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(71) Applicants *(for all designated States except US)*: **EYEGATE PHARMACEUTICALS, INC.** [US/US]; 100 Beaver Street, Waltham, MA 02453 (US). **LIQUIDIA TECHNOLOGIES** [US/US]; P.O. Box 110085, Research Triangle Park, NC 27709 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FROM, Stephen** [CA/US]; 473 Chestnut Hill Avenue, Brookline, MA 02445 (US). **PATANE, Michael** [US/US]; 6 Westwind Road, Andover, MA 01810 (US). **SCHUBERT, William** [US/US]; 10 Gallup Drive, Chelmsford, MA 01824 (US). **ROTHROCK, Ginger** [US/US]; c/o Liquidia Technologies, P.O. Box 110085, Research Triangle Park, NC 27709 (US).

(74) Agents: **MORENCY, Michel** et al.; Foley & Lardner LLP, 111 Huntington Avenue, Boston, MA 02199 (US).

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(54) **Title**: IONTOPHORETIC DELIVERY OF A CONTROLLED-RELEASE FORMULATION IN THE EYE

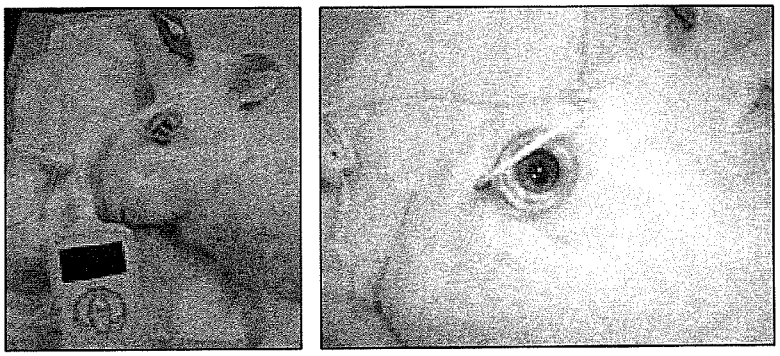


FIG. 21

(57) **Abstract**: Disclosed are compositions of the molded nanoparticle comprising an active agent, methods for manufacturing the molded nanoparticle, and method for delivering the molded nanoparticle through ocular iontophoresis for treating ocular conditions.

IONTOPHORETIC DELIVERY OF A CONTROLLED-RELEASE FORMULATION IN THE EYE

RELATED APPLICATION

This application claims benefit of prior to US provisional patent application USSN 61/081013, filed on July 15, 2008, the content of which is incorporated herein by reference in its entirety.

BACKGROUND

Conventional ophthalmic drug delivery methods employ topical and injection modes of administration. These methods have a number of limitations. For example, these methods encounter transfer problems at the anatomical corneo-conjunctival epithelium barrier, increase tear flow, reflex blinking, and pre-corneal factors, resulting in a short residence time of the delivered active agent "drug". Furthermore, the injection mode of delivery is invasive and involves frequent dosing which results in low patient compliance. The drug formulation also suffers poor solubility, low permeability, rapid elimination and short half-life. Also significantly, current ophthalmic delivery lacks a universal drug delivery and controlled-release system.

Iontophoresis can be used in conjunction with topical or injection delivery methods to improve delivery; however, conventional ocular iontophoresis has limitations. For example, other than drug deporting within ocular tissue(s), there is a lack of controlled-release system to enhance the durability of drug efficacy. Additionally, certain properties of molecules make them less- and/or unsuitable for iontophoresis; for example, molecules that lack a charge, water solubility, and/or stability across a range of pHs.

From the foregoing, it will be appreciated that it would be an advancement in the art to provide an efficient method that combines ocular iontophoresis delivery and controlled-release strategy that would extend the durability of *in vivo* delivery of all therapeutics while offering development opportunities for molecules less- and/or un-suitable for iontophoresis.

SUMMARY

The present invention is directed to compositions and methods for controlled-release delivery of an active agent to the eye of a subject using iontophoresis. More specifically, the active agent is molded in the form of a nanoparticle comprising desired physical (size and shape), chemical (surface charge), and biological (biocompatibility) properties that will allow controlled-release delivery of a wide range of active agents that are either suitable for iontophoresis, or otherwise unsuitable for iontopheresis, such as, *e.g.*, water insoluble, pH sensitive, or neutral; thereby maximizing the efficacy of the active agent. Furthermore, iontophoresis is a non-invasive, pain-free method of administering a drug into the diseased eye. By combining iontophoresis delivery with controlled-release compositions, the present invention shortens the treatment time, has minimized or no side effects, and improves patient compliance by decreasing treatment frequencies.

In one embodiment, the present invention is directed to a molded nanoparticle, comprising an active agent and one or more excipients, such that the molded nanoparticle is suitable for ocular iontophoresis. In a particular embodiment, the active agent is useful for treating one or more ocular conditions. In a particular embodiment, the molded nanoparticle is capable of releasing the active agent in a desired tissue of a subject. In a particular embodiment, the molded nanoparticle is delivered to the desired tissue by iontophoresis. In a particular embodiment, the molded nanoparticle is delivered into the eye of the subject.

In one embodiment, the present invention is directed to a molded nanoparticle, comprising an active agent and one or more excipients, such that the molded nanoparticle is suitable for ocular iontophoresis.

In one embodiment, the present invention is directed to a method of delivering an active agent to the eye of a subject, comprising applying the active agent to the eye of the subject, wherein the active agent is molded alone or with one or more excipients into a nanoparticle; and applying an electric current such that the nanoparticle is iontophoresed into the subject's eye, thereby causing the active agent to be delivered to the eye of the subject. In a particular embodiment, the electric current is between about -10 mA and about +10 mA. In a particular embodiment,

the iontophoresis is performed for a duration of up to 10 minutes. In a particular embodiment, the molded nanoparticle is prepared as a suspension at a concentration between about 0.01 mg/mL and about 100 mg/mL. In a particular embodiment, the molded nanoparticle is applied topically. In a particular embodiment, the molded nanoparticle is injected.

