeutic agent may be a small molecule or biomolecule. The therapeutic agent may be released to the patient over the course of up to four, six, or even up to twelve months after administration. In some embodiments, the therapeutic agent is released to the patient over the course of 1 month to 6 months. In preferred embodiments, the therapeutic agent is released to the patient over the course of 2 days to 2 weeks. In preferred embodiments, the therapeutic agent is released to the patient over the course of 4 days to 12 days. In preferred

embodiments, the therapeutic agent is released to the patient over the course of 6 days to 10 days. In preferred embodiments, the therapeutic agent is released to the patient over the course of 7days.

In certain embodiments, the composition is injected or surgically inserted subcutaneously. In other embodiments, the composition is delivered to the patient intravenously or intraarticularly.

In some embodiments, the composition is administered orally. In some embodiments, the composition is orally administered and comprises a vaccine. Oral administration can be used, for instance, to deliver active agents to the stomach, small intestine, or large intestine. Formulations for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, and the like, each containing a predetermined amount of an active ingredient. Solid dosage forms for oral administration (capsules, tablets, pills, dragees, powders, granules, and the like), may comprise the device and one or more pharmaceutically acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose, and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, cetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like. The oral compositions can also include sweetening, flavoring, perfuming, and preservative agents.

In certain embodiments, multiple particle populations are delivered to the patient such as two particle populations, three particle populations, four particle populations or five particle populationsor more. The particle populations may be substantially identical in size or composition or may have different sizes, a make up of different carrier materials or be loaded with different therapeutic agents. The multiple particle populations may be administered to

the patient simultaneously or over a period of time, and at one or more locations of the patient's body.

In certain embodiments, the therapeutic agent is released from the composition into the surrounding biological system over a duration of days, weeks, months or years. In certain such embodiments, the therapeutic agent is released over the course of time selected from one day to two years, such as from two weeks to about one year, such as about one month to about one year. The composition may release the drug into the eye over the course of 1 day to 12 months, such as 1 day to 6 months, such as over the course of 1 week to 3 months. In certain embodiments, the therapeutic agent is released within two years, such as with 18 months, within 15 months, within one year, within 6 months, within three months, or even within two months. In certain embodiments, the release of the therapeutic agent from the composition occurs in a controlled manner such that a large percentage of the total impregnated therapeutic agent is not released immediately or within a short time span, e.g., within minutes or hours of administration. For example if the desired drug delivery time is 2 months, the total impregnated therapeutic agent may, for example, be released at a rate of approximately 1/60th of the impregnated therapeutic agent per day. In certain embodiments, controlled release involves the release of a therapeutic agent over the course of, for example, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, or 8 months, wherein the amount of the agent released charts linearly with respect to the full course of delivery. In some embodiments, there may be a burst effect of the therapeutic agent shortly after administration, followed by a substantially constant release over a subsequent period of time. The burst effect may last, for example, from 1-10 days during which a percentage of the loaded drug is released. After the burst, the remainder of the therapeutic agent may be released constantly over a certain period of time. For example, in certain embodiments, less than 10% of the therapeutic agent is released over the first day following administration, and a further 50% is constantly released over the subsequent 2-30 days, e.g. at a substantially constant rate of release. In another exemplary embodiment, less than 10% of the therapeutic agent is released in the first 5 days following administration, followed by constant release of 50% of the therapeutic agent over the subsequent 25 days. By substantially constant release, it is meant that that rate of release of the therapeutic agent from the composition is essentially constant over a certain period of time.

In certain embodiments, the therapeutic agent begins being released immediately after being administered. In certain embodiments, the therapeutic agent is released over the course of approximately 3 to 8 months, such as over the course of about 6 months. In certain

embodiments, additional compositions of the invention are administered to a patient at appropriate periods to ensure a substantially continuous therapeutic effect. For example, successive doses of an composition that releases a drug for a period of six months may be administered biannually, i.e., once every six months.

The release of drug from the composition and into the body can be assessed by serum and vitreous analyses, e.g., using ELISA.

In certain embodiments, the composition may completely or partially bioerode within a biological system. In certain embodiments, the composition may be resorbed by the biological system. In certain embodiments, the composition may be both bioerodible and resorbable in the biological system. In certain embodiments, the carrier material may be partially bioactive such that the material incorporates into living tissue. In some embodiments, after implantation, the carrier material does not substantially mineralize or attract mineral deposits. For instance, in some embodiments, the carrier material does not substantially calcify when placed *in situ* in a site where calcification is undesirable.

In certain embodiments, the composition may bioerode in a biological system. In certain embodiments, greater than about 80% of the carrier material will bioerode in a biological system, such as greater than about 85%, greater than about 90%, greater than about 92%, greater than about 95%, greater than about 96%, greater than about 97%, greater than about 98%, greater than about 99%, greater than 99.5%, or even greater than 99.9%. In certain embodiments, where the carrier material bioerodes, it is partially or completely resorbed.

In certain embodiments, the composition may substantially bioerode of the course of 1 week to 3 years. In certain embodiments, substantially bioerosion refers to erosion of greater than 95% of the carrier material. In certain embodiments, substantial bioerosion occurs of the course of about 1 month to about 2 years, such as about 3 months to 1 year. In certain embodiments, substantial bioerosion occurs within about 3 years, such as within about 2 years, within about 21 months, within about 18 months, within about 15 months, within about 1 year, within about 11 months, within about 10 months, within about 9 months, within about 8 months, within about 7 months, within about 6 months, within about 5 months, within about 4 months, within about 3 months, within about 2 months, within about 1 month within about 3 weeks, within about 2 weeks, within about 1 week, or even within about 3 days. In certain embodiments, where the carrier material bioerodes, it is partially or completely resorbed.

In certain embodiments, the extent of bioerosion may be evaluated by any suitable technique used in the art. In exemplary embodiments, the bioerosion is evaluated through an *in vitro* assay to identify degradation products or *in vivo* histology and analysis. The biodegradability kinetics of the porous carrier material may be assessed *in vitro* by analyzing the concentration of the principle degradation product in the relevant body fluid. For porous silicon-based carrier materials in the back of the eye, for example, the degradation product may include orthosilicic acid, quantified, for example, by the molybdate blue assay, and the body fluid may be simulated or real vitreous humor. The biodegradability kinetics *in vivo* may be determined by implanting a known quantity of the porous silicon-based material into the relevant body site and monitoring its persistence over time using histology combined with, for example, standard microanalytical techniques.

Examples

Materials

Specifications of commercial porous silica

Supplier	Trade Name	Nominal Pore Size (Å)	Surface Area (m^2/g)	Pore Volume (mL/g)
Grace Davison Discovery Sciences	Davisil	60	550	0.9
		150	330	1.2
		250	285	1.8
		500	80	1.1
		1000	40	1.1
SiliCycle	SiliaSphere PC	300	100	1.1

Example 1: Preparation of sugar and porous silica formulation

The co-formulations of mannitol, sorbitol or xylitol with 60Å porous silica (such as Davisil) can be achieved through melt loading. Approximately equal weights of silica and sugar are mixed by hand in a zip-lock bag; then transferred to a suitable sample vial. The mixture is heated at the melting point of the sugar for a period of five minutes.

Example 2: Preparation of trehalose and porous silica formulation

The co-formulation of trehalose with 60Å porous silica (such as Davisil) can be achieved through immersion loading. Approximately 1000 mg of porous silica is immersed in 5mL of a concentrated solution of trehalose (500 mg/mL) and incubated for a period of two hours at room temperature and pressure, under continual agitation. This loading solution can

be prepared using trehalose dihydrate crystals. The starting weight of these crystals must therefore be adjusted, so that the final concentration of the solution is approximately 500 mg/mL. Following incubation, the co-formulation is recovered from the loading solution via spin filtration using a PVDF filter (2 minutes at 13000 rpm), frozen to minus 20°C and freeze-dried. To prevent re-crystallisation of the sugar during centrifugation, samples are heated to approximately 40°C during this process. After the sugar has been loaded, the formulation is dried.

Example 3: Preparation of sucrose and porous silica formulation

The co-formulation of sucrose with 60Å porous silica (such as Davisil) can be achieved through immersion loading. Approximately 1000 mg porous silica is immersed in 5 mL of a saturated solution of sucrose (2 g/mL) and incubated for a period of two hours at room temperature, pressure and under continual agitation. The sample is recovered via spin filtration using a PVDF filter, frozen to minus 20°C and freeze-dried. To prevent re-crystallisation of the sugar during centrifugation, this procedure is completed at a temperature of approximately 40°C. After the sugar has been loaded, the formulation is dried.

Example 4: Preparation of sucrose and porous silica formulations

Bevacizumab (2 mL of a 1 mg/mL solution) was incubated with porous silica 250 Å (e.g., Davisil) (40mg) for 18 hours at room temperature. Sucrose (2 g) was added and the composition was incubated for 20 hours. After incubation the material was recovered via centrifugation through a 0.45μm centrifugal filter at 16,000 g. The composition was freeze-dried for 18 hours.

A control formulation was prepared by freeze drying 100 μL of 1mg/mL bevacizumab in phosphate buffer 50 mM pH 6.2 (without silica). A bevacizumab-sucrose co-formulation control was also prepared by freeze drying 100 μL of 1 mg/mL bevacizumab and 300 μL 1 g/mL sucrose in phosphate buffer 50 mM pH 6.2.

After drying, triplicate samples of each composition were extracted with 200 mM carbonate buffer pH 9.6 for 6 hours. After extraction the samples were centrifuged and the supernatant assayed via SEC to assay for recovery of bevacizumab. Results are shown in figure 2.

Example 5: Preparation of formulations comprising sucrose and mesoporous oxidized anodized silicon material

Mesoporous oxidized anodized silicon material, as disclosed in U.S. Patent 8,318,194 and U.S. 20120177695, was successively incubated with bevacizumab and sucrose as disclosed herein, followed by vacuum drying to remove excess water. Results are shown in figure 2.

Example 6: Release of Myoglobin from Polymer-Coated Particles

Mesoporous oxidized anodized silicon material (60 Å) was loaded with myoglobin and sucrose in analogy to the previous examples and the loaded particles were coated with PLA or PLGA. Release of myoglobin from these coated particles is depicted in Figure 3.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the compounds and methods of use thereof described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims. Those skilled in the art will also recognize that all combinations of embodiments described herein are within the scope of the invention.

While the above described embodiments are in some cases described in terms of preferred characteristics (e.g., preferred ranges of the amount of effective agent, and preferred thicknesses of the preferred layers) these preferences are by no means meant to limit the invention. As would be readily understood by one skilled in the art, the preferred characteristics depend on the method of administration, the beneficial substance used, the shell and carrier materials used, the desired release rate and the like.

All of the foregoing U.S. patents and other publications are expressly incorporated by reference herein in each of their entireties.

We claim:

1. A composition comprising a bioerodable porous silicon-based carrier material wherein the carrier material comprises at least one large molecule therapeutic agent and an amorphous sugar.

2. The composition of any of claim 1, wherein the carrier material is resorbable.

3. The composition of any one of claims 1 to 2, in which the therapeutic agent is distributed through a volume of the carrier material.

4. The composition according to claim 3, in which the therapeutic agent is distributed through substantially the whole volume of the carrier material.

5. The composition of any one of claims 1 to 4, wherein the therapeutic agent is selected from proteins, peptides, antibodies, carbohydrates, polymers and polynucleotides.

6. The composition of claim 5, wherein the therapeutic agent is an antibody.

7. The composition of any one of claims 1 to 6, wherein the amorphous sugar is selected from trehalose, trehalose dihydrate, sucrose, mannitol, sorbitol, xylitol or glycerol, or a combination thereof.

8. The composition of any one of claims 1 to 7, wherein the silicon-based carrier material is amorphous.

9. The composition of any one of claims 1 to 8, in which the carrier material has a porosity of at least about 40%.

10. The composition of claim 9, in which the carrier material has a porosity of at least about 70%.

11. The composition of any one of claims 1 to 8, wherein the carrier material has a porosity in the range of about 40% to about 80%.

12. The composition of any one of claims 1 to 11, wherein the average pore size is in the range 2-50 nm.

13. The composition of claim 12, wherein the average pore size is in the range of 10-50 nm.

14. The composition of any one of claims 1 to 13, wherein the surface area of the carrier material is between 20 and 1000 m^2/g.

15. The composition of claim 14, wherein the surface area of the carrier material is between 100 and 300 m^2/g.

16. The composition of any one of claims 1 to 15, wherein the average width of the walls in the carrier material which separate the pores is less than 5 nm.

17. The composition of claim 16, wherein the average width of the walls is less than 3 nm.

18. The composition of any one of claims 1 to 17, wherein a length of the carrier material measured at its longest point is between 1 and 500 microns.

19. The composition of claim 18, wherein the length of the carrier material as its longest point is between 5 and 100 microns.

20. The composition of any one of claims 1-19, wherein the load level of the carrier material is less than 80% by weight based on the combined weight of the carrier material and therapeutic agent.

21. The composition of any one of claims 1-19, wherein the load level of the carrier material is from about 1% to about 70% by weight based on the combined weight of the carrier material and therapeutic agent.

22. The composition of any one of claims 1-19, wherein the load level of the carrier material is from about 3% to about 50% by weight based on the combined weight of the carrier material and therapeutic agent.

23. The composition of any one of claims 1-19, wherein the load level of the carrier material is from about 5% to about 40% by weight based on the combined weight of the carrier material and therapeutic agent.

24. The composition of any one of claims 1-19, wherein the load level of the carrier material is less than about 40% by weight based on the weight of the composition.

25. The composition of any one of claims 1-19, wherein the load level of the carrier material is from about 1% to about 40% by weight based on the weight of the composition.

26. The composition of any one of claims 1-25, wherein the porous carrier material comprising a therapeutic agent and an amorphous sugar is coated with a polymer.

27. The composition of claim 26, wherein the polymer is a controlled release polymer.

28. A method of preparing a composition of any one of claims 1-27, comprising contacting a porous silicon-based carrier material with a therapeutic agent and a non-crystalline sugar.