In one embodiment, the present invention is directed to a molded nanoparticle comprising an active agent and one or more excipients, wherein the active agent is useful for treating one or more ocular conditions, and wherein the molded nanoparticle is capable of releasing the active agent in a desired tissue of a subject. In a particular embodiment, the excipient provides a net surface charge. In a particular embodiment, the excipient is a biodegradable compound selected from the group consisting of a biodegradable polymer, a crosslinker compound, and a matrix forming compound, and/or a plurality of their co-polymerized forms. In a particular embodiment, the biodegradable polymer comprises a synthetic polyelectrolyte and a polar polymer selected from the group consisting of: poly(acrylic acid), poly(styrene sulfonate), carboxymethylcellulose, poly(vinyl alcohol), poly(ethylene oxide), poly(vinyl pyrrolidone), dextran, and the like, reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, branched polyethylene glycols, combinations thereof, poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone), and combinations thereof. In a particular embodiment, the biodegradable polymer is hydrophilic and comprises a compound selected from the group consisting of poly(vinyl pyrrolidinone), reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, hydroxyethyl methacrylate, branched polyethylene glycols, and combinations thereof. In a particular embodiment, the biodegradable polymer is hydrophobic and comprises a compound selected from the group consisting of poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone). In a particular embodiment, the biodegradable polymer comprises monomeric subunits selected from the group consisting of butadienes, styrenes, propene, acrylates, methacrylates, vinyl ketones, vinyl esters, vinyl

acetates, vinyl chlorides, vinyl fluorides, vinyl ethers, vinyl pyrrolidone, acrylonitrile, methacrylnitrile, acrylamide, methacrylamide allyl acetates, fumarates, maleates, ethylenes, propylenes, tetrafluoroethylene, ethers, isobutylene, fumaronitrile, vinyl alcohols, acrylic acids, amides, carbohydrates, esters, urethanes, siloxanes, formaldehyde, phenol, urea, melamine, isoprene, isocyanates, expoxides, bisphenol A, chlorsianes, dihalides, dienes, alkyl olefins, ketones, aldehydes, vinylidene chloride, anhydrides, saccharide, acetylenes, naphthalenes, pyridines, lactams, lactones, acetals, thiiranes, episulfide, peptides, derivatives thereof, and combinations thereof. In a particular embodiment, the biodegradable polymer further comprises one or more charged monomeric subunits selected from the group consisting of: cationically charged [2-(acryloyloxy)ethyl]trimethyl ammonium chloride, 2-aminoethyl methacrylate hydrochloride, anionically charged monomers, and combinations thereof. In a particular embodiment, the biodegradable polymer further comprises specific polymers selected from the group consisting of polyamides, proteins, polyesters, polystyrene, polyethers, polyketones, polysulfones, polyurethanes, polysiloxanes, polysilanes, cellulose, amylase, polyacetals, polyethylene, glycos, poly(acrylate)s, poly(methacrylate)s, poly (vinyl alcohol), poly(vinylidene chloride), poly(vinyl acetate), poly(ethylene glycol), polystyrene, polyisoprene, polyisobutylenes, poly(vinyl chloride), polyvinyl pyrrolidone, poly(propylene), poly(lactic acid), polyisocyanates, polycarbonates, alkyds, phenolics, epoxy resins, polysulfides, polyimides, liquid crystal polymers, heterocyclic polymers, polypeptides, conducting polymers including polyacetylene, polyquinoline, polyaniline, polypyrrole, polythiophene, and poly(p-phenylene), dendimers, fluoropolymers, derivatives thereof, and combinations thereof. In a particular embodiment, the biodegradable polymer comprises a compound selected from the group consisting of: a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, and combinations thereof. In a particular embodiment, the polyester comprises a compound selected from the group consisting of: a polylactic acid, a polyglycolic acid, a poly(hydroxybutyrate), a poly(ε-caprolactone), a poly(β-malic acid), a poly(dioxanone), and combinations thereof. In a particular embodiment, the crosslinker is biocompatible and/or

biodegradable. In a particular embodiment, the crosslinker comprises a hydrolytically labile component selected from the group consisting of: a carbonate, an ester, a ketal, an acetal, a silyl, an orthoester, a hydrazone, a silicon based hydrolyzable crosslinker, a phosphazene linker, a lactide, a glycolide, a succinic acid, an alpha hydroxy acid, a glycolic acid, and a lactic acid. In a particular embodiment, the crosslinker further comprises a biodegradable moiety selected from the group consisting of: an anhydride, a ketal, an acetal, an orthoester, and a phosphoester. In a particular embodiment, the crosslinker further comprises a compound selected from the group consisting of: an ethylene glycol oligomer, an oligo(ethylene glycol), a poly(ethylene oxide), a poly(vinyl pyrolidone), a poly(propylene oxide), a poly(ethyloxazoline), and combinations thereof. In a particular embodiment, the crosslinker further comprises a reduction/oxidation-cleavable crosslinker comprising a disulfide bridge or an azo linkage. In a particular embodiment, the nanoparticle further comprises a non-wetting agent. In a particular embodiment, nanoparticle exists in a single phase or in multiple phases. In a particular embodiment, the active agent is useful for treating one or more ocular conditions. In a particular embodiment, the active agent is selected from the group consisting of an anti-inflammatory agent, an anti-viral agent, an anti-neovascularization agent, and an antibiotic agent. In a particular embodiment, the nanoparticle further comprises a composition selected from the group consisting of: targeting ligands (activating and/or inactivating), enzyme inhibitors, allosteric modulators, oligonucleotides, bioprocess activators, cell-targeting peptides, cell-penetrating peptides, integrin receptor peptides, hormones, antibodies, vitamins, viruses, polysaccharides, cyclodextrins, liposomes, proteins, fluorescent tags, imaging agents, and combinations thereof. In a particular embodiment, the molded nanoparticle has a hydraulic diameter between about 1 nm and about 1000 nm, between about 200 nm and about 400 nm or between about 50 nm and about 400 nm. In a particular embodiment, the molded nanoparticle has a zeta potential between about -60 mV and about +60 mV. In a particular embodiment, the molded nanoparticle forms a shape selected from the group consisting of cylinder, trapezoid, bar, cone, boomerang, and arrow.

In one embodiment, the molded nanoparticle is delivered to the desired tissue by iontophoresis. In another embodiment, the molded nanoparticle is delivered into the eye of the subject.

In one embodiment, the present invention is directed to a system for treating an ocular condition, comprising: a) a device comprising a first reservoir configured and dimensioned to be positioned adjacent to an eye of a subject and deliver a controlled electric current to the eye; and b) a plurality of charged nanoparticles within the first reservoir wherein each nanoparticle comprises a predetermined three dimensional shape and an active agent, whereby delivering the controlled electric current to the eye drives the plurality of nanoparticles from the first reservoir into tissue of the eye.

In one embodiment, the charged nanoparticle further comprises a biodegradable polymer or a polyelectrolyte.

In one embodiment, the biodegradable polymer is selected from the group consisting of: chitosan, polylacticcoglycolic acid, polyvinylalcohol, crosslinked cationic polyethyleneglycol and crosslinked anionic polyethyleneglycol. In a particular embodiment, the biodegradable polymer comprises a synthetic polyelectrolyte and a polar polymer selected from the group consisting of: poly(acrylic acid), poly(styrene sulfonate), carboxymethylcellulose, poly(vinyl alcohol), poly(ethylene oxide), poly(vinyl pyrrolidone), dextran, and the like, reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, branched polyethylene glycols, combinations thereof, poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone), and combinations thereof.

In one embodiment, the biodegradable polymer comprises poly(lactic-co-glycolic acid) and polylactic acid.