29. The method of claim 28, wherein the average pore size of the carrier material is selected to allow for entry of the therapeutic agent and controlled release of the therapeutic agent into a biological medium over at least about three days.

30. The method of claim 29, wherein the average pore size is from about 15 nm to about 40 nm and the therapeutic agent has a molecular weight from about 100,000 to about 200,000 amu.

31. The method of claim 29, wherein the average pore size is from about 25 nm to about 40 nm and the therapeutic agent has a molecular radius from about 6 to about 8 nm.

32. The method of claim 29, wherein the average pore size is from about 2 nm to about 10 nm and the therapeutic agent has a molecular weight from about 5,000 to about 50,000 amu.

33. The method of claim 28, further comprising using a vacuum-assisted drying process.

34. A method of treating or preventing a condition in a patient comprising administering the composition of any one of claims 1-27 to a patient.

35. The method of claim 34, wherein the composition is administered to the surface or the skin or eye of a patient.

36. The method of claim 34, wherein the composition is administered intravitreally, subcutaneously, subconjunctivally, intraperitoneally, intramuscularly or subretinally.

37. The method of claim 34, wherein the composition is administered into the eye.

38. The method of claim 37, wherein the composition is administered within the aqueous of the eye.

39. The method of claim 37, wherein the composition is administered within the vitreous of the eye.

40. The method of claim 34, wherein the condition is selected from conditions of the eye.

41. The method of claim 40, wherein the condition is selected from glaucoma, macular degeneration, diabetic macular edema, geographic atrophy and age-related macular degeneration.

42. The method of any one of claims 34 to 41, wherein the composition releases the drug into the eye over the course of 1 day to 6 months.

43. The method of claim 42, wherein the composition releases the therapeutic agent over the course of 1 week to 3 months.

44. The method of any one of claims 34-43, wherein the porous silicon-based carrier material is contacted with a solution comprising the therapeutic agent.

45. A composition comprising an amorphous sugar and a biomolecule loaded within the pores of a porous silicon-based carrier material, wherein the biomolecule within the carrier material has a half-life at room temperature that is at least twice the half-life of the biomolecule without the carrier material under the same conditions.

46. The composition of claim 45, wherein the half-life of the biomolecule within the carrier material is equal to or greater than 10 times the half-life of the biomolecule without the carrier material under the same conditions.

47. A composition comprising an amorphous sugar and a biomolecule loaded within the pores of a porous silicon-based carrier material, wherein the biomolecule within the carrier material has a shelf life at room temperature that is at least twice as long as the shelf life of the biomolecule without the carrier material under the same conditions.

48. The composition of claim 47, wherein the biomolecule within the carrier material has a shelf life that is at least 10 times as long as the shelf life of the biomolecule without the carrier material under the same conditions.

49. The composition of claim 47 or 48, wherein the biomolecule within the carrier material is stable at 25 °C for at least 6 months.

50. The composition of any one of claims 45 to 49, wherein the biomolecule is an antibody.

5

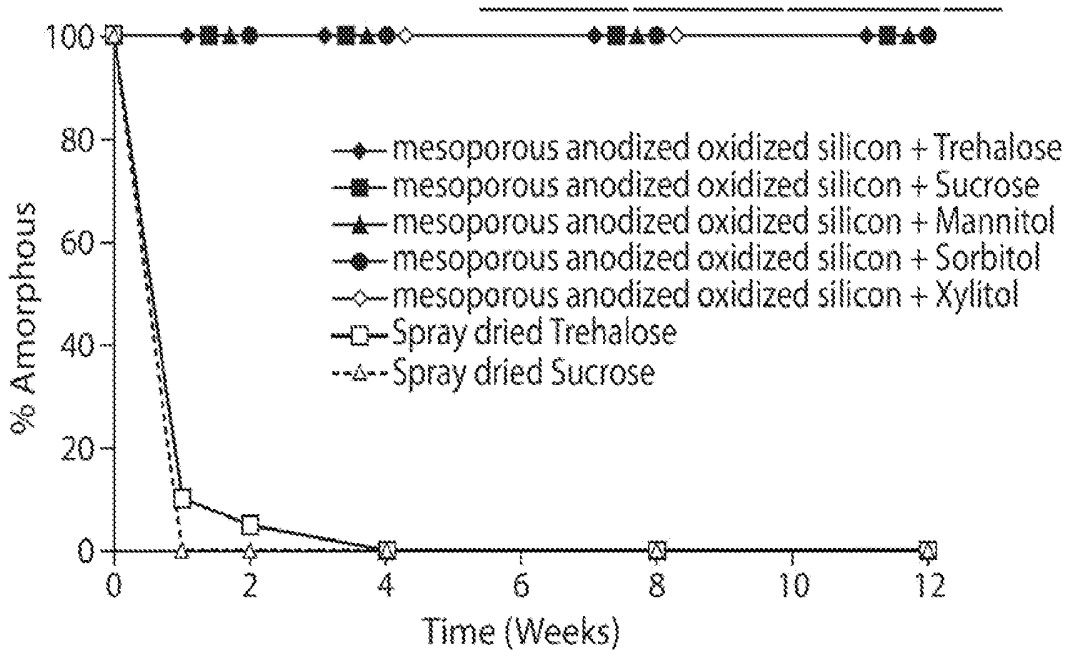

100
80
60
40
20
0
% Amorphous
0
2
4
6
8
10
12
Time (Weeks)
mesoporous anodized oxidized silicon + Trehalose
mesoporous anodized oxidized silicon + Sucrose
mesoporous anodized oxidized silicon + Mannitol
mesoporous anodized oxidized silicon + Sorbitol
mesoporous anodized oxidized silicon + Xylitol
Spray dried Trehalose
Spray dried Sucrose

Fig. 1

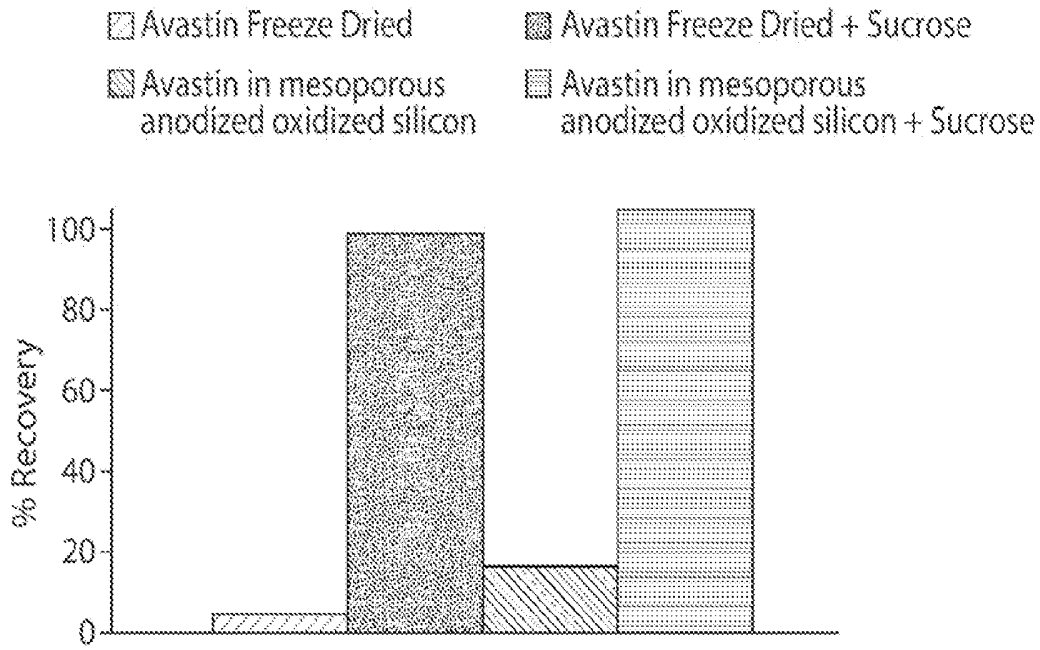
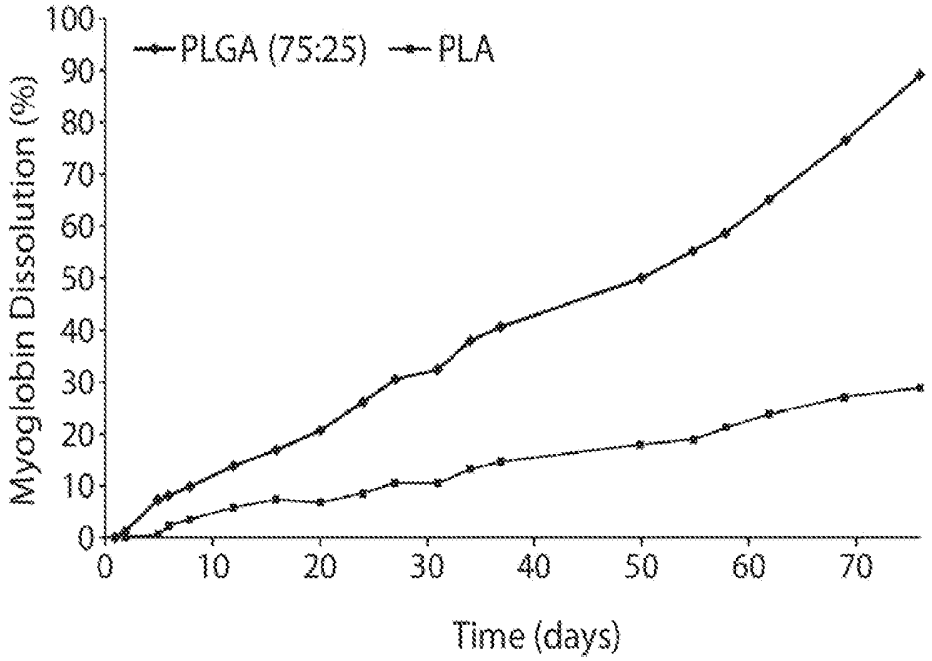

Avastin Freeze Dried
Avastin Freeze Dried + Sucrose
Avastin in mesoporous anodized oxidized silicon
Avastin in mesoporous anodized oxidized silicon + Sucrose
% Recovery
100
80
60
40
20
0

Fig. 2


PLGA (75:25)
PLA
Myoglobin Dissolution (%)
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90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
Time (days)

Fig. 3

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 2014/025612

A. CLASSIFICATION OF SUBJECT MATTER

(see extra sheet)

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 47/00-47/28, 38/00-38/42, 9/00, A61P 27/00-27/06

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)

Espacenet, PAJ, DWPI, Patentscope, USPTO, EAPATIS, RUPAT, RUPAT OLD, PatSearch (RUPTO internal), PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2012/0177695 A1 (PAUL ASHTON et al.) 12.07.2012, claims, examples	1-4, 28-43, 45-49
A	AMORIJ J-P. et al. Development of stable influenza vaccine powder formulations challenges and possibilities. Pharmaceutical Research, vol.25, № 6, June 2008, p.1261	1-4, 28-43, 45-49
A	Clive A. Prestidge et al. Mesoporous silicon: a platform for the delivery of therapeutics. Review, Expert Opinion. Drug Deliv., 2007, 4(2), pp. 101-110, paragraph 8, pp. 105-107	1-4, 28-43, 45-49
A	WO 2012/061377 A1 (PSIVIDA US, INC. et al.) 10.05.2012, claims	1-4, 28-43, 45-49
A	WO 2009/009563 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.) 15.01.2009, claims	1-4, 28-43, 45-49

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

☐ 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 document 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
31 July 2014 (31.07.2014)	14 August 2014 (14.08.2014)
Name and mailing address of the ISA/RU: FIPS, Russia, 123995, Moscow, G-59, GSP-5, Berezhkovskaya nab., 30-1 Facsimile No. +7 (499) 243-33-37	Authorized officer N. Luzina Telephone No. (495)531-64-81

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 2014/025612

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-27, 44, 50
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Classification of subject matter

International application No.
PCT/US 2014/025612

A61K 47/04 *(2006.01)*
A61K 47/26 *(2006.01)*
A61K 38/42 *(2006.01)*
A61K 9/00 *(2006.01)*
A61P 27/02 *(2006.01)*
A61P 27/06 *(2006.01)*

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(71)申请人 普西维达公司
地址 美国马萨诸塞州
申请人 PSI医疗有限公司

(72)发明人 C·巴奈特 P·阿什通 L·T·坎汉

(74)专利代理机构 北京市铸成律师事务所 11313
代理人 孟锐

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A61K 38/42(2006.01)
A61K 9/00(2006.01)
A61P 27/02(2006.01)
A61P 27/06(2006.01)

权利要求书3页 说明书18页 附图2页

(54)发明名称

用于递送治疗剂的可生物蚀解的硅基组合物

(57)摘要

本发明包括一种组合物，所述组合物包含可生物蚀解的多孔硅基载体材料，其中所述载体材料携带至少一种大分子治疗剂和至少一种无定形糖，任选地进一步包含结晶抑制剂。所述组合物可以用于在体外或体内递送治疗剂，优选地以控制方式历经预定时段如多天、多周或多月来递送。所述组合物可以用于治疗或预防患者的病状如慢性疾病。

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1.一种组合物，其包含可生物蚀解的多孔硅基载体材料，其中所述载体材料包含至少一种大分子治疗剂和无定形糖。

2.如权利要求1中任一项所述的组合物，其中所述载体材料是可再吸收的。

3.如权利要求1至2中任一项所述的组合物，其中所述治疗剂在所述载体材料的一定体积上分布。

4.根据权利要求3所述的组合物，其中所述治疗剂在所述载体材料的大致上整个体积上分布。

5.如权利要求1至4中任一项所述的组合物，其中所述治疗剂选自蛋白质、肽、抗体、碳水化合物、聚合物和多核苷酸。

6.如权利要求5所述的组合物，其中所述治疗剂是抗体。

7.如权利要求1至6中任一项所述的组合物，其中所述无定形糖选自海藻糖、二水合海藻糖、蔗糖、甘露醇、山梨醇、木糖醇或甘油，或其组合。

8.如权利要求1至7中任一项所述的组合物，其中所述硅基载体材料是无定形的。

9.如权利要求1至8中任一项所述的组合物，其中所述载体材料的孔隙率为至少约40％。

10.如权利要求9所述的组合物，其中所述载体材料的孔隙率为至少约70％。

11.如权利要求1至8中任一项所述的组合物，其中所述载体材料的孔隙率在约40％至约80％的范围内。

12.如权利要求1至11中任一项所述的组合物，其中平均孔尺寸在2-50nm的范围内。

13.如权利要求12所述的组合物，其中所述平均孔尺寸在10-50nm的范围内。

14.如权利要求1至13中任一项所述的组合物，其中所述载体材料的表面积在20m^2/g和1000m^2/g之间。

15.如权利要求14所述的组合物，其中所述载体材料的表面积在100m^2/g和300m^2/g之间。

16.如权利要求1至15中任一项所述的组合物，其中所述载体材料中分隔所述孔的壁的平均宽度小于5nm。

17.如权利要求16所述的组合物，其中所述壁的所述平均宽度小于3nm。

18.如权利要求1至17中任一项所述的组合物，其中所述载体材料在其最长点处测量的长度在1微米和500微米之间。

19.如权利要求18所述的组合物，其中所述载体材料在其最长点处测量的长度在5微米和100微米之间。

20.如权利要求1-19中任一项所述的组合物，其中以所述载体材料与治疗剂的组合重量计，所述载体材料的负载水平小于80重量％。

21.如权利要求1-19中任一项所述的组合物，其中以所述载体材料与治疗剂的所述组合重量计，所述载体材料的所述负载水平为约1重量％至约70重量％。

22.如权利要求1-19中任一项所述的组合物，其中以所述载体材料与治疗剂的所述组合重量计，所述载体材料的所述负载水平为约3重量％至约50重量％。

23.如权利要求1-19中任一项所述的组合物，其中以所述载体材料与治疗剂的所述组合重量计，所述载体材料的所述负载水平为约5重量％至约40重量％。

24. 如权利要求 1-19 中任一项所述的组合物，其中以所述组合物的重量计，所述载体材料的所述负载水平小于约 40 重量%。

25. 如权利要求 1-19 中任一项所述的组合物，其中以所述组合物重量计，所述载体材料的所述负载水平为约 1 重量%至约 40 重量%。

26. 如权利要求 1-25 中任一项所述的组合物，其中用聚合物涂布包含治疗剂和无定形糖的所述多孔载体材料。

27. 如权利要求 26 所述的组合物，其中所述聚合物为控制释放聚合物。

28. 一种制备如权利要求 1-27 中任一项所述的组合物的方法，其包括使多孔硅基载体材料与治疗剂和非晶糖接触。

29. 如权利要求 28 所述的方法，其中选择所述载体材料的平均孔尺寸以允许历经至少约 3 天使所述治疗剂进入并使所述治疗剂控制释放到生物培养基中。

30. 如权利要求 29 所述的方法，其中所述平均孔尺寸为约 15nm 至约 40nm 并且所述治疗剂的分子量为约 100,000amu 至约 200,000amu。

31. 如权利要求 29 所述的方法，其中所述平均孔尺寸为约 25nm 至约 40nm 并且所述治疗剂的分子半径为约 6nm 至约 8nm。

32. 如权利要求 29 所述的方法，其中所述平均孔尺寸为约 2nm 至约 10nm 并且所述治疗剂的分子量为约 5,000amu 至约 50,000amu。

33. 如权利要求 28 所述的方法，其进一步包括使用真空辅助干燥方法。

34. 一种治疗或预防患者的病状的方法，其包括向患者施用如权利要求 1-27 中任一项所述的组合物。

35. 如权利要求 34 所述的方法，其中向患者的表面或皮肤或眼睛施用所述组合物。

36. 如权利要求 34 所述的方法，其中通过玻璃体内、皮下、结膜下、腹膜内、肌肉内或视网膜下施用所述组合物。

37. 如权利要求 34 所述的方法，其中向眼睛内施用所述组合物。

38. 如权利要求 37 所述的方法，其中在眼房水内施用所述组合物。

39. 如权利要求 37 所述的方法，其中在眼睛的玻璃体内施用所述组合物。

40. 如权利要求 34 所述的方法，其中所述病状选自眼睛的病状。

41. 如权利要求 40 所述的方法，其中所述病状选自青光眼、黄斑变性、糖尿病性黄斑水肿、地图样萎缩和年龄相关性黄斑变性。

42. 如权利要求 34 至 41 中任一项所述的方法，其中所述组合物历经 1 天至 6 个月的过程将所述药物释放到所述眼睛中。

43. 如权利要求 42 所述的方法，其中所述组合物历经 1 周至 3 个月的过程释放所述治疗剂。

44. 如权利要求 34-43 中任一项所述的方法，其中使所述多孔硅基载体材料与包含所述治疗剂的溶液接触。

45. 一种组合物，其包含负载在多孔硅基载体材料的孔内的无定形糖和生物分子，其中所述载体材料内的所述生物分子在室温下的半衰期是在相同条件下无所述载体材料的所述生物分子的半衰期的至少两倍。

46. 如权利要求 45 所述的组合物，其中所述载体材料内的所述生物分子的半衰期等于

或大于在相同条件下无所述载体材料的所述生物分子的半衰期的 10 倍。

47. 一种组合物，其包含负载在多孔硅基载体材料的孔内的无定形糖和生物分子，其中所述载体材料内的所述生物分子在室温下的保质期是在相同条件下无所述载体材料的所述生物分子的保质期的至少两倍长。

48. 如权利要求 47 所述的组合物，其中所述载体材料内的所述生物分子的保质期是在相同条件下无所述载体材料的所述生物分子的保质期的 10 倍长。

49. 如权利要求 47 或 48 所述的组合物，其中所述载体材料内的所述生物分子在 25℃下稳定至少 6 个月。

50. 如权利要求 45 至 49 中任一项所述的组合物，其中所述生物分子为抗体。

用于递送治疗剂的可生物蚀解的硅基组合物

[0001] 相关申请

[0002] 本申请要求 2013 年 3 月 15 日提交的美国临时专利申请序列号 61/798,324 的优先权益，所述美国临时专利申请以引用的方式整体并入本文中。

[0003] 背景

[0004] 在制药工业内在开发在一段时间内提供治疗剂的控制释放的剂型方面一直存在相当大的关注。以此方式释放活性物质可帮助提高生物利用率并且确保适当浓度的药剂被提供持续的时段而不需要重复给药。进而，这还有助于将患者不顺从的影响减小到最低程度，在其它形式的施用情况下患者不顺从经常是一个问题。

[0005] 患者可能不愿顺从其治疗方案，因为顺从可能疼痛并且有创伤。例如，目前存在可在良好的临床成功下治疗眼科病状如年龄相关性黄斑变性、糖尿病性黄斑水肿、糖尿病性视网膜病变、脉络膜新血管形成和可引起失明或近乎失明的其它病状的治疗剂。通常受折磨的群体是年长患者群，其必须调整其日常活动以应付这些疾病的早期。然而，随着疾病进展，会发生永久性眼睛损伤，并且许多临床上有效的治疗仅具预防性并且不具恢复性。因此，为了预防失明几乎必须要强制性地一贯顺从治疗方案。

[0006] 不幸的是，治疗方案典型地需要在医师用皮下注射针刺穿患者的眼睛以将治疗剂递送到眼中，典型地递送到眼睛的玻璃体中的同时，患者保持不动。这可具有创伤性和疼痛，因此患者可能不愿接受注射。每次注射提供较长期益处，因此减小患者所遭受的疼痛和创伤的能力取决于治疗剂和携带和释放所述药剂的递送媒介物的所需药代动力学。

[0007] 一些已知的递送媒介物具有通过在基质相合成期间截留而并入聚合物和溶胶－凝胶体系中的活性成分。用于可生物降解的聚合物的微胶囊化技术包括如下方法，诸如薄膜铸塑、模制、喷雾干燥、挤压、熔体分散、界面沉积、通过乳化和溶剂蒸发进行相分离、空气悬浮涂布、盘涂布和原位聚合。熔体分散技术例如描述在美国专利号 5,807,574 和美国专利号 5,665,428 中。

[0008] 在一个替代性方法中，在多孔基质形成完全之后负载活性成分。这些载体体系通常具有微米级而非纳米级孔以允许药剂进入孔中。美国专利号 6,238,705 例如描述了通过简单浸入活性成分溶液中来负载大孔聚合物组合物，并且美国专利号 5,665,114 和 6,521,284 公开了使用压力来负载由聚四氟乙烯 (PTFE) 制成的可植入假肢的孔。虽然这种方法可能对有机小分子有效，但是较大分子如蛋白质倾向于在大孔中聚集并且不会以受控的方式有效地体内释放。

[0009] 在较小孔的情况下，已经证明由于窄孔阻挡，因此难以并入高浓度的治疗剂。向孔的开口沉积材料易于防止高比例的材料占据孔系统。实现活性成分的高负载的问题限制了许多目前已知的递送系统的效力。

[0010] 通过递送媒介物递送治疗剂时的另一问题是在药物释放之后递送媒介物的生物相容性。可生物蚀解的或可再吸收的递送媒介物材料将为需要在药物释放之后去除的递送媒介物的有吸引力的替代方案。已经开始探索携带治疗剂的可生物蚀解的递送媒介物的设计和制备。PCT 公布号 WO2009/009563 描述了包含多孔硅材料的药物递送系统。

[0011] 因此，持续需要开发用于控制释放治疗剂的具有生物相容性并且能够以持续方式递送大分子的改进剂型。

[0012] 概要

[0013] 本发明包括一种组合物，所述组合物包含可生物蚀解的多孔硅基载体材料，其中所述载体材料携带至少一种大分子治疗剂和至少一种无定形糖，任选地进一步包含结晶抑制剂。在某些实施方案中，使用真空辅助急骤干燥来制备所述组合物。

[0014] 所公开的组合物用于以受控的方式递送治疗剂，尤其是大分子如蛋白质、肽、抗体、碳水化合物、聚合物、疫苗、小干扰 RNA(siRNA) 或多核苷酸。组合物包含负载有治疗剂和无定形糖的多孔硅基载体材料。在一些实施方案中，组合物包含负载有治疗剂以及无定形糖混合物的多孔硅基载体材料。在一些实施方案中，组合物包含负载有治疗剂以及糖与结晶抑制剂的混合物的多孔硅基载体材料。所述组合物可以用于在体外或体内递送治疗剂，优选地以控制方式历经预定时段如多天、多周或多月来递送。载体材料优选地由可生物蚀解的或可再吸收的材料例如硅基材料如元素硅或二氧化硅形成，以在治疗剂释放之后不必进行去除。在某些这种实施方案中，载体材料和其分解产物具有生物相容性，以使载体材料的生物蚀解所致的生物副作用最小或无害。

[0015] 在某些实施方案中，载体材料包括多孔二氧化硅，如中孔二氧化硅。典型地选择载体材料的平均孔尺寸以使其可以携带治疗剂，并且示例性孔尺寸为直径 2-50nm，如直径约 15nm 至约 40nm、直径约 20nm 至约 30nm、直径约 2nm 至约 15nm，或者直径约 5nm 至约 10nm。以引用的方式并入本文中的美国专利 20120177695 中也公开了硅基材料。

[0016] 在某些实施方案中，治疗剂为分子量在约 500amu 与约 200,000amu 之间，且或许在约 800amu 与约 200,000amu 之间、约 1000amu 与约 200,000amu 之间、约 1500amu 与约 200,000amu 之间、约 2,000amu 与约 200,000amu 之间、约 5,000amu 与约 200,000amu 之间、约 10,000amu 至约 150,000amu、在约 10,000amu 与约 50,000amu 之间、在约 50,000amu 与约 100,000amu 之间或者在约 100,000amu 与约 200,000amu 之间的蛋白质。

[0017] 治疗剂的尺寸或者可以通过分子半径来表征，而分子半径可以例如通过 X 射线结晶分析或者通过流体动力学半径来测定。治疗剂可为蛋白质，例如分子半径选自 0.5nm 至 20nm，如约 0.5nm 至 10nm、甚至约 1nm 至 8nm。优选地，根据孔 - 治疗剂（药剂）差别来选择允许具体药剂例如蛋白质进入的合适的孔半径，所述差别在本文中定义为药剂半径与孔半径之间的差异。例如，流体动力学半径为 1.3nm 的胰岛素与最小半径为 4.8nm 的孔的孔 - 药剂差别具有 3.5nm 的孔 - 蛋白质差别。孔 - 药剂差别可用于确定用于容纳特定半径的蛋白质的最小合适平均孔尺寸。孔 - 蛋白质差别可以典型地选自约 3.0 至约 5.0nm。

[0018] 典型地选择平均孔尺寸容纳治疗剂的组合物。可以基于待负载到载体材料孔中的治疗剂的分子量或分子半径来选择载体材料的平均孔尺寸。例如，分子量选自 100,000amu 至 200,000amu 的治疗剂可以与较大平均孔尺寸如约 15nm 至约 40nm 的载体材料一起使用。在某些实施方案中，分子量选自 5,000amu 至 50,000amu 的治疗剂可以与较小平均孔尺寸如约 2nm 至约 10nm 的载体材料一起使用。

[0019] 在某些实施方案中，糖无论是单独使用或组合使用都选自蔗糖、果糖、葡萄糖、赤藻醇、麦芽糖醇、乳糖醇、山梨醇、甘露醇、木糖醇、D- 塔格糖、海藻糖、脱水海藻糖、半乳糖、甘油、鼠李糖、环糊精、棉子糖、核酮糖、核糖、苏阿糖、阿拉伯糖、木糖、来苏糖、阿洛糖、