In another embodiment, the biodegradable polymer comprises a compound selected from the group consisting of poly(vinyl pyrrolidinone), reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, hydroxyethyl methacrylate, branched polyethylene glycols, and combinations thereof.

In one embodiment, the biodegradable polymer comprises a compound selected from the group consisting of poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone).

In one embodiment, the biodegradable polymer comprises one or more monomeric subunits selected from the group consisting of: butadienes, styrenes, propene, acrylates, methacrylates, vinyl ketones, vinyl esters, vinyl acetates, vinyl chlorides, vinyl fluorides, vinyl ethers, vinyl pyrrolidone, acrylonitrile, methacrylnitrile, acrylamide, methacrylamide allyl acetates, fumarates, maleates, ethylenes, propylenes, tetrafluoroethylene, ethers, isobutylene, fumaronitrile, vinyl alcohols, acrylic acids, amides, carbohydrates, esters, urethanes, siloxanes, formaldehyde, phenol, urea, melamine, isoprene, isocyanates, expoxides, bisphenol A, chlorsianes, dihalides, dienes, alkyl olefins, ketones, aldehydes, vinylidene chloride, anhydrides, saccharide, acetylenes, naphthalenes, pyridines, lactams, lactones, acetals, thiiranes, episulfide, peptides, derivatives thereof, and combinations thereof.

In a further embodiment, the biodegradable polymer comprises one or more monomeric subunits selected from the group consisting of: cationically charged [2 (acryloyloxy)ethyl]trimethyl ammonium chloride, 2-aminoethyl methacrylate hydrochloride, anionically charged monomers, and combinations thereof.

In one embodiment, the polyelectrolyte is selected from the group consisting of: crosslinked cationic poly allylamine hydrochloric acid and anionic polystyrene sodium sulfonate.

The nanoparticle described herein and suitable for use in the methods described herein further comprises a surfactant.

The active agent described herein and suitable for use in the methods described herein is useful for treating one or more ocular conditions. In one embodiment, the active agent is selected from the group consisting of an anti-inflammatory agent, an anti-viral agent, an anti-neovascularization agent, and an antibiotic agent.

The nanoparticle described herein and suitable for use in the methods described herein further comprises a composition selected from the group consisting of: targeting ligands (activating and/or inactivating), enzyme inhibitors, allosteric

modulators, oligonucleotides, bioprocess activators, cell-targeting peptides, cell-penetrating peptides, integrin receptor peptides, hormones, antibodies, vitamins, viruses, polysaccharides, cyclodextrins, liposomes, proteins, fluorescent tags, imaging agents, and combinations thereof.

The nanoparticle described herein and suitable for use in the methods described herein has a hydraulic diameter between about 1 nm and about 1000 nm.

The nanoparticle described herein and suitable for use in the methods described herein has a hydraulic diameter between about 200 nm and about 400 nm.

The nanoparticle described herein and suitable for use in the methods described herein has a hydraulic diameter between about 50 nm and about 400 nm.

The nanoparticle described herein and suitable for use in the methods described herein has a zeta potential between about -60 mV and about +60 mV.

In one embodiment, the predetermined three dimensional shape is selected from the group consisting of cylinder, trapezoid, bar, cone, boomerang, and arrow.

In one embodiment, the present invention is directed to a method for delivering an active agent to the eye of a subject, comprising: a) positioning a delivery device comprising a reservoir adjacent to an eye of a subject, wherein the delivery device is configured to deliver a controlled electric current to the eye, wherein the reservoir is configured and dimensioned to house a plurality of charged nanoparticles, and wherein the charged nanoparticles comprise a predetermined three dimensional shape and an active agent; b) applying a controlled current to the eye through the device; and c) iontophoretically delivering the charged nanoparticles into tissue of the eye.

In one embodiment, the electric current is applied at between about -10 mA and about +10 mA.

In one embodiment, the charged nanoparticles are iontophoretically delivered to the eye for between one minute and about ten minutes.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is an illustration of the Particle Replication in Non-Wetting Templates (PRINT) process according to an embodiment of the present invention.

FIG. 2 are micrographs of examples of molded nanoparticles.

FIG. 3 are images of molded nanoparticles showing manipulation of shape. Panel A: 200 nm trapezoidal PEG particles; Panel B: 200 nm × 800 nm bar PEG particles; Panel C: 500 nm conical PEG particles that are <50 nm at the tip; Panel D: 3 mm arrow PEG particles.

FIG. 4 depicts cross sections of scleral tissue samples following *in vitro* screening in an Ussing chamber following iontophoresis. The donor and acceptor chambers are labeled. The particles are characterized by the red intensities displayed by their fluorescent cargo.

FIG. 5 depicts cross sections of scleral tissue samples following in vitro screening in an Ussing chamber following iontophoresis. The donor and acceptor chambers are labeled. The particles are characterized by the red intensities displayed by their fluorescent cargo.

FIG. 6 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #1 under different drug administration conditions. Particles penetrate the conjuctiva at +4 mA for 10 minutes, and can be spotted in the lamina propria, which is the layer of tissue in between the conjuctiva and sclera.

FIG. 7 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #1 under different drug administration conditions. Particles penetrate the conjuctiva at +4 mA for 10 minutes, and can be spotted in the lamina propria.

FIG. 8 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #1 under different drug administration conditions. Particles penetrate the conjuctiva at +4 mA for 10 minutes, and can be spotted in the lamina propria.

FIG. 9 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #1 under different drug administration conditions. Particles penetrate the conjuctiva at +4 mA for 10 minutes, and can be spotted in the lamina propria.

FIG. 10 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #2 and #3 under different drug administration

conditions. Particles penetrate the conjuctiva and can be spotted in the lamina propria.

FIG. 11 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #2 and #3 under different drug administration conditions. Particles penetrate the conjuctiva and can be spotted in the lamina propria.

FIG. 12 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #2 and #3 under different drug administration conditions. Particles penetrate the conjuctiva and can be spotted in the lamina propria.

FIG. 13 shows fluorescent microscopy images of histological sections of rabbit eye tissue treated with particle #2 and #3 under different drug administration conditions. Particles penetrate the conjuctiva and can be spotted in the lamina propria.

FIG. 14 shows images depicting particle passive distribution in freshly loaded foam (left panel) and after one hour in water (right panel). No difference in distribution was observed.

FIG. 15 shows particle size and zeta potential comparison of "Batch B" particles.

FIG. 16 is an image showing particles adhering to the surface of return electrode.

FIGS. 17a through 17c show images of different magnification showing particles on and embedded in agarose after iontophoresis. FIG. 17a is at 2× magnification (agarose surface). FIG. 17b is at 10× magnification (agarose surface). FIG. 17c is at 10× magnification (electrode surface embedded in salt bridge).

FIGS. 18a and 18b show images of two different magnification showing particles sticking to the plastic cap used as receptor chamber. FIG. 18a is at 2× magnification. FIG. 18b is at 10× magnification. Both are images of the inner surface of the cap wall.

FIG. 19 shows a scanning electron micrograph of FITC-PEG PRINT® nanoparticles; PEG: polyethylene glycol.

FIG. 20 shows a schematic diagram of an ocular iontophoretic drug delivery device. The device has an annular shape and consists of the following main elements: (1) proximal part, which provides rigid support for the device; (2) source connector pin, which provides a connection point between the current generator and the electrode; (3) electrode, which transfers the current to the formulation reservoir; (4) agarose hydrogel membrane (Type II applicator); (5) reservoir, which contains a polyurethane-based foam insert saturated with the nanoparticle suspension to be delivered; and (6) distal part, which is a soft plastic that interfaces with the eye.

FIG. 21 shows details of the setup for iontophoretic delivery of nanoparticles in New Zealand White rabbit eyes with the ocular device and a low-voltage generator.

FIGS. 22a through 22c show representative microscopic images of rabbit Conjunctiva (Conj) and Lamina Propria (LP) tissues immediately after administration of a single dose of a 1 mg/mL suspension of positively charged PRINT® nanoparticles under: (a) anodal iontophoresis (+4 mA, 5 min) using Type I applicator (n = 2 eyes); (b) anodal iontophoresis (+4 mA, 5 min) using Type II applicator (n = 2 eyes); and (c) passive (no current, 5 min) condition with Type II applicator (n = 2 eyes). Sections were counter-stained with DAPI. Scale bar: 25 microns.

FIG. 23 is a schematic depiction of a device for application of an ocular active substance.

FIG. 24 is a depiction in top view of a main electrode.

FIG. 25 is a schematic depiction in section of he main electrode of FIG. 24.

FIG. 26 is a schematic depiction in section of the main electrode of FIG. 24 according to a variant implementation.

FIGS. 27a and 27b depict a first variant implementation of a device for application of an ocular active substance.

FIGS. 28a, 28b and 28c depict a second variant implementation of a device for application of an ocular active substance.

FIG. 29 depicts a third variant implementation of a device for application of an ocular active substance.

DETAILED DESCRIPTION

Several considerations come into play when delivering an active agent to the eye of a subject. Considerations include, but are not limited to, efficacy of the delivered active agent, biocompatibility and biodegradability of carriers and excipients (*e.g.*, the charged nanoparticles), ease of administration, combinations thereof and the like. Using nanoparticles comprising the active agent, permeability, penetration, and/or the durability of the active agent can be enhanced. Additionally, charge of the nanoparticle, as well as precise and controlled size and shape of the nanoparticle can afford delivery of poorly and/or insoluble and low iontophoretically mobile therapeutics (*e.g.*, active agents) in a controlled manner that results in improved delivery, bioavailability, prolonged release and less frequent dosing regimens. The compositions of the charged particles can include an active agent combined with or complexed into a nanoparticle, which can further include biocompatible and biodegradable matrix or carrier materials. Administration of the nanoparticle can be achieved by the use of iontophoresis, a non-invasive, pain-free method of administering a drug into, for example, a diseased eye. Iontophoresis allows reduced duration of therapy, has minimized or no side effects and improves patient compliance. The present invention utilizes ocular iontophoresis delivery with controlled nanoparticle fabrication and drug-release technologies. Additionally, although iontophoresis is useful for increasing the efficiency of delivery, other methods, including, but not limited to, topical application of the molded nanoparticle, are envisioned as useful for delivery of an active agent at lower efficiencies, although at a controlled-release rate.

Definitions

As used herein, the term "nanoparticle" means a particle having a submicron dimension. The dimension can be measured across the largest portion of the particle. The dimension can be a length, width or diameter of the particle.

As used herein, the term "active agent" refers to a drug or pro-drug. A suitable drug or pro-drug, for example, can be useful for treating one or more ocular conditions.

As used herein, the term "excipient" refers to a neutral or charged substance used as a carrier for the active agent. An excipient is typically biologically inert or nearly so.

As used herein, the term "molded nanoparticle" refers to a particle comprising an active agent that can contain one or more excipients molded as a nanoparticle.

As used herein, the term "biodegradable" means the ability to be broken down into water soluble non-toxic components that can be excreted from cells, tissues, organs, and/or an organism under selected intracellular or biologic conditions.

As used herein, the term "polymer" refers to a natural or synthetic compound with large molecules made up of smaller molecules (called monomers). Some polymers, like cellulose, occur naturally, while others, like nylon, are artificial. Polymers have a range of polarity, water solubility, volatility, structure, and molecular weight with varied and versatile uses.

As used herein, the term "subject" refers to an animal, in particular, a mammal, *e.g.*, a human. A "patient" is a subject afflicted with a particular condition, disease or disorder, or who is being treated for or in need of treatment for a particular condition, disease or disorder.

Particle replication in non-wetting template (PRINT) particles

The molded nanoparticle of the present invention comprises an active agent molded with one or more excipients. The active agent of the molded nanoparticle is a drug or pro-drug that can be useful for treating, for example, one or more ocular conditions. In one embodiment, the active agent is selected from the group consisting of an anti-inflammatory agent, an anti-viral agent, an anti-neovascularization agent, and an antibiotic agent. In some embodiments, the compositions of the present invention are provided in the form of discrete nanoparticles. The present invention is further directed to methods of preparing and using such discrete nanoparticles suitable for controlled-release across sclera by iontophoresis.

The process for making the nanoparticles includes the use of a mold, which optionally includes a plurality of cavities. The mold cavities can have a substantially predetermined and desired size and shape.

Materials that can be useful with and/or as the mold materials used in the present invention include, in some embodiments, substantially solvent resistant, low surface energy polymeric materials. In other embodiments, the mold can be or include a solvent resistant elastomer-based material, such as but not limited to a fluoropolymer, a fluorinated elastomer-based material, a fluoropolyether, perfluoropolyether, combinations thereof, or the like. In some embodiments, the mold is designed to have a specific surface energy, *e.g.*, below a predetermined or defined value.

Representative substantially solvent-resistant elastomer-based materials include, but are not limited to, fluorinated elastomer-based materials. As used herein, the term "substantially solvent-resistant" refers to a material such as, for example, an elastomeric material that neither swells nor dissolves beyond a nominal amount in common hydrocarbon-based organic solvents or acidic or basic aqueous solutions. Representative fluorinated elastomer-based materials include, but are not limited to, fluoropolyether and perfluoropolyether (collectively PFPE) based materials. PFPE materials and modified PFPE materials that are useful for making the molds are described herein. It will be appreciated that the materials described herein can be combined in numerous ways to form different mold materials for use in the present invention.

According to some embodiments, compositions are introduced into the mold and subsequently hardened. Compositions include materials, solutions, dispersions, or the like. Hardening can be achieved, for example, by a chemical reaction, such as polymerization or moisture cure, or a physical transition such as a phase change, melting/cooling transition, or evaporation, combinations thereof, and the like.