阿卓糖、甘露糖、艾杜糖、乳糖、麦芽糖、转化糖、异海藻糖、新海藻糖、帕拉金糖或异麦芽酮糖、赤藓糖、脱氧核糖、古洛糖、艾杜糖、塔罗糖、赤藓酮糖、木酮糖、阿洛酮糖、松二糖、纤维二糖、葡萄糖胺、甘露糖胺、海藻糖、葡糖醛酸、葡萄糖酸、葡萄糖酸－内酯、阿比可糖、半乳糖胺、木寡糖、苦杏仁寡糖 (gentio-oligoscaccharide)、半乳寡糖、山梨糖、尼哥洛寡糖 (nigero-oligosaccharide)、果寡糖、麦芽四醇、麦芽三醇、麦芽糖糊精、麦芽寡糖、乳果糖、蜜二糖或其任何组合。在优选实施方案中，糖选自海藻糖、二水合海藻糖、蔗糖、甘露醇、山梨醇、木糖醇或甘油，或其组合。

[0020] 在某些实施方案中，如下制备组合物：首先形成多孔载体材料，然后用治疗剂和无定形或溶液形式的糖或者多种糖或者糖与结晶抑制剂的组合负载孔。在优选实施方案中，在无定形或溶液形式的糖或者结晶抑制剂之前负载治疗剂。

[0021] 本发明包括将治疗剂负载到多孔硅基载体材料的孔中的方法，其包括使多孔硅基载体材料与治疗剂接触。一种将治疗剂负载到多孔硅基载体材料的孔中的示例性方法包括选择具有在尺寸上适于允许单一蛋白质负载到孔中以使蛋白质的相对侧接合孔的相对侧的孔尺寸的多孔硅基载体。一种将治疗剂负载到多孔硅基载体材料的孔中的方法包括选择具有在尺寸上适于容许仅单一药剂一次负载到单一孔的宽度（即不排除沿着孔长度的纵向系列）（例如，如果并行（横向）放在孔中，那么不能容纳两种药剂）的孔尺寸的多孔硅基载体。以引用的方式并入本文中的美国专利 20120177695 中还公开了将药剂负载到硅基材料的孔中的方法以及选择适用于相关药剂的载体材料的方法。

[0022] 可以将组合物安置在皮肤或眼表面上。或者，可以将组合物安置在哺乳动物体内，如在患者眼内或在患者身体的任何其它组织或器官内。在具体应用中，将组合物安置在皮下、结膜下或在眼睛玻璃体中。组合物可以用于治疗或预防患者的病状如慢性疾病。在某些实施方案中，组合物用于治疗或预防眼睛疾病如青光眼、黄斑变性、糖尿病性黄斑水肿和年龄相关性黄斑变性。治疗剂可以用受控的方式历经数周或数月的时段释放，例如以治疗或预防眼睛疾病如黄斑变性。

[0023] 本发明包括包含无定形糖的稳定化制剂以及使如本文所述的多孔载体材料中的治疗剂稳定的方法。在某些实施方案中，本发明包括在载体材料的孔中的稳定化生物分子如抗体，以使生物分子的半衰期或保质期优于在载体材料外的生物分子的半衰期或保质期。在某些实施方案中，对于在减压下在室温环境条件下的干燥，稳定化制剂的蛋白质是稳定的。在某些实施方案中，包含治疗剂和无定形糖的多孔载体材料涂有聚合物。在优选实施方案中，包含治疗剂和无定形糖的多孔载体材料涂有控制释放聚合物。

[0024] 在如本文所述的组合物的某些实施方案中，本文所述的组合物的无定形形式的糖当与本文所述的多孔载体材料接触时在 25℃ /60％相对湿度下在 90 天之后会比在没有多孔载体材料的类似条件下保持其无定形特征。在某些实施方案中，在 25℃的温度下，无定形糖会使生物分子例如抗体稳定至少 15 天、至少 1 个月、至少 6 个月、至少 1 年、至少 1.5 年、至少 2 年、至少 2.5 年、至少 3 年或至少 4 年。

[0025] 在一些实施方案中，本发明的稳定化制剂当暴露于非水溶剂如二氯甲烷或任何不能溶解糖的溶剂时是稳定的。

具体实施方式

[0026] 图1示出了无定形糖在氧化阳极化的中孔硅(例如,由实施例1-3制备)中在25℃和60%相对湿度下稳定90天。

[0027] 图2示出了相对于商业冷冻干燥,贝伐单抗(bevacizumab)在真空干燥之后在氧化阳极化的中孔硅上的稳定性。

[0028] 图3示出了与蔗糖共同配制的肌红蛋白在涂布的60 Å氧化阳极化的中孔硅粒子中的分解率。

[0029] 综述

[0030] 在现代医学疗法中,向患者尤其是患有慢性病状如青光眼或癌症的患者持续并控制递送治疗剂正变得越来越重要。许多疗法当以频繁间隔施用时最有效,以在体内维持近乎持续的活性剂存在。虽然可以推荐频繁的施用,但是不便和患者顺从性的相关困难可能会以这种方式有效阻止治疗。因此,以受控的方式释放治疗剂的持续释放组合物在如癌症疗法和其它慢性疾病治疗的领域极具吸引力。

[0031] 体内或体外释放治疗剂的组合物可以由多种生物相容性或至少基本上生物相容性材料形成。一种类型的组合物采用硅基载体材料。硅基载体材料可以包括例如元素硅,和呈如二氧化硅(硅石)形式的氧化硅,或者硅酸盐。一些硅基组合物已经证明在生物系统中的高生物相容性和有利降解,从而消除了在释放治疗剂之后去除载体材料的需要。

[0032] 测试显示高孔隙率例如80%孔隙率的硅基材料被再吸收得快于中等孔隙率例如50%孔隙率的硅基材料,而中等孔隙率的硅基材料又被再吸收得快于大块硅基材料,所述大块硅基材料在生物系统中显示极少至没有生物侵蚀或再吸收迹象。此外,应了解载体材料的平均孔尺寸将影响再吸收速率。通过调节载体材料的平均孔尺寸以及材料的孔隙率,可以调整和选择生物蚀解速率。

[0033] 通常如下制备硅基载体材料:使用高温和有机溶剂或酸性介质以形成多孔材料,并且将治疗剂负载在孔内。这些条件可能适于某些分子如盐、元素和某些高度稳定的有机小分子。然而,对于负载大有机分子如蛋白质或抗体,在制备或负载模板期间的苛性和/或严苛条件可引起活性剂变性和去活化,如果不完全降解的话。在温和条件下将大分子如抗体负载到载体材料中是本文所述的方法的特征,其尤其有利于有机大分子如蛋白质。

[0034] 硅基载体材料的粒度也可能影响载体材料的孔可以负载有治疗剂的速率。较小粒子例如最大直径是20微米或更小的粒子可以比最大直径大于20微米的粒子负载得更快。当孔径的尺寸类似于治疗剂的分子直径或尺寸时,这尤其显而易见。较小粒子的快速负载可以归因于治疗剂必须在较小粒子中穿透的较短平均孔深度。

[0035] 定义

[0036] 如本文说明书中使用,“一个(种)”可以意指一个(种)或多个(种)。如本文权利要求书中使用,当与措词“包含”、措词“一个(种)”共同使用时可以意指一个(种)或一个(种)以上。如本文中使用,“另一个”可以意指至少第二个或更多个。

[0037] 术语“抗体”广泛地涵盖天然存在形式的抗体和重组抗体,如单链抗体、骆驼化抗体、嵌合和人源化抗体和多特异性抗体以及所有前述各物的片段和衍生物,优选地具有至少一个抗原结合位点的片段和衍生物。抗体衍生物可以包含与抗体缀合的蛋白质或化学部分。术语“抗体”以广义使用并且完全涵盖组装抗体,以及包含其的重组肽。

[0038] “抗体片段”包含完整抗体的一部分,优选地完整抗体的抗原结合区或可变区。抗

体片段的实例包括 Fab、Fab'、F(ab')2 和 Fv 片段；双体抗体 (diabody)；线性抗体 (Zapata 等，(1995)Protein Eng.8(10):1057-1062))；单链抗体分子；和由抗体片段形成的多特异性抗体。对抗体的木瓜蛋白酶消化产生两个相同的抗原结合片段，称为“Fab”片段，其中每一个具有单一抗原结合位点，和残余“Fc”片段，其名称反映了其容易地结晶的能力。胃蛋白酶处理产生具有两个抗原组合位点并且仍能交联抗原的 F(ab')2 片段。

[0039] 如本文中使用，生物蚀解是指在生物系统中结构或包壳随时间逐渐崩解或崩溃，例如通过一种或多种物理或化学降解方法，例如酶促作用、水解、离子交换，或者通过溶解、乳液形成或胶束形成来分解而实现。

[0040] 术语“预防”是本领域公认的，并且当与病状如局部复发（例如疼痛）、疾病如癌症、复杂综合征 (syndrome complex) 如心力衰竭或任何其它医学病状关联使用时在本领域中得到了充分的了解，并且包括施用组合物，相对于不接受组合物的受试者，所述组合物会降低受试者医学病状的症状的发作频率或者延迟所述发作。因此，预防癌症包括例如相对于未经过治疗的对照群体减少接受预防性治疗的患者群体的可检测癌性生长的数目，和 / 或相对于未经过治疗的对照群体延迟经过治疗的群体的可检测癌性生长的出现，所述减少和 / 或延迟是统计上和 / 或临床上大量的。预防感染包括例如相对于未经过治疗的对照群体减少经过治疗的群体的感染诊断数目，和 / 或相对于未经过治疗的对照群体延迟经过治疗的群体的感染症状的发作。疼痛的预防包括例如，相对于未经过治疗的对照群体，减少经过治疗的群体中的受试者所经历的痛觉的程度或可选地延迟所述痛觉。

[0041] 术语“防治性或治疗性”治疗是本领域公认的并且包括向宿主施用一种或多种主题组合物。如果其在不期望的病状（例如宿主动物的疾病或其它不期望的状态）有临床表现之前施用，那么治疗具有防治性（即其会保护宿主以免产生不期望的病状），而如果其在不期望的病状表现之后施用，那么治疗具有治疗性（即其旨在减小、改善或稳定现有的不期望的病状或其副作用）。

[0042] 如本文中使用的再吸收是指当引入活的人或动物的生理器官、组织或液体内部或上方时材料的蚀解。

[0043] 关于主题治疗方法的化合物的“治疗有效量”是指根据待治疗的病症或病状或美容目的的临床上可接受的标准例如在适用于任何医学治疗的合理的益处 / 风险比下，当作为所需给药方案的部分（向哺乳动物，优选地人）施用时减轻症状、改善病状或减缓疾病状况发作的制剂中化合物的量。

[0044] 如本文中使用，术语“治疗”包括以改善或稳定受试者的病状的方式逆转、减少或停滞病状的症状、临床征兆和潜在病理。

[0045] 除非另有指示，否则术语治疗性大分子是指分子量等于或大于 2000amu 或甚至大于 3000amu 的分子。

[0046] 除非另有指示，否则术语“小分子”是指分子量小于约 2000amu、优选地小于约 1500amu、更优选地小于约 1000amu 或最优选地小于约 750amu 的有机分子。优选地，小分子含有一个或多个杂原子。

[0047] 除非另有指示，否术语“糖”是指单糖、二糖、寡糖或糖醇。术语“糖”的实例是但不限于蔗糖、果糖、葡萄糖、赤藻醇、麦芽糖醇、乳糖醇、山梨醇、甘露醇、木糖醇、D- 塔格糖、海藻糖、脱水海藻糖、半乳糖、甘油、鼠李糖、环糊精、棉子糖、核酮糖、核糖、苏阿糖、阿拉伯

糖、木糖、来苏糖、阿洛糖、阿卓糖、甘露糖、艾杜糖、乳糖、麦芽糖、转化糖、异海藻糖、新海藻糖、巴拉金糖或异麦芽酮糖、赤藓糖、脱氧核糖、古洛糖、艾杜糖、塔罗糖、赤藓酮糖、木酮糖、阿洛酮糖、松二糖、纤维二糖、葡萄糖胺、甘露糖胺、海藻糖、葡糖醛酸、葡萄糖酸、葡萄糖酸－内酯、阿比可糖、半乳糖胺、木寡糖、苦杏仁寡糖、半乳寡糖、山梨糖、尼哥洛寡糖、果寡糖、麦芽四醇、麦芽三醇、麦芽糖糊精、麦芽寡糖、乳果糖、蜜二糖或其任何组合。

[0048] 硅基载体材料

[0049] 本文所述的装置和方法尤其提供包含多孔硅基载体材料的组合物，其中将至少一种治疗剂和无定形糖安置在载体材料的孔中。所述方法使用这些组合物以治疗或预防疾病，尤其是慢性疾病。此外，所述制备组合物的方法提供特征在于持续和控制释放治疗剂尤其是大分子如蛋白质或抗体的组合物。

[0050] 组合物典型地包含硅基载体材料，如元素硅、二氧化硅（硅石）、一氧化硅、硅酸盐（含有携带硅的阴离子例如 SiF_6^{2-}、$Si_2O_7^{6-}$或 SiO_4^{4-}的化合物），或这些材料的任何组合。在某些实施方案中，载体材料包含元素硅的完整或部分构架，并且所述构架大致上或完全由二氧化硅表面层覆盖。在其它实施方案中，载体材料完全是或基本上完全是硅石。

[0051] 在某些实施方案中，载体材料包含硅石，如大于约 50％硅石、大于约 60 重量％硅石、大于约 70 重量％硅石、大于约 80 重量％硅石、大于约 90 重量％硅石、大于约 95 重量％硅石、大于 99 重量％硅石，或甚至大于 99.9 重量％硅石。可以从供应商如 Davisil、Salicycle 和 Macherey-Nagel 购得多孔硅石。

[0052] 在某些实施方案中，载体材料包含元素硅，大于 60 重量％硅、大于 70 重量％硅、大于 80 重量％硅、大于 90 重量％硅，或甚至大于 95％硅。可以从供应商如 Vesta Ceramics 购得硅。

[0053] 可使用技术如能量分散 X 射线分析、X 射线荧光、感应耦合光学发射光谱或辉光放电质谱来定量地评估硅基材料的纯度。

[0054] 载体材料可以包含其它组分如金属、盐、矿物或聚合物。载体材料可以具有安置在表面的至少一部分上例如以提高载体材料的生物相容性和／或影响释放动力学的涂层（如聚合物涂层）。