The mold and/or substrate materials can be, for example, one or more of the following: flexible, non-toxic, substantially UV transparent, highly gas permeable, highly fluorinated, have a high free volume, tough, have a low surface energy, are highly permeable to oxygen, are highly permeable to carbon dioxide, are highly permeable to nitrogen, or are substantially resistant to swelling, combinations

thereof, and the like. The properties of these materials can be tuned over a wide range through the judicious choice of additives, fillers, reactive co-monomers, and functionalization agents.

Further embodiments of molds of the present invention are disclosed in the following references, which are incorporated herein by reference in their entirety: PCT Publication WO 2007/021762, filed August 9, 2006; PCT Publication WO 2005/084191, filed February 14, 2005; and U.S. Patent Application Publication No. 2007/0275193, filed August 11, 2006.

Particles can be formed in cavities of the molds described above. A substantially liquid composition can be applied, for example, to the mold to form particles. In some embodiments, a substantially liquid composition includes a liquid precursor. Particles prepared according to the present invention can be molded in low surface energy molds, for example, according to methods and materials described in PCT Publication WO 2007/030698, filed September 7, 2006 and PCT Publication WO 2007/094829, filed November 7, 2006, both of which are incorporated herein by reference in their entirety including all references cited therein.

A particle prepared according methods described herein can have a size and shape that substantially mimics the size and shape of the cavity of the mold in which the particle is formed. In some embodiments, a particle has a substantially predetermined size and shape. Moreover, the manufacturing process can produce particles with inherent variations in shape. In some embodiments, the shape of the particles varies from the shape of the mold, and the shape of the particles can also vary from the shape of other particles in the plurality of particles. In certain embodiments, the variations of the shape of the particles are nanoscale variations. In other embodiments, the particles have identical or substantially identical shapes.

As previously pointed out, particles prepared using the compositions of the invention can be of a size so as to be characterized as microparticles or nanoparticles (*i.e.*, nanoscale particles). As used herein, "nanoscale" refers to a submicron scale. Since particles according to the invention can be formed to have a shape corresponding to a mold (*i.e.*, the particle has a shape reflecting the shape of the mold within which the particle was formed), the mold used to prepare the particle

can have a desired shape and/or a desired size. In particular embodiments, particles according to the present invention have an average dimension of less than about 500 μm, less than about 400 μm, less than about 300 μm, less than about 200 μm, or less than about 100 μm. The nanoparticle can be, for example, less than about 25 μm, less than about 10 μm, less than about 5 μm, less than about 1 μm, less than about 900 nm, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 90 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, less than about 50 nm, less than about 40 nm, less than about 30 nm, less than about 20 nm, or less than about 10 nm. In still other embodiments, the dimension of the nanoparticle is between about 1 nm and about 1,000 nm, between about 1 nm and about 900 nm, between about 1 nm and about 800 nm, between about 1 nm and about 700 nm, between about 1 nm and about 600 nm, between about 1 nm and about 500 nm, between about 1 nm and about 400 nm, between about 1 nm and about 300 nm, between about 1 nm and about 200 nm, between about 1 nm and about 100 nm, between about 1 nm and about 50 nm, between about 10 nm and about 500 nm, between about 10 nm and about 400 nm, between about 10 nm and about 300 nm, between about 10 nm and about 200 nm, between about 10 nm and about 100 nm, between about 20 nm and about 200 nm, between about 20 nm and about 100 nm, between about 50 nm and about 500 nm, between about 50 nm and about 250 nm, between about 50 nm and about 200 nm, or between about 100 nm and about 500 nm. The dimension of the mold cavity and/or the nanoparticle can be a predetermined dimension,length, width, height, a cross-sectional diameter, a circumferential dimension, etc.

Matrix Material

Nanoparticles are composed of materials that are hardened into the shape- and size-specific nanoparticles described herein. The nanoparticle is designed, for example, to release a therapeutic at a given rate or degrade in a specific environment. The nanoparticle can comprise a biodegradable or labile compound as described herein. In some embodiments, the active agent and/or biodegradable compound can be used as the matrix material itself.

The nanoparticle is typically biodegradable under selected intracellular or biologic conditions into water soluble non-toxic components that can be excreted from cells, tissues, organs, and/or an organism. Depending on which polymer is selected as the polymer component of the nanoparticle matrix materials, the particle can have different properties such as, for example, varying biodegradation conditions, varying hydrophobic/hydrophilic conditions, ionic nature, water solubility, non-water solubility, and the like. The biodegradable compound can be formed, for example, using a high molecular weight PEG compound or other similar compound useful to form a matrix, particularly a matrix that can be formed into discrete particles. In other embodiments, a biodegradable compound can be a crosslinker compound that is combined with one or more further matrix-forming compounds to form a biodegradable matrix (the degradation being made possible by the presence of the biodegradable compound in the form of a crosslinker compound). Likewise, the biodegradable compound of the invention can be a polymeric material that can be co-polymerized with another material to form the matrix of the composition.

The polymer can include natural or synthetic polymers. In some embodiments, the matrix materials of the present invention can include synthetic polyelectrolytes and polar polymers, such as poly(acrylic acid), poly(styrene sulfonate), carboxymethylcellulose (CMC), poly(vinyl alcohol), poly(ethylene oxide) (PEO), poly(vinyl pyrrolidone) (PVP), dextran, and the like. In some embodiments, water insoluble polymers are made water soluble by ionization or protonation of a pendant group. As will be appreciated by one skilled in the art, water insoluble polymers containing pendent anhydride or ester groups can be solubilized when the anhydride or esters hydrolyze to form ionized acids on the polymer chain. Water soluble polymers can be used as polymers for the polymer component of the nanoparticle because the polymers can be solubilized in cellular and body fluids and excreted therefrom. In some embodiments, the polymers of the matrix are selected or tuned to degrade upon encountering a dissolution condition, which in some embodiments, can be a condition selected from a cellular or biologic environment, such as, for example, pH. Further polymers, water soluble polymers, solubilization of polymers and the like are described in Biodegradable Hydrogels for

Drug Delivery, Park K., Shalaby W., Park H., Taylor & Francis Group, LLC., 1993, the entire contents of which are incorporated herein by reference.

According to some embodiments, the water soluble polymer useful in the particles can include poly(vinyl pyrrolidinone), reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(DMAEMA), HEA, HEMA, branched PEGs, combinations thereof, and the like. In some embodiments, the polymer is a non-water soluble polymer such as, for example poly(beta-aminoesters), PLGA, PLA, poly(caprolactone), etc.

In some embodiments, the synthesis of well-defined polymers having controlled molecular structures can be essential to the preparation of certain intracellular delivery nanoparticles. Depending on the polymer material of interest and the processing conditions and environment, the nanoparticle can be fabricated from prepolymers having well-defined pre-determined molecular weight, low volatility, high volatility, narrow molecular weight distribution, combinations thereof, etc. In certain embodiments polymers for forming the nanoparticle can be prepolymerized from volatile or otherwise unstable monomers. The present invention includes prepolymerization techniques to reduce evaporation, reactivity, or other loss of the volatile component by initially forming a prepolymer or oligomer of the volatile or unstable monomer.

The monomer can be, but is not limited to, e.g., butadienes, styrenes, propene, acrylates, methacrylates, vinyl ketones, vinyl esters, vinyl acetates, vinyl chlorides, vinyl fluorides, vinyl ethers, vinyl pyrrolidone, acrylonitrile, methacrylnitrile, acrylamide, methacrylamide allyl acetates, fumarates, maleates, ethylenes, propylenes, tetrafluoroethylene, ethers, isobutylene, fumaronitrile, vinyl alcohols, acrylic acids, amides, carbohydrates, esters, urethanes, siloxanes, formaldehyde, phenol, urea, melamine, isoprene, isocyanates, expoxides, bisphenol A, chlorsianes, dihalides, dienes, alkyl olefins, ketones, aldehydes, vinylidene chloride, anhydrides, saccharide, acetylenes, naphthalenes, pyridines, lactams, lactones, acetals, thiiranes, episulfide, peptides, derivatives thereof, combinations thereof, and the like. Such monomeric units can be combined with biodegradable compounds according to the invention and co-polymerized to form a biodegradable co-polymer matrix material. Likewise, the degradable compounds can be used as

crosslinkers to facilitate crosslinking of one or more of the monomers illustrated above to form a matrix having biodegradable crosslinkers.

The matrix of the composition can be described, for example, in terms of specific polymers used to form the matrix. Non-limiting examples of polymers that can be used to form the matrix include polyamides, proteins, polyesters, polystyrene, polyethers, polyketones, polysulfones, polyurethanes, polysiloxanes, polysilanes, cellulose, amylose, polyacetals, polyethylene, glycols, poly(acrylate)s, poly(methacrylate)s, poly(vinyl alcohol), poly(vinylidene chloride), poly(vinyl acetate), poly(ethylene glycol), polystyrene, polyisoprene, polyisobutylenes, poly(vinyl chloride), polyvinyl pyrrolidone, poly(propylene), poly(lactic acid), polyisocyanates, polycarbonates, alkyds, phenolics, epoxy resins, polysulfides, polyimides, liquid crystal polymers, heterocyclic polymers, polypeptides, conducting polymers including polyacetylene, polyquinoline, polyaniline, polypyrrole, polythiophene, and poly(p-phenylene), dendimers, fluoropolymers, derivatives thereof, combinations thereof. Such polymers can be combined with degradable compounds according to the invention to form a degradable matrix material. Likewise, such polymers can be formed using the degradable compounds as crosslinkers so that that disclosed polymers include degradable crosslinks.

Matrix materials can be chosen to meet specific needs or use conditions. Different monomers and polymers, for example, are known to be hydrophilic or lipophilic. It is possible to customize the hydrophilic or lipophilic nature of the matrix material by choosing the type or ratio of monomer and polymers used to form the matrix. When desirable to prepare compositions that may cross a lipophilic cell membrane, for example, the matrix material can be chosen to have a desired lipophilic nature. Likewise, for use under conditions where hydrophilic properties are desired, the matrix material can be chosen to have a desired hydrophilic nature.

The nanoparticle can optionally include one or more charged monomers. The charged monomers can include cationically charged monomers such as, for example, [2-(acryloyloxy)ethyl]trimethyl ammonium chloride (AETMAC), 2-aminoethyl methacrylate hydrochloride (AEM-HCl), anionically charged monomers, combinations thereof, or the like. In some embodiments the charged monomer contributes one positive charge for each molecule of the charged monomer added to

the composition. The charged monomers can be included to offset the charge of the cargo, *i.e.*, the active agent to be delivered, or other matrix components and configure the particle with a localized or overall charge that can assist the particle in transport, crossing a cell membrane and entering a cell or otherwise delivering or maintaining a charged cargo within the particle through ionic interactions. The measurement or effect of including the charged monomer with the particle matrix is to create a zeta potential of the particle that will assist the particle in transport.

The nanoparticles can be configured, for example, to have a positive or negative zeta potential. The nanoparticles can have a zeta potential of between about negative 60 mV to about positive 60 mV, between about 5 mV and about 150 mV, between about 15 mV and about 100 mV, between about 20 mV and about 75 mV or between about 25 mV and about 50 mV.

The particles of the present invention further include a biologically compatible and/or biodegradable crosslinker. The crosslinker can be selected or tuned to degrade under specific conditions, such as for example, in response to a selected pH, in response to a selected enzyme, after a selected time in an aqueous environment, combinations thereof, or the like. Degradable crosslinkers can degrade, for example, through hydrolysis, enzymatic cleavage, a change of temperature, pH, or other environments such as oxidation or reduction.

Crosslinking groups can include hydrolytically labile carbonate, ester, ketal, acetal, silyl, orthoester, hydrazone, silicon based hydrolyzable crosslinkers, and phosphazene linkers, lactide or glycolide, and succinic acid and alpha hydroxy acids such as glycolic, or lactic acid. Crosslinkers can also include a degradable region containing one or more groups such as anhydride, a ketal, an acetal, an orthoester and/or a phosphoester. In certain cases the biodegradable region can contain at least one amide functionality. The crosslinker can also include an ethylene glycol oligomer, oligo(ethylene glycol), poly(ethylene oxide), poly(vinyl pyrolidone), poly(propylene oxide), poly(ethyloxazoline), or combinations of these substances. In some embodiments, crosslinkers include reduction/oxidation cleavable cross linkers, such as, for example, a disulfide bridges, azo linkages, combinations thereof, etc. Crosslinkers susceptible to pH changes are also included; these systems can be

stable under acidic or basic conditions and start to degrade at blood pH or can be base or acid catalyzed.

In addition to the use of the biodegradable compounds of the invention, the matrix material used to form the nano-compositions can also include one or more biodegradable polymers. The use of biodegradable polymers can facilitate delivery of the cargo component. The use of biodegradable polymers can facilitate the removal of the matrix material from the delivery site after delivery of the cargo component *via* degradation of the biodegradable compound of the invention. In some embodiments, the biodegradable polymer includes, without limitation, one or more of a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, a polyacetal, combinations thereof, or the like. In some embodiments, specific non-limiting examples of a polyester include one or more of polylactic acid, polyglycolic acid, poly(hydroxybutyrate), poly(ϵ-caprolactone), poly(β-malic acid), poly(dioxanones), combinations thereof, or the like. Specific, non-limiting examples of a polyanhydride include one or more of poly(sebacic acid), poly(adipic acid), poly(terpthalic acid), combinations thereof, or the like. Specific, non-limiting examples of a polyamide include one or more of poly(imino carbonates), polyaminoacids, combinations thereof, or the like. Specific, non-limiting examples of a phosphorous-based polymer include one or more of a polyphosphate, a polyphosphonate, a polyphosphazene, combinations thereof, or the like.