[0055] 硅基载体材料可以包含呈无定形形式的元素硅或其化合物，例如二氧化硅或硅酸盐。在一些实施方案中，硅基载体材料包含气相硅石。在某些实施方案中，元素硅或其化合物以结晶形式存在。在其它实施方案中，载体材料包含无定形硅石和／或无定形硅。在某些实施方案中，硅基材料大于约 60 重量％无定形、大于约 70 重量％无定形、大于约 80 重量％无定形、大于约 90 重量％无定形、大于约 92 重量％无定形、大于约 95 重量％无定形、大于约 99 重量％无定形，或甚至大于 99.9 重量％无定形。

[0056] 可使用 X 射线衍射分析以鉴别硅基材料的晶相。粉末衍射可例如在例如配备有液氮冷却锗固态检测器的 Scintag PAD-X 衍射仪上使用 Cu K-α 辐射来进行。

[0057] 硅基材料的孔隙率可为约 40％至约 95％，如约 60％至约 80％。如本文中使用，孔隙率是材料中孔隙空间的度量，并且是孔隙体积相对于材料总体积的分数。在某些实施方案中，载体材料的孔隙率为至少约 10％、至少约 20％、至少约 30％、至少约 40％、至少约 50％、至少约 60％、至少约 70％、至少约 80％或甚至至少约 90％。在具体实施方案中，孔隙率大于约 40％，如大于约 50％、大于约 60％或甚至大于约 70％。

[0058] 组合物的载体材料的表面积与重量比可选自约 20m^2/g 至约 2000m^2/g，如约 20m^2/g 至约 1000m^2/g，或甚至约 100m^2/g 至约 300m^2/g。在某些实施方案中，表面积大于约 200m^2/g、大于约 250m^2/g 或大于约 300m^2/g。

[0059] 在某些实施方案中，治疗剂从载体材料表面分布到至少约 10 微米、至少约 20 微米、至少约 30 微米、至少约 40 微米、至少约 50 微米、至少约 60 微米、至少约 70 微米、至少约 80 微米、至少约 90 微米、至少约 100 微米、至少约 110 微米、至少约 120 微米、至少约 130 微米、至少约 140 微米或至少约 150 微米的孔深度。在某些实施方案中，治疗剂基本上均匀地分布在载体材料的孔中。

[0060] 治疗剂可以负载到载体材料中一定深度，所述深度被测量为与载体材料总宽度的比率。在某些实施方案中，治疗剂分布到载体材料中至少约 10％、载体材料中至少约 20％、载体材料中至少约 30％、载体材料中至少约 40％、载体材料中至少约 50％或载体材料中至少约 60％的深度。

[0061] 无定形糖可以负载到载体材料中一定深度，所述深度被测量为与载体材料总宽度的比率。在某些实施方案中，无定形糖分布到至少约 1％至至少约 9％、载体材料中至少 10％、载体材料中至少约 20％、载体材料中至少约 30％、载体材料中至少约 40％、载体材料中至少约 50％，或载体材料中至少约 60％的深度。在一些实施方案中，无定形糖可密封所述孔。

[0062] 无定形糖可以负载到载体材料中达一定重量，所述重量被测量为与载体材料和治疗剂的组合重量的比率。在某些实施方案中，将无定形糖负载至至少约 1％至至少约 80％、至少约 1％至至少约 70％、至少约 1％至至少约 60％、至少约 1％至至少约 50％、至少约 1％至至少约 40％、至少约 1％至至少约 30％、至少约 1％至至少约 20％，至至少约 1％至至少约 15％、约 1％至至少约 10％、约 1％至至少约 5％、约 1％至至少约 4％、至少约 1％至至少约 3％，或至少约 1％至至少约 2％的重量。在某些实施方案中，将无定形糖负载至至少约 5％至至少约 10％、至少约 10％至至少约 20％、至少约 10％至至少约 30％、至少约 30％至至少约 40％、至少约 40％至至少约 50％、至少约 50％至至少约 60％、至少约 60％至至少约 70％，或至少约 70％至至少约 80％的重量。在某些实施方案中，可以将无定形糖负载至约 30％的重量。总负载的定量可以通过许多分析方法，例如药物组合物的重力测量、EDX（通过 x 射线进行能散分析）、傅里叶变换红外光谱 (FTIR) 或拉曼光谱，或通过溶液中洗脱治疗剂的紫外分光光度测定法、滴定分析、HPLC 或质谱分析来实现。负载均匀性的定量可以通过能够进行空间分辨的组合技术如截面 EDX、欧杰纵深分析 (Auger depth profiling)、微拉曼和微 FTIR 来实现。

[0063] 本发明的多孔硅基材料可以依据孔尺寸的平均直径来分类。微孔硅基材料的平均孔尺寸小于 2nm、中孔硅基材料平均孔尺寸在 2-50nm 之间，并且大孔硅基材料的孔尺寸大于 50nm。在某些实施方案中，大于 50％的硅基材料孔的孔尺寸为 2-50nm、大于 60％的硅基材料孔的孔尺寸为 2-50nm、大于 70％的硅基材料孔的孔尺寸为 2-50nm、大于 80％的硅基材料孔的孔尺寸为 2-50nm，或甚至大于 90％的硅基材料孔的孔尺寸为 2-50nm。

[0064] 在某些实施方案中，载体材料包含多孔二氧化硅，如中孔二氧化硅。在某些实施方案中，载体材料的平均孔尺寸选自 2-50nm，如约 15nm 至约 40nm，如约 20 至约 30nm。在某些实施方案中，平均孔尺寸选自约 2nm 至约 15nm，如约 5nm 至约 10nm。在某些实施方案中，平

均孔尺寸为约 30nm。

[0065] 可以针对治疗剂的尺寸特征预选孔尺寸以控制生物系统中治疗剂的释放速率。典型地，太小的孔尺寸会阻碍治疗剂负载，而过大的孔不会与治疗剂足够强烈地相互作用以控制释放速率。例如，载体材料的平均孔径可以选自较大孔，例如 15nm 至 40nm，对于高分子量分子，例如 200,000-500,000amu，以及较小孔，例如 2nm 至 10nm，对于较低分子量分子，例如 10,000-50,0000amu。例如，约 6nm 直径的平均孔尺寸可能适于具有约 14,000amu 至 15,000amu 如约 14,700amu 的分子量的分子。对于具有约 45,000amu 至 50,000amu 如约 48,000amu 的分子量的分子，可以选择约 10nm 直径的平均孔尺寸。对于具有约 150,000nm 的分子量的分子，可以选择约 25-30nm 直径的平均孔尺寸。

[0066] 可以预选孔尺寸以适于治疗剂的分子半径以控制生物系统中治疗剂的释放速率。例如，约 25nm 至约 40nm 直径的平均孔尺寸可以适于最大分子半径为约 6nm 至约 8nm 的分子。可以通过任何合适的方法如通过使用基于 X 射线结晶学分析数据的分子外形尺寸或使用表示分子的溶液状态尺寸的流体动力学半径来计算分子半径。因为溶液状态计算依赖于进行计算的溶液的性质，所以对于一些测量可能优选的是，使用基于 X 射线结晶学分析数据的分子外形尺寸。如本文中使用，最大分子半径反映了治疗剂的最大尺寸的一半。

[0067] 在某些实施方案中，选择平均孔直径以限制分子（例如蛋白质）在孔内聚集。防止生物分子（诸如蛋白质）在载体材料中聚集将是有利的，因为这种聚集被认为阻碍分子向生物系统中的控制释放。因此，归因于孔的尺寸与生物分子的尺寸之间的关系允许例如仅一个生物分子在任一时刻进入孔的孔将优于允许多个生物分子一起进入孔并且在孔内聚集的孔。在某些实施方案中，可将多个生物分子加载至孔中，但归因于孔的深度，在孔的整个这一深度中分配的蛋白质将在较小程度上聚集。

[0068] 在某些实施方案中，治疗剂选自适用于疾病治疗或预防的任何药剂。在某些实施方案中，药剂选自小分子治疗剂，即分子量小于 1000amu 的化合物。在优选实施方案中，治疗剂选自分子量等于或大于 1000amu 的大分子。在某些实施方案中，本发明的治疗剂是生物分子。如本文中使用，生物分子是指由活生物体产生的任何分子，包括聚合大分子如蛋白质、多糖和核酸以及小分子如初级代谢产物、次级代谢产物，和其天然产物或合成变体。具体来说，蛋白质如抗体、配体和酶可以用作本发明的治疗剂。在具体实施方案中，本发明的生物分子的分子量在约 10,000amu 至约 500,000amu 的范围内。在某些实施方案中，治疗剂选自一种或多种单克隆抗体如雷珠单抗 (ranibizumab)(Lucentis) 和贝伐单抗 (Avastin)。

[0069] 在某些实施方案中，治疗剂的分子量在 10,000amu 和 50,000amu 之间、在 50,000amu 和 100,000amu 之间或者在 100,000amu 和 150,000amu 之间。在某些实施方案中，治疗剂是分子量在 5,000amu 和 200,000amu 之间如约 10,000amu 至约 150,000amu 的蛋白质。

[0070] 治疗剂的尺寸或者可以通过分子半径来表征，而分子半径可以例如通过 X 射线结晶分析或者通过流体动力学半径来测定。治疗剂可为蛋白质，例如分子半径选自 0.5nm 至 20nm，如约 0.5nm 至 10nm、甚至约 1nm 至 8nm 的蛋白质。

[0071] 分子半径为 1nm 至 2.5nm 的治疗剂可以有利地与最小孔半径为 4.5nm 至 5.8nm 的载体材料一起使用。分子半径为 7nm 的治疗剂可以有利地与最小孔半径为 11nm 至 13nm 如

约 12nm 的载体材料一起使用。例如，流体动力学半径为 1.3nm 的胰岛素可以与平均最小孔半径为 4.8nm 的载体材料一起使用。

[0072] 蛋白质 - 孔差别可用于选择适于容纳治疗剂的载体材料。这种计算从孔半径减去分子半径。典型地，治疗剂的半径将为流体动力学半径或通过 x 射线结晶分析测定的最大半径。孔半径将典型地为载体材料的平均孔径。例如，流体动力学半径为 1.3nm 的胰岛素与最小半径为 4.8nm 的孔的孔 - 蛋白质差别具有 3.5nm 的蛋白质 - 孔差别。在某些实施方案中，蛋白质 - 孔差别选自 3nm 至 6nm，如 3.2nm 至 4.5nm。蛋白质 - 孔差别可为约 3.2nm、约 3.3nm、约 3.4nm、约 3.5nm、约 3.6nm、约 3.7nm、约 3.8nm、约 3.9nm、约 4.0nm、约 4.1nm、约 4.2nm、约 4.3nm、约 4.4nm 或约 4.5nm。

[0073] 在某些实施方案中，治疗剂为抗体并且载体材料的平均孔尺寸选自约 20nm 至约 40nm，如约 25nm 至 35nm，如约 30nm。在某些实施方案中，治疗剂为选自贝伐单抗或雷珠单抗的抗体，并且载体材料的平均孔尺寸选自约 20nm 至约 40nm，如约 25nm 至 35nm，如约 30nm。在某些实施方案中，治疗剂为贝伐单抗并且载体材料的平均孔尺寸为约 30nm。

[0074] 在某些实施方案中，分隔孔的载体材料的壁的平均宽度小于 5nm，如约 4.8nm、约 4.6nm、约 4.4nm、约 4.2nm、约 4.0nm、约 3.8nm、约 3.6nm、约 3.4nm、约 3.2nm、约 3.0nm、约 2.8nm 或甚至约 2.6nm。在某些实施方案中，分隔孔的载体材料的壁的平均宽度小于 3nm，如约 2.8nm、约 2.6nm、约 2.4nm、约 2.2nm、约 2.0nm、约 1.8nm、约 1.6nm、约 1.4nm、约 1.2nm、约 1.0nm 或甚至约 0.8nm。

[0075] 可例如通过使用例如在 200keV 下操作的 2000JEOL 电镜的透射电子显微镜术 (TEM) 来测量载体材料的维度和形态。TEM 样品可通过稀释浆液将大量多孔载体材料粒子分配到金属格栅上的有孔碳膜上来制备。

[0076] 在某些实施方案中，载体材料的孔界定了载体材料的体积为约 0.1mL/g 至约 5mL/g 的空间。在某些实施方案中，孔体积为约 0.2mL/g 至约 3mL/g，如约 0.4mL/g 至约 2.5mL/g，如约 1.0mL/g 至约 2.5mL/g。

[0077] 在某些实施方案中，以载体材料与治疗剂的组合重量计，载体材料的负载水平高达 80 重量%。通过将负载治疗剂的重量除以负载治疗剂与载体材料的组合重量再乘以 100 来计算负载水平。在某些实施方案中，载体材料的负载水平大于 1%，如大于 3%、如大于 5%、如大于 10%、如大于 15%、大于 20%、大于 25%、大于 30%、大于 35%、大于 40%、大于 45%、如大于 50%、如大于 60%或大于 70%。负载水平可以在约 5%与约 10%之间。在某些实施方案中，载体材料的负载水平在约 10 重量%和约 20 重量%之间、在约 20 重量%和约 30 重量%之间、在约 30 重量%和约 40 重量%之间、在约 40 重量%和约 50 重量%之间、在约 50 重量%和约 60 重量%之间、在约 60 重量%和约 70 重量%之间或在约 70 重量%与约 80 重量%之间。

[0078] 在某些实施方案中，以组合物的重量计，载体材料的负载水平高达 40 重量%。在某些实施方案中，载体材料的负载水平大于 1%，如大于 3%、如大于 5%、如大于 10%、如大于 15%、大于 20%、大于 25%、大于 30%或大于 35%。负载水平可以在约 5%与约 10%之间。在某些实施方案中，载体材料的负载水平在约 10 重量%和约 20 重量%之间、在约 20 重量%和约 30 重量%之间、在约 30 重量%和约 40 重量%之间。通过将负载治疗剂的重量除以组合物重量再乘以 100 来计算负载水平。组合物可以包含载体材料、治疗剂、无定形糖