In still further embodiments, the material used to prepare the matrix of the inventive composition can include a non-wetting agent. The material can be described in terms of a specific phase, such as being a liquid material in a single phase or a liquid material including a plurality of phases. In some embodiments, the liquid material includes, without limitation, one or more of multiple liquids, multiple immiscible liquids, surfactants, dispersions, emulsions, micro-emulsions, micelles, particulates, colloids, porogens, active ingredients, combinations thereof, etc.

Ocular iontophoresis delivering of an active agent to the eye of a subject

The present invention provides a method of delivering an active agent to the eye of a subject, comprising applying the active agent to the eye of the subject, wherein the active agent is molded alone or with one or more excipients into a

nanoparticle; and applying an electric current such that the nanoparticle is iontophoresed into the subject's eye, thereby causing the active agent to be delivered to the eye of the subject.

In one embodiment, the ocular iontophoresis system used in the methods of the present invention is a device selected in the group consisting of the devices disclosed in the following patents: U.S. Patent No. 4,141,359, issued February 27, 1979; U.S. Patent No. 4,250,878, issued January 17, 1981; U.S. Patent No. 4,301,794, issued November 24, 1981; U.S. Patent No. 4,747,819, issued April 31, 1988; U.S. Patent No. 4,752,285, issued June 21, 1988; U.S. Patent No. 4,915,685, issued April 10, 1990; U.S. Patent No. 4,979,938, issued December 25, 1990; U.S. Patent No. 5, 252,022, issued October 5, 1993; U.S. Patent No. 5,374,245, issued December 20, 1994; U.S. Patent No. 5,498,235, issued March 12, 1996; U.S. Patent No. 5,730,716, issued March 24, 1998; U.S. Patent No. 6,001,088, issued December 14, 1999; U.S. Patent No. 6,018,679, issued January 25, 2000; U.S. Patent No. Patent 6,139,537, issued October 31, 2000; U.S. Patent No. 6,148,231, issued November 14, 2000; U.S. Patent No. 6,154,671, issued November 28, 2000, and U.S. Patent No. 6,167,302, issued December 26, 2000, the entire contents of each of which are herein incorporated by reference.

In one embodiment, the ocular iontophoresis system used in the methods of the present invention is a device selected in the group consisting of the devices disclosed in the U.S. Patent No. 6,154,671, issued November 28, 2000, said device being characterized in that it comprises a reservoir configured to receive an aqueous solution, optionally buffered and including various electrolytes, and having an internal wall, an external wall, and an end wall bridging the internal wall and the external wall, the internal wall and the external wall being annular and having a free end configured to be applied to an eyeball, said device further comprising at least one active electrode arranged in the reservoir, a passive electrode and a current generator, wherein the at least one active electrode is a surface electrode arranged on an interior surface of the end wall and wherein the internal wall has an outer diameter that is configured to be at least equal to a predetermined diameter, whereby the predetermined diameter represents a diameter of a human cornea.

Ocular iontophoresis can be performed with a current between about -10 mA and about +10 mA for about 1 - 10 min. In one embodiment, the iontophoresis is performed for a duration of 10 minutes. In one embodiment, the nanoparticle is prepared as a suspension at a concentration between about 0.01 mg/mL and about 100 mg/mL. In one embodiment, the molded active agent is applied topically. In another embodiment, the molded active agent is injected. Transpalpebral iontophoresis can be performed with a current of about 1-5 mA for about 1-7 min; with a current of about 1-3 mA for about 3-6 min; or with a current of about 2 mA for up to about 5 min.

In another embodiment, a transpalpebral iontophoresis system is used in the methods of the present invention is a transpalpebral iontophoresis device such as the device disclosed in the US nonprovisional patent application USSN 10/492,494 (U.S. Pub. No. US 2004/0267188) filed on December 30, 2004 and as described herein.

Transpalpebral Iontophoresis Device of US Pub. No. US 2004/0267188 Useful in the System/Methods of the Present Invention

The device comprises a main electrode having an insulating layer and an adhesive layer able to bond the insulating layer to a conductive layer characterized in that the main electrode has an area able to come into contact with an eyelid. The transpalpebral iontophoresis can be an anionic or cationic iontophoresis, although cationic iontophoresis is preferred for transpalpebral iontophoresis. The main electrode is placed directly on the eyelid of the eye to be treated. It thus makes it possible to treat the area of the sclera that is the most permeable and has the least risk for vision, since there is no functional retina inside the eye around the cornea. Furthermore, the functional electrode does not come into direct contact with the eyeball. The device relies on the fact that the thickness of the skin at the eyelid is the smallest in the organism. Thus, the patient can use the device on their own without requiring the presence of a doctor, which is advantageous for iontophoreses of very long duration (up to 18 hours). This makes it possible to treat pathologies of the ocular adnexa and glands of the eyelids. Abnormalities of the palpebral glands are responsible for abnormalities or reduction of the quality of the tear film, and responsible for pathologies of the ocular surface.

The device for ocular application of an active substance can have at least one of the following characteristics: the main electrode is of oval overall shape; the oval shape of the electrode has a large external diameter equal at most to approximately 40 mm and a small external diameter equal at most to approximately 35 mm; the area has a non-functional central area surrounded by a functional peripheral area; the non-functional central area is of circular shape; the circular shape of the non-functional central area has a diameter equal at most to approximately 13 mm; the non-functional central area is a hole passing through the electrode; the main electrode is flexible; the main electrode has in addition a cutaneous adhesive layer; the main electrode has in addition a foam layer bonded to the conductive layer by a conductive adhesive layer; the foam layer is an absorbent layer able to act as a reservoir for the active substance; the insulating layer is a rigid casing; and the active substance is applied by iontophoresis.

An electrode comprising an insulating layer and an adhesive layer able to bond the insulating layer to a conductive layer, characterized in that it has a non-functional central area surrounded by a functional peripheral area able to come into contact with an eyelid, is also provided. There is also provided a method of ocular application of an active substance comprising steps of placing a medication reservoir comprising a main electrode on the eyelids, placing a return electrode on the tissues adjacent to the eyeball to be treated, and penetration through the eyelid of the active substance (*e.g.*, a molded nanoparticle or a nanoparticle "charged" with an active agent) under the effect of a current of energy circulating between the electrodes. The method can have one of the following characteristics: prior to the placing of the active electrode on the eyelids, the active substance is disposed under the eyelids; the main electrode has at least one of the aforementioned characteristics; the active substance is in a topical form (liquid, suspension, gel); the active substance is in the form of an insert.