和任选地其它组分如结晶抑制剂。在一些实施方案中，组合物包含：

[0079] 在 1 重量％至 40 重量％范围内的治疗剂（如蛋白质）、

[0080] 在 1 重量％至 50 重量％的范围内的无定形糖，以及

[0081] 在 10 重量％至 30 重量％的范围内的载体材料。

[0082] 可以就多孔材料中由治疗剂占据的孔体积来评价本文所述的载体材料的负载体积。根据本发明的载体材料的由治疗剂占据的最大负载容量的百分比（也即多孔载体材料中由治疗剂占据的孔的总体积百分比）可为约 30％至约 100％，如 50％至约 90％。对于任何给定的载体材料，这个值可以通过将在负载期间吸收的治疗剂的体积除以在负载之前的载体材料孔隙体积再乘以一百来确定。

[0083] 在某些实施方案中，本发明的载体材料为在最大直径处测量具有约 1 微米至约 500 微米如约 5 微米至约 100 微米的平均尺寸的粒子。在某些实施方案中，在最大直径处测量的单个载体材料粒子为约 1 微米至约 500 微米如约 5 微米至约 500 微米。

[0084] 为了提高本发明的粒子的负载率，可能有利的是使用相对小的粒子。因为较小粒子具有供治疗剂穿透的较小深度的孔，所以负载粒子所需时间的量减少。当孔径的尺寸类似于治疗剂的分子直径或尺寸时，这可能尤其有利。在最大尺寸处测量，较小粒子可为 1-20 微米，如约 10-20 微米，例如约 15-20 微米。

[0085] 在一些方面，在最大尺寸处测量，大于 60％、大于 70％、大于 80％或大于 90％的粒子的粒度为 1-20 微米、优选地 5-15 微米。粒子的平均粒度可以在 1 微米和 20 微米之间，如在 5-15 微米之间或约 15 微米、约 16 微米、约 17 微米、约 18 微米、约 19 微米。

[0086] 可例如使用来自 Malvern Instruments,UK 的 Malvern 粒度分析仪（型号 Mastersizer）来测量粒度分布，包括平均粒径。氦氖气体激光束可发射穿过含有载体材料的悬浮液的光学池。撞击载体材料的光线通过与粒度成反比的角度散射。光检测器矩阵测量在若干预定角度的光强度，并且然后通过微型计算机系统针对由样品载体材料和水性分散剂的折射率预测的散射模式处理与所测量的光通量值成比例的电信号。

[0087] 还设想将较大载体材料粒子或植入物用于控制递送治疗剂。在最大尺寸处测量，本发明的粒子／植入物的平均尺寸可为约 1mm 至约 5cm。在某些实施方案中，在最大尺寸处测量，粒子／植入物的平均尺寸为约 5mm 至约 3cm。如在最大尺寸处测量，大于 1mm 的粒子可能适用于肌肉内、皮下、玻璃体内或真皮下药物递送。

[0088] 在某些实施方案中，孔中存在的本文所述的无定形糖用于使敏感的治疗化合物如生物分子例如抗体稳定。在某些实施方案中，可使在高温如室温或更高温度下部分或完全不稳定的生物分子在室温下长期稳定。例如，与载体材料内的无定形糖一起配制的生物分子对在减压下在室温下的干燥稳定。

[0089] 在某些实施方案中，本文所述的多孔载体材料用于使敏感的治疗化合物如生物分子例如抗体稳定。在某些实施方案中，可使在高温如室温或更高温度下部分或完全不稳定的生物分子在室温下长期稳定。可以将生物分子负载到载体材料中以使负载到载体材料中的生物分子的水性混悬液比生物分子的相应水溶液（即添加和没有添加多孔载体材料的相同水溶液）更稳定。例如，载体材料内的生物分子的室温（例如约 23℃）下半衰期可以大于在相同条件下没有载体材料的生物分子的半衰期。在某些实施方案中，在相同条件下，载体材料的孔中的生物分子的半衰期是在载体材料外的生物分子的至少两倍长，更优选地

是在载体材料外的生物分子的至少 5 倍、至少 10 倍、至少 15 倍、至少 20 倍、至少 30 倍、至少 40 倍、至少 50 倍、至少 60 倍或至少 100 倍长。例如，载体材料的孔中的抗体的半衰期可为在载体材料外的抗体的至少 10 倍长，更优选地至少 20 倍长。

[0090] 类似地，与无定形糖一起配制的生物分子在载体材料的孔内的保质期比在相应水溶液中长，优选地至少长 2 倍、至少长 5 倍、至少长 10 倍、至少长 20 倍、至少长 30 倍、至少长 40 倍、至少长 50 倍、至少长 60 倍或至少长 100 倍。例如，载体材料的孔中的抗体的半衰期可比在载体材料外的抗体长，优选地至少 10 倍长、至少 20 倍长。

[0091] 在某些实施方案中，包含载体材料和生物分子如抗体以及无定形糖的多孔组合物在温度 25℃下显示稳定性持续至少 15 天或甚至约 1 个月。另外地或可选地，在某些实施方案中，负载抗体的载体材料在 25℃下稳定至少 6 个月、至少 1 年、至少 1.5 年、至少 2 年、至少 2.5 年、至少 3 年或至少 4 年。可以例如通过高效尺寸排阻色谱 (HPSEC) 或通过将所存储的负载生物分子的组合物的生物活性与新鲜制备的负载生物分子的组合物或与在存储之前测量的组合物活性相比来评估稳定性。抗体的活性例如可通过各种免疫学分析（包括例如酶联免疫吸附测定 (ELISA) 和放射免疫测定）来评估。优选地，在存储期结束时，所存储组合物的活性是相应新鲜制备的组合物的活性的至少 75％、至少 80％、至少 85％、至少 90％、至少 95％、至少 98％、至少 99％、至少 99.5％、至少 99.8％或甚至至少 99.9％。因此，本发明涵盖如下治疗方法，其中在向患者施用组合物之前，负载生物分子的组合物在 25℃下存储至少 6 个月、至少 1 年、至少 1.5 年、至少 2 年、至少 2.5 年、至少 3 年或至少 4 年。

[0092] 本发明进一步包括使生物分子稳定的方法。本发明的方法包括通过任何适于形成本发明的组合物的方法将生物分子负载到载体材料的孔中。

[0093] 制备方法

[0094] 本发明还提供制备硅基载体材料的方法。在某些实施方案中，可以合成的方式制备多孔硅基载体材料。例如，可以通过使正硅酸四乙酯与由胶束棒制成的模板反应来合成多孔硅石。在某些实施方案中，结果为填充有规则排列的孔的一批球或棒。然后例如通过用调节至适当 pH 值的溶剂洗涤来除去模板。在某些实施方案中，多孔硅基载体材料可以使用溶胶 - 凝胶法或喷雾干燥法来制备。在某些实施方案中，多孔硅基载体材料可以通过四氯化硅在氢氧焰中火焰水解来制备。在某些实施方案中，载体材料的制备包括一种或多种适于制备多孔硅基材料的技术。

[0095] 可以通过技术如阳极化、染色蚀刻或电化学蚀刻将孔引入硅基载体材料中。在一个示例性实施方案中，阳极化采用浸渍于氟化氢 (HF) 电解质中的铂阴极和硅晶片阳极。通过使电流流经电池而发生在材料中产生孔的阳极蚀解。在具体实施方案中，通常实施恒定 DC 的流动以确保 HF 稳定的尖端浓度，从而得到更均质的孔隙层。

[0096] 在某些实施方案中，通过用氢氟酸、硝酸和水进行染色蚀刻将孔引入硅基载体材料中。在某些实施方案中，使用一种或多种染色蚀刻试剂的组合，如氢氟酸与硝酸。在某些实施方案中，使用氢氟酸和硝酸的溶液以在硅基材料中形成孔。

[0097] 可通过重量测量来测定材料的孔隙率。可使用 BET 分析来测定载体材料的孔体积、孔尺寸、孔尺寸分布和表面积中的任何一项或多项。以理论的作者的姓氏首字母组合命名的 BET 理论适用于固体表面上气体分子的物理吸附，并且充当测量材料比表面积的重要分析技术的基础 (J. Am. Chem. Soc. 第 60 卷，第 309 页 (1938))。可以例如用可获

自 Micromeritics Instrument Corporation,Norcross,Georgia 的 Micromeritics ASAP 2000 仪器进行 BET 分析。在一个示例性程序中，在进行测量之前，载体材料的样品可以在真空下在例如大于 200℃的温度下除气一段时间如约 2 小时或更多。在某些实施方案中，孔尺寸分布曲线来源于对等温线输出的吸附分支的分析。可以在 P/P0 ＝ 0.985 单点采集孔体积。

[0098] 一种或多种干燥技术可以用于制备本发明的多孔硅基材料。例如，为了防止多孔硅基材料裂开，可以通过超临界干燥、冷冻干燥、戊烷干燥、缓慢蒸发、喷雾干燥或真空辅助急骤干燥来干燥材料。超临界干燥包括在临界点以上过度加热液体孔以避免界面张力。冷冻干燥包括在真空下冷冻和升华任何溶剂。戊烷干燥使用戊烷而非水作为干燥液体，因此可以减少由较低表面张力造成的毛细管应力。缓慢蒸发是一种可在水或乙醇冲洗之后实施并且可以有效降低材料中溶剂的捕捉密度的技术。喷雾干燥是一种藉此蛋白质和糖的溶液以使水足够快速地蒸发以允许糖从溶液来到固体而不重排成晶体的技术。真空辅助急骤干燥是一种藉此多孔基质辅助制剂在减压下快速干燥同时使无定形糖稳定的技术。真空辅助急骤干燥可以在室温下进行，其为物理稳定的无定形体系如生物分子和糖所需。

[0099] 可以对多孔硅基材料的表面改性以显示诸如提高的稳定性、细胞粘附力或生物相容性的性质。任选地，材料可以暴露于氧化条件，如通过热氧化来实现。在一个示例性实施方案中，热氧化方法包括将硅基材料加热至高于 1000℃的温度以促进硅基材料完全氧化。或者，可以氧化载体材料的表面以使载体材料包含部分、大致上或完全由氧化表面如二氧化硅表面覆盖的元素硅骨架。

[0100] 多孔硅基材料的表面或其部分可以衍生化。在一个示例性实施方案中，多孔硅基材料的表面可以用有机基团如烷烃或烯烃进行衍生。在一个具体实施方案中，载体材料的表面可以通过硅的硅氢化作用来衍生。在具体实施方案中，衍生的载体材料可以用作并入活组织中的生物材料。

[0101] 静电相互作用、毛细管作用和疏水相互作用中的任何一种或多种可以使治疗剂能够负载到载体材料的孔中。在某些实施方案中，将载体材料和治疗分子置于溶液中，并且将大分子例如蛋白质或其它抗体从溶液抽吸到载体材料的孔中，这就联想到分子筛从有机液体抽吸水的能力。疏水药物可以较好地适于负载到主要由硅形成的载体材料（例如，大于 50％的材料为硅）中，而亲水药物可以较好地适于负载到特征大部分为硅石的载体材料（例如，大于 50％的载体材料为硅石）中。在某些实施方案中，由外部因素如超声处理或热来驱动大分子负载到载体材料的孔中。载体材料可以具有静电荷和 / 或治疗剂可以具有静电荷。优选地，载体材料具有与治疗剂相反的静电荷，以通过有吸引力的静电力帮助治疗剂吸附到载体材料的孔中。在某些实施方案中，治疗剂或载体材料本身在中性条件下不具有的静电荷，但是可被极化或电离。例如，在这些实施方案中，载体材料和 / 或治疗剂可以电离以有助于治疗剂吸附在载体材料的孔中。例如，在体内，在生理 pH 值下，二氧化硅显示带负电的表面，其会促进带正电的肽的静电吸附。类似地，具有羧酸、磷酸和 / 或磺酸的分子随着递增的 pH 值而电离为带负电的羧酸盐、磷酸盐和 / 或磺酸盐，而氮化的分子（例如携带胺、胍或其它碱性取代基）随着递减的 pH 值而质子化为铵、胍盐或其它带正电的盐。

[0102] 载体材料可以包含涂层或表面改性以将治疗剂吸引到孔中。在某些实施方案中，用包含带电的部分的材料对载体材料进行完全或部分涂布或改性以将蛋白质或抗体吸引

到载体材料的孔中。在其它实施方案中，所述部分可以直接附接于载体材料。例如，胺基可以共价附接到载体材料的表面上，以当在生理 pH 值下质子化时，载体材料的表面携带正电荷，从而例如用带负电的表面吸引蛋白质或抗体。在其它实施方案中，可以用羧酸部分对载体材料改性，以当在生理 pH 值下去质子化时，载体材料携带负电荷，从而用带正电的表面将蛋白质或抗体吸引到孔中。

[0103] 在某些实施方案中，可以在完全形成载体材料之后将治疗剂并入载体材料中。或者，可以在载体材料的一个或多个制备阶段将治疗剂并入载体材料中。例如，可以在载体材料的干燥阶段之前，或者在干燥载体材料之后，或者在这两个阶段将治疗剂引入载体材料中。在某些实施方案中，可以在载体材料的热氧化步骤之后将治疗剂引入载体材料中。

[0104] 可以将一种以上治疗剂并入载体材料中。在某些这种实施方案中，每一种治疗剂可以个别地选自有机小分子以及大分子如蛋白质和抗体。例如，可以用两种治疗青光眼的治疗剂，或者用一种治疗黄斑变性的治疗剂和另一种治疗青光眼的药剂浸渗眼用载体材料。

[0105] 在某些方面，例如当小分子治疗剂与较大分子治疗剂如蛋白质都并入载体材料中时，可以在组合物的不同制备阶段将治疗剂并入载体材料中。例如，可以在氧化或干燥步骤之前将小分子疗法引入载体材料中，并且可以在氧化或干燥步骤之后并入大分子治疗剂。类似地，可以将相同或不同类型的多种不同治疗剂以不同顺序或基本上同时引入成品载体材料中。

[0106] 当载体材料包含单一材料或多种具有多种孔尺寸的材料的组合时，优选在添加较小治疗剂之前将较大治疗剂添加到载体材料中，以避免较小治疗剂填充较大孔并且干扰较大治疗剂的吸附。例如，如果载体材料包含单一材料或多种材料的组合、所述材料具有一些界限分明的直径为约 6nm 的孔（即适于分子量为约 14,000amu 至 15,000amu 的分子）和一些界限分明的直径为约 10nm 的孔（即适于分子量为约 45,000amu 至 50,000amu 的分子），那么优选地在添加较小治疗剂（即具有分子量为约 14,000amu 至 15,000amu 的分子的治疗剂）之前将后一治疗剂（即具有分子量为约 45,000amu 至 50,000amu 的分子的治疗剂）添加到载体材料中。或者且另外地，在两种不同多孔材料一起构成装置的实施方案中，每一载体材料可以单独地负载有不同治疗剂，然后载体材料可以组合得到所述装置。

[0107] 治疗剂可以与一种或多种药学上可接受的赋形剂呈混合物或溶液的形式来引入载体材料中。治疗剂可以配制为以任何合适的方式施用，典型地以适于皮下、肌肉内、腹膜内或经表皮引入或适于植入器官（如肝、肺或肾）内的组合物的形式施用。根据本发明的治疗剂可以配制为以注射液形式胃肠外施用，例如眼内、静脉内、血管内、皮下、肌肉内或输注施用，或用于口服施用。

[0108] 在负载治疗剂之前，载体材料可以呈任何合适的形式，如呈干燥粉末或微粒的形式，或例如用缓冲溶液或其它药学上可接受的液体配制成水性浆液。在负载到载体材料中之前，治疗剂可以呈任何合适的形式，如呈溶液、浆液或固体如冻干物。载体材料和 / 或所述治疗剂可以与其它组分如赋形剂、防腐剂、稳定剂例如糖，或治疗剂例如抗生剂配制。

[0109] 可以将治疗剂配制（且包装和 / 或分散）成浓度 >50mg/mL，如 >60mg/mL，如 >75mg/mL 的溶液。在一个示例性实施方案中，治疗剂是贝伐单抗，并且可以将贝伐单抗在例如磷酸盐缓冲液中配制为具有浓度 >50mg/mL，如 >60mg/mL，如 >75mg/mL。治疗剂可以于表面活

性剂和/或稳定剂例如糖配制(且包装和/或分散),其中治疗剂的最大浓度为50mg/mL。可以将蛋白质片段如抗体片段配制(且包装和/或分散)成浓度>10mg/mL、>15mg/mL或>20mg/mL的溶液。

[0110] 治疗剂可以于稳定剂、赋形剂、表面活性剂或防腐剂配制(且包装和/或分散)。在一些实施方案中,稳定剂、赋形剂、表面活性剂或防腐剂是糖。在具体实施方案中,糖选自海藻糖、蔗糖、甘露醇、山梨醇、木糖醇或甘油。在其它实施方案中,治疗剂在基本上没有稳定剂、赋形剂、表面活性剂和防腐剂中的任何一种或多种的情况下配制(且包装和/或分散),例如其含有小于1mg/mL或优选地小于0.1mg/mL的稳定剂、赋形剂、表面活性剂或防腐剂。治疗剂的制剂可以含有小于1mg/mL的表面活性剂,如小于0.1mg/mL的表面活性剂。

[0111] 在某些实施方案中,组合物可以包含包围粒子(例如载体材料/药剂/糖复合物)的涂层以调控治疗剂的释放。例如,可以用聚合涂层即赋形剂如可可脂涂布(例如通过喷雾干燥)粒子以获得治疗剂从递送媒介物所需的释放概况。聚合涂层可为可生物降解的或不可生物降解的、可渗透的或不可渗透的以释放药剂。本领域技术人员将认识到,聚合物优选是可渗透的、可生物降解的或两者,以使药剂从粒子释放。

[0112] 在某些实施方案中,组合物的粒子可涂有一系列聚合物/溶剂,如聚氨酯、聚硅氧烷、乙烯-乙酸乙烯酯共聚物、聚乙烯醇、聚酸酐、聚乳酸(PLA)、乳酸-羟基乙酸共聚物(PLGA)、聚原酸酯、聚氰基丙烯酸烷酯、聚己内酯、衍生的纤维素基聚合物以及其衍生物和共聚物,如基于聚甲基丙烯酸酯的共聚物,以获得治疗剂从载体材料所需的释放概况。

[0113] 使用方法

[0114] 在某些实施方案中,组合物用于预防或治疗患者的病状。本文提供的各个实施方案通常提供以局部递送治疗有效量的治疗剂,即递送到患者的疼痛、疾病等部位。在某些实施方案中,可以将本发明的组合物递送到患者表面或体内的任何部位。例如,本发明的组合物可以用于皮肤或眼睛表面或者可以植入皮肤下、肌肉内、器官内、骨头旁、眼内或治疗剂的控制释放将有利的任何其它位置。组合物可以在玻璃体内、皮下、结膜下、腹膜内、肌肉内或视网膜下施用。在某些实施方案中,将本发明的组合物递送到眼表面或者眼内,如眼睛的巩膜内或者眼睛的玻璃体内。

[0115] 在某些实施方案中,本发明的组合物用于治疗眼内疾病,如眼后疾病。示例性眼内疾病包括青光眼、年龄相关性黄斑变性比如湿性年龄相关性黄斑变性、糖尿病性黄斑水肿、地图样萎缩、脉络膜新血管形成、葡萄膜炎、糖尿病性视网膜病变、视网膜血管疾病和其它类型的视网膜变性。

[0116] 在某些实施方案中,本发明的组合物用于治疗眼表面疾病。示例性疾病包括病毒性角膜炎和慢性过敏性结膜炎。

[0117] 在某些实施方案中,治疗眼部病状的方法包括将组合物安置在眼表面或者眼内,如眼睛的玻璃体或眼房水内。在某些实施方案中,将组合物注射或手术插入患者的眼内。在某些实施方案中,将组合物注射到患者的眼内,例如注射到眼睛的玻璃体中。在某些实施方案中,组合物作为组合物来注射。在某些实施方案中,组合物包含多种载体材料粒子。组合物可以包含平均尺寸在约1微米至约500微米之间的粒子。在某些实施方案中,组合物包含平均粒度在5微米和300微米之间如在约5微米和100微米之间的粒子。

[0118] 在某些方面,本发明的组合物可用于以持续方式向有需要的患者施用任何治疗

剂。本发明的组合物不限于眼部和眼内使用并且可以用于身体的任何部分。例如，本发明的组合物可用于类似于 Norplant 避孕器来真皮下施用治疗剂。在其它实施方案中，本发明的组合物可用于在持续时段内施用生物分子以便治疗慢性疾病如关节炎。例如，本发明的组合物可用于向需要这种疗法的患者递送治疗剂如依那西普 (etanercept) 或阿达木单抗 (adalimumab)。本发明的组合物可以位于体内任何地方如在肌肉内。组合物可以包含多种小粒子如多种 500 微米或更小的粒子。组合物可以包含如尺寸大于 500 微米的较大粒子，或一种或多种尺寸大于 1mm 如大于 10mm 的粒子。

[0119] 治疗剂可为小分子或生物分子。治疗剂可以历经施用之后多达 4 个月、6 个月或甚至多达 12 个月的过程释放到患者。在一些实施方案中，治疗剂历经 1 个月至 6 个月的过程释放到患者。在优选实施方案中，治疗剂历经 2 天至 2 周的过程释放到患者。在优选实施方案中，治疗剂历经 4 天至 12 天的过程释放到患者。在优选实施方案中，治疗剂历经 6 天至 10 天的过程释放到患者。在优选实施方案中，治疗剂历经 7 天的过程释放到患者。

[0120] 在某些实施方案中，将组合物注射或通过手术皮下插入。在其它实施方案中，将组合物静脉内或关节内递送到患者。

[0121] 在一些实施方案中，组合物通过口服施用。在一些实施方案中，组合物通过口服施用并且包括疫苗。可使用口服施用例如以将活性剂递送到胃、小肠或大肠。用于口服施用的制剂可以呈胶囊、扁囊剂、丸剂、片剂、锭剂（使用调味基，通常是蔗糖和阿拉伯胶或黄芪胶）、粉末、颗粒等形式，每一个都含有预定量的活性成分。用于口服施用的固体剂型（胶囊、片剂、丸剂、糖衣丸、粉末、颗粒等）可以包含装置和一种或多种药学上可接受的载体，如柠檬酸钠或磷酸二钙，和 / 或任何以下物质 :(1) 填充剂或增量剂，如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和 / 或硅酸 ;(2) 粘合剂如羧甲基纤维素、海藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和 / 或阿拉伯胶 ;(3) 保湿剂，如甘油 ;(4) 崩解剂，如琼脂、碳酸钙、马铃薯或木薯淀粉、海藻酸、某些硅酸盐和碳酸钠 ;(5) 溶解阻滞剂，如石蜡 ;(6) 吸收加速剂，如季铵化合物 ;(7) 润湿剂，如十六醇和单硬脂酸甘油酯 ;(8) 吸收剂，如高岭土和膨润土 ;(9) 润滑剂，如滑石、硬脂酸钙、硬脂酸镁、固体聚乙二醇、十二烷基硫酸钠和其混合物 ;以及 (10) 着色剂。在胶囊、片剂和丸剂的情况下，药物组合物还可以包含缓冲剂。类似类型的固体组合物还可以用作软填充和硬填充明胶胶囊中的填充剂，所述胶囊使用赋形剂如乳糖，以及高分子量聚乙二醇等。口服组合物还可包括甜味剂、调味剂、香料和防腐剂。

[0122] 在某些实施方案中，将多个粒子群体递送到患者，如两个粒子群体、三个粒子群体、四个粒子群体或五个粒子群体或更多。粒子群体可以在尺寸或组成方面大致上相同，或者可以具有不同尺寸、由不同载体材料组成或负载有不同治疗剂。可以向患者同时或者在一定时段内并且在患者身体的一个或多个位置施用多个粒子群体。

[0123] 在某些实施方案中，治疗剂历经数天、数周、数月或数年的持续时间从组合物释放到周围生物系统中。在某些这种实施方案中，治疗剂历经选自一天至两年，如两周至约一年，如约一个月至约一年的时间过程释放。组合物可以历经 1 天至 12 个月，如 1 天至 6 个月的过程，如历经 1 周至 3 个月的过程将药物释放到眼中。在某些实施方案中，在 2 年内，如在 18 个月内、在 15 个月内、在 1 年内、在 6 个月内、在 3 个月内，或甚至在 2 个月内释放治疗剂。在某些实施方案中，治疗剂以受控的方式从组合物释放，以使较大百分比的总浸渗治疗剂不会立即或在短时间跨度内，例如在施用数分钟或数小时内释放。例如，如果所需的

递送时间是 2 个月，那么总的浸渗的治疗剂可以例如以每天浸渗治疗剂的约 1/60 的速率释放。在某些实施方案中，控制释放涉及历经例如 1 个月、2 个月、3 个月、4 个月、5 个月、6 个月、7 个月或 8 个月的过程释放治疗剂，其中释放的药剂的量相对于整个递送过程在图上呈线性关系。在一些实施方案中，在施用之后不久治疗剂的效果爆发，接着大致上恒定的随后的时段的释放。爆发效果可以持续例如 1-10 天，期间释放一定百分比的负载药物。在爆发之后，治疗剂的其余部分可以历经某一时段恒定释放。例如，在某些实施方案中，小于 10％的治疗剂在施用之后第一天释放，并且另外 50％在随后 2-30 天内例如以大致上恒定的释放速率恒定释放。在另一示例性实施方案中，小于 10％的治疗剂在施用之后前 5 天内释放，接着 50％的治疗剂在随后 25 天内恒定释放。大致上恒定的释放意指治疗剂从组合物的释放速率在某一时段内基本上恒定。

[0124] 在某些实施方案中，治疗剂在施用之后立即开始释放。在某些实施方案中，治疗剂历经约 3 至 8 个月的过程，如历经约 6 个月的过程释放。在某些实施方案中，在适当时段向患者施用本发明的其它组合物以确保大致上连续的治疗效果。例如，可以一年两次，即每六个月一次地施用释放药物持续六个月的时段的连续剂量的组合物。

[0125] 药物从组合物释放到体内可通过血清和玻璃体分析，例如使用 ELISA 来评估。

[0126] 在某些实施方案中，组合物可以在生物系统内是完全或部分可生物蚀解的。在某些实施方案中，组合物可以由生物系统再吸收。在某些实施方案中，组合物可以在生物系统中是可生物蚀解的与可再吸收的。在某些实施方案中，载体材料可以具有部分生物活性以使材料并入活组织中。在一些实施方案中，在植入之后，载体材料不会大幅矿化或引起矿物沉积。例如，在一些实施方案中，当原位放入不需要钙化的部位时，载体材料不会大幅钙化。

[0127] 在某些实施方案中，组合物在生物系统中会进行生物蚀解。在某些实施方案中，大于约 80％，如大于约 85％、大于约 90％、大于约 92％、大于约 95％、大于约 96％、大于约 97％、大于约 98％、大于约 99％、大于 99.5％或甚至大于 99.9％的载体材料将在生物系统中进行生物蚀解。在某些实施方案中，当载体材料进行生物蚀解时，其被部分或完全再吸收。