With reference to FIG. 23, a description will be given of the device for ocular application of an active substance by iontophoresis according to the invention. The device **1** comprises a current generator **4** connected on the one hand to a return electrode **3** and on the other hand to a main electrode **2**. The current generator **4** can deliver, for example, a direct current between 0.5 mA and 5 mA, or

up to 10 mA, for a time between approximately 0.5 and approximately 30 minutes, perhaps even up to approximately 18 hours. According to the electrical resistance of the tissues forming the circuit, a resistance liable to change during the iontophoresis, the voltage delivered by the generator is adapted according to Ohm's law, $U = R \times I$, where U is the voltage in volts and R the total resistance of the circuit in ohms and I the chosen current in amperes. The voltage delivered by this current generator, however, can never exceed 80 V. The use can be envisaged of an alternating current generator so as to avoid an increase in pH under the effect of an oxidation-reduction phenomenon at the electrode, in particular in the case of lengthy treatment. The frequency range of these currents is chosen in order to allow a maximum increase in the permeability of the tissues of the active substance. In this particular case, the return electrode **3** is of electrocardiogram type and consisting of an adhesive and an Ag/AgCl film of low impedance. Finally, the generator can use a current profile having very high voltage peaks, between 50 V and 2500 V approximately, over very short durations of the order of 0.01 to 0.1 seconds, at low current (like those described for electroporation).

It is possible to use other modes making it possible to improve the permeability of biological membranes: magnetophoresis which uses magnetic fields, radiofrequency and microwave electromagnetic energy, or ultrasound energy. Preferably, a device according to the invention uses iontophoresis or electroporation.

With reference to FIG. 24, the overall shape of the main electrode **2** is oval. Its large external diameter corresponds substantially to the large diameter of the eye socket, that is approximately 40 mm. Similarly, its small external diameter corresponds to the small diameter of the socket, that is approximately 35 mm. These dimensions correspond to the standard adult size of the socket. Other sizes and shapes can be envisaged depending on the age and morphology of the patient to be treated. The electrode **2** has a central non-functional area **21**. This non-functional central area can be circular in shape with a diameter corresponding substantially to the diameter of the cornea, which is approximately 13 mm. The electrode can comprise a central through hole acting as a non-functional area **21**. The functional peripheral area surrounding the central non-functional area has two sub-areas **22** and **23**. The functional sub-area **22** is able to come opposite the surface of the sclera

situated around the cornea. Similarly, the functional sub-area **23** is able to cover the surface of the eyelid where the oculomotor muscle attachments are situated. This particular shape of the main electrode **2** makes it possible, during use, to cover the maximum surface area of the sclera around the cornea and the maximum surface area of the eyelid where the oculomotor muscle attachments are situated. It should be noted that the surface of the sclera situated around the cornea is the most permeable and presents the least risk for vision since there is no retina inside the eye around said cornea. In general terms the main electrode **2** is flexible. Thus, the main electrode **2** conforms to the eyelid when it is put in place so as to assume the shape of the palpebral tissues as closely as possible, allowing for good electrical contact with the eyelid.

In another embodiment depicted in FIG. 26, the electrode **2** can be rigid. In this case, it is in the form of a casing 320 whose internal face is coated with a flexible material 360 able to absorb anatomical differences so as to assume the shape of the palpebral tissues as closely as possible and thus allow good electrical contact.

FIG. 25 depicts, in section, the six layers of material possibly constituting the electrode, certain of these layers being optional as will be seen. Each of these layers of material has a precise function. The first layer **32** is an insulating layer. This is the part of the main electrode **2** that is able to be in contact with the operator. It makes it possible to insulate the rest of the electrode therefrom. This layer can be flexible and flat or else rigid and in the form of a casing. The layer **33** is an adhesive layer that provides bonding between the insulating layer **32** and a conductive layer **34**. Furthermore, this adhesive layer **33** makes it possible to hold on the conductive layer **34** an electric lead **31** connecting the main electrode **2** to the generator **4**.

The layer **34** is a conductive layer. This layer can consist of, for example, a silver film and a carbon film and has the function of distributing the electric current over the entire surface of the functional area of the main electrode **2**. The silver film of this conductive layer is situated facing the adhesive layer **33**. It allows good distribution of the current over the surface of the carbon film and provides an optimum electrical contact with the electric lead **31**. For its part, the carbon film is disposed facing an absorbent foam layer **36**. This carbon layer resists oxidation in an aqueous medium under a direct electric current. The most suitable material is

silver/carbon film with a thickness of 0.2 mm (Rexam conductive film, reference 2252, from Rexam Image Products).

The layer **36** is an optional layer. It is an absorbent foam layer able to be impregnated with the active substance or with a solution comprising the active substance before use. For this reason, this absorbent foam layer is highly absorbent and comprises pores of small dimensions on the order of 100 to 500 micrometers. Suitable materials include, for example, open-cell hydrophilic polyurethane foam of low density on the order of 0.05 to 0.1 g/cm^3 (Hydrocrest™ from Crest Foam Ind, Capu-cell® from TMP Technologies inc., Amrel® from Rynel, Medicell™ Foam from Hydromer). This layer is optional depending on whether the active substance is placed in the foam before use or placed directly under the eyelid before the electrode is put in place on the eyelid. This absorbent foam layer **36** is bonded to the carbon film of the conductive layer 34 by a conductive adhesive layer **35**. This conductive adhesive must not be soluble in water. Finally, the layer **37** is a cutaneous adhesive layer. This layer is optional depending on whether the main electrode is adhesive or not. The type of adhesive chosen must conduct electric current on the one hand and must allow the passage of the active substance whilst adhering as little as possible to the skin to allow for easy removal after use. This cutaneous adhesive layer can be situated on the absorbent foam layer if the latter is present or else directly on the carbon film of the conductive layer **34**.

One of the variant implementations consists of replacing the adhesive layer **33**, conductive layer **34** and conductive adhesive layer **35** by a single conductive adhesive layer (ARcare® 8881 from Adhesive Research Inc.), which fulfills all the aforementioned finctions.

One embodiment of the main electrode **2** is a flexible electrode comprising a cutaneous adhesive layer **37**, a conductive layer **34**, an adhesive layer **33** and an insulating layer **32**.

Another embodiment of the main electrode **2** is a flexible electrode comprising a cutaneous adhesive layer **37**, an absorbent foam layer **36**, a conductive adhesive layer **35**, a conductive layer **34**, an adhesive layer **33** and an insulating layer **32**.

Another embodiment of the main electrode **2** is a rigid electrode in the form of a casing comprising a cutaneous adhesive layer **37**, a foam layer **36** acting as a flexible material intended to absorb anatomical differences and assume the shape of the palpebral tissues as closely as possible during use, a conductive adhesive layer **35**, a conductive layer **34**, an adhesive layer **33** and a rigid insulating layer **32** forming the casing.

Another embodiment of the main electrode **2** is identical to the third embodiment described above, the foam layer being replaced by the absorbent foam layer **36**.

Within the context of the first and third embodiments of the main electrode **2** above