[0128] 在某些实施方案中，组合物可以在 1 周至 3 年的过程中大致上生物蚀解。在某些实施方案中，大致上生物蚀解是指蚀解大于 95％的载体材料。在某些实施方案中，大致生物蚀解发生约 1 个月至约 2 年，如约 3 个月至 1 年的过程。在某些实施方案中，大致生物蚀解发生在约 3 年内，如在约 2 年内、在约 21 个月内、在约 18 个月内、在约 15 个月内、在约 1 年内、在约 11 个月内、在约 10 个月内、在约 9 个月内、在约 8 个月内、在约 7 个月内、在约 6 个月内、在约 5 个月内、在约 4 个月内、在约 3 个月内、在约 2 个月内、在约 1 个月内、在约 3 周内、在约 2 周内、在约 1 周内或甚至在约 3 天内。在某些实施方案中，当载体材料进行生物蚀解时，其被部分或完全再吸收。

[0129] 在某些实施方案中，可以通过本领域中使用的任何合适技术来评价生物蚀解的程度。在示例性实施方案中，通过鉴别降解产物的体外测定或者体内组织学和分析来评价生物蚀解。可以通过分析有关体液中主要降解产物的浓度来体外评估多孔载体材料的生物降解能力动力学。对于眼后多孔硅基载体材料，例如，降解产物可以包括原硅酸（例如通过钼酸蓝测定进行定量），并且体液可以是模拟的或者是真正的玻璃状液。可以如下测定体内生物降解能力动力学：将已知量的多孔硅基材料植入相关身体部位并且使用组织学结合例如

标准微量分析技术来监测其随时间的持久性。

[0130] 实施例

[0131] 材料

[0132] 商业多孔硅石的规格

[0133]

供应商	商品名	标称孔尺寸 (Å)	表面积 (m^2/g)	孔体积 (mL/g)
Grace Davison Discovery Sciences	Davisil	60	550	0.9
		150	330	1.2
		250	285	1.8
		500	80	1.1
		1000	40	1.1
SiliCycle	SiliaSphere PC	300	100	1.1

[0134] 实施例 1：制备糖和多孔硅石制剂

[0135] 甘露醇、山梨醇或木糖醇与60 Å多孔硅石（如 Davisil）的共同制剂可通过熔融负载来实现。在自封袋（zip-lock）中手工混合近似相等重量的硅石和糖；然后转移到合适的样品小瓶中。在糖的熔点下加热混合物持续 5 分钟的时段。

[0136] 实施例 2：制备海藻糖和多孔硅石制剂

[0137] 海藻糖与60 Å多孔硅石（如 Davisil）的共同制剂可通过浸渍负载来实现。将约 1000mg 多孔硅石浸渍于 5mL 海藻糖浓溶液（500mg/mL）中，并且在室温和室压下在连续搅拌下孵育 2 小时的时段。这个负载溶液可使用二水合海藻糖晶体来制备。因此必须调节这些晶体的起始体重，以使溶液的最终浓度为约 500mg/mL。在孵育之后，使用 PVDF 过滤器（2 分钟，在 13000rpm 下）通过旋转过滤从负载溶液回收共同制剂，冻结至 -20℃并且冷冻干燥。为了防止糖在离心期间再结晶，在这个过程期间将样品加热至约 40℃。在已经负载糖之后，干燥所述制剂。

[0138] 实施例 3：制备蔗糖和多孔硅石制剂

[0139] 蔗糖与60 Å多孔硅石（如 Davisil）的共同制剂可通过浸渍负载来实现。将约 1000mg 多孔硅石浸渍于 5mL 饱和蔗糖溶液（2g/ml）中，并且在室温和室压下并且在连续搅拌下孵育 2 小时的时段。使用 PVDF 过滤器通过旋转过滤回收样品，冻结至 -20℃并且冷冻干燥。为了防止糖在离心期间再结晶，在约 40℃的温度下完成这个程序。在已经负载糖之后，干燥所述制剂。

[0140] 实施例 4：制备蔗糖和多孔硅石制剂

[0141] 在室温下将贝伐单抗（2mL 的 1mg/mL 溶液）与250 Å多孔硅石（例如 Davisil）（40mg）一起孵育 18 小时。添加蔗糖（2g）并且孵育组合物 20 小时。在孵育之后，通过 0.45μm 离心过滤器在 16,000g 下通过离心回收材料。冷冻干燥所述组合物 18 小时。

[0142] 通过冷冻干燥在 50mM(pH 6.2) 磷酸盐缓冲液（没有硅石）中的 100μL 的 1mg/mL

贝伐单抗来制备对照制剂。还通过冷冻干燥在 50mM(pH 6.2) 磷酸盐缓冲液中的 100μL 的 1mg/mL 贝伐单抗和 300μL 的 1g/mL 蔗糖来制备贝伐单抗 - 蔗糖共同制剂对照。

[0143] 在干燥之后，用 200mM 碳酸盐缓冲液 (pH 9.6) 萃取每一组合物的一式三份的样品持续 6 小时。在萃取之后，对样品离心并且通过 SEC 分析上清液以分析贝伐单抗的回收率。将结果示于图 2 中。

[0144] 实施例 5：制备包含蔗糖和氧化阳极化的中孔硅材料的制剂

[0145] 将如在美国专利 8,318,194 和美国专利 20120177695 中所公开的氧化阳极化的中孔硅材料相继与如本文所公开的贝伐单抗和蔗糖孵育，接着真空干燥以去除过量的水。将结果示于图 2 中。

[0146] 实施例 6：从聚合物涂布的粒子释放肌红蛋白

[0147] 与前述实施例类似地使氧化阳极化的中孔硅材料（60 Å）负载有肌红蛋白和蔗糖，并且用 PLA 或 PLGA 涂布负载的粒子。图 3 中描绘了从这些涂布的粒子释放肌红蛋白。

[0148] 等效物

[0149] 本领域技术人员将认可，或仅仅使用常规实验就能够确定本文所描述的化合物和其使用方法的许多等效物。此类等效物被视为在本发明范围内并且由以下权利要求书覆盖。本领域技术人员还将认识到本文所描述的实施方案的所有组合均在本发明范围内。

[0150] 虽然上文所描述的实施方案在一些情况下是根据优选特征（例如，优选范围的有效药剂量和优选厚度的优选层）的进行描述，但这些优选项决不意在限制本发明。如本领域技术人员将容易理解，优选特征取决于施用方法、所用有益物质、所用外壳和载体材料、所需释放速率等。

[0151] 所有前述美国专利和其它公布各自明确地以全文引用的方式并入本文中。

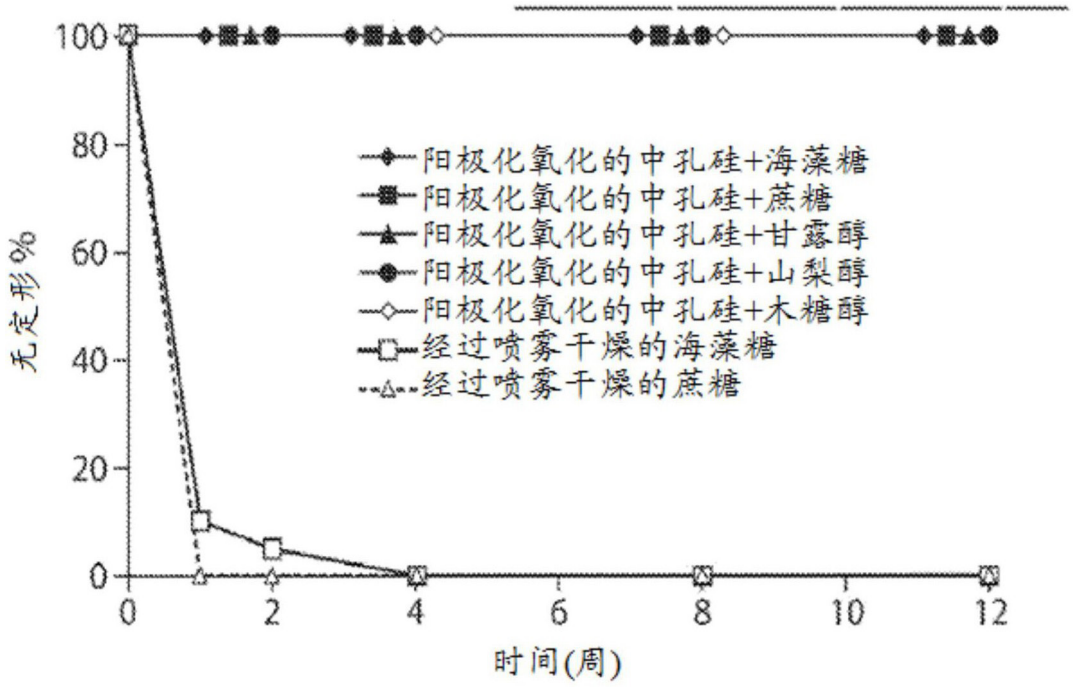
100
80
60
40
20
0
无定形%
0
2
4
6
8
10
12
时间(周)
阳极化氧化的中孔硅+海藻糖
阳极化氧化的中孔硅+蔗糖
阳极化氧化的中孔硅+甘露醇
阳极化氧化的中孔硅+山梨醇
阳极化氧化的中孔硅+木糖醇
经过喷雾干燥的海藻糖
经过喷雾干燥的蔗糖

图 1

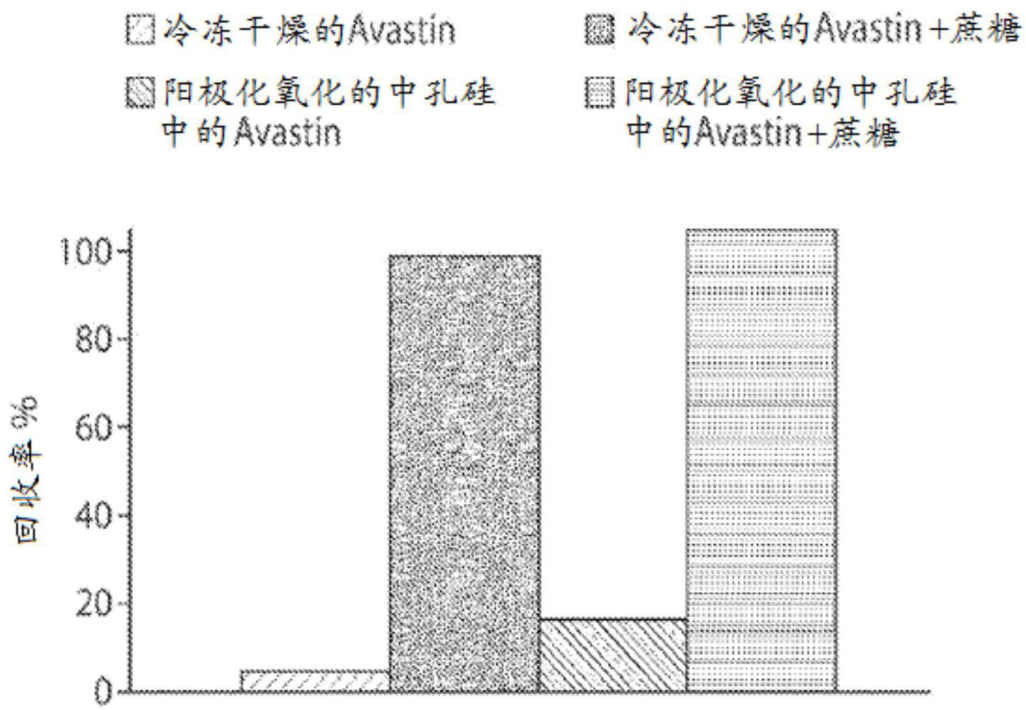
冷冻干燥的Avastin
冷冻干燥的Avastin+蔗糖
阳极化氧化的中孔硅中的Avastin
阳极化氧化的中孔硅中的Avastin+蔗糖
回收率%
100
80
60
40
20
0

图 2

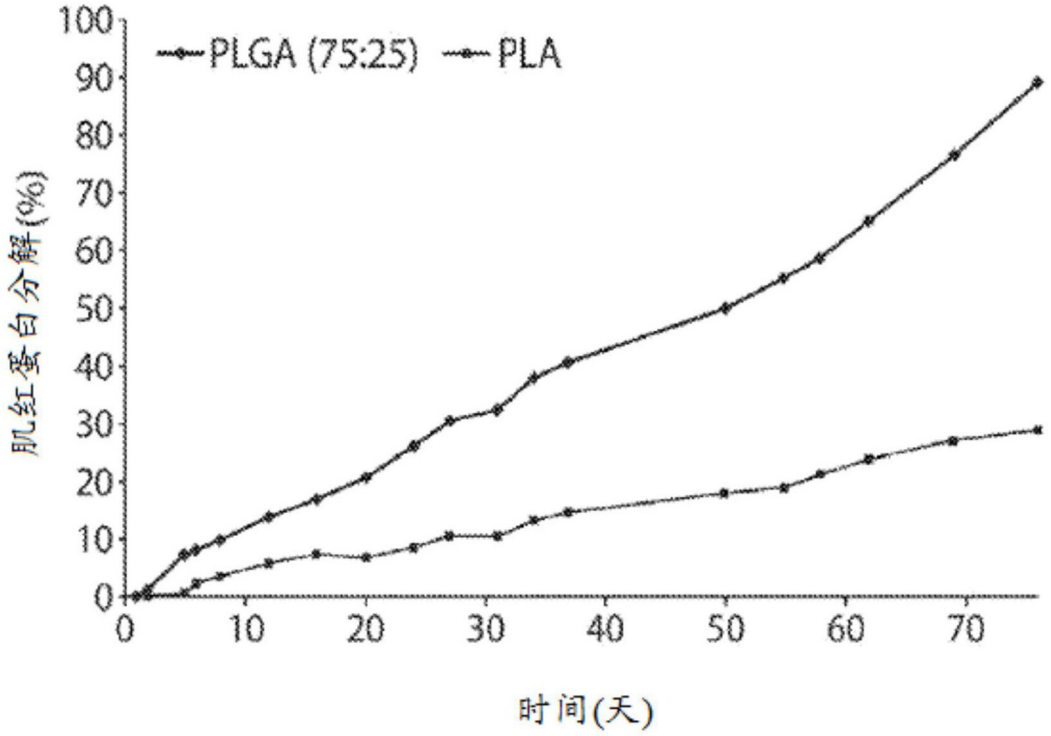

图 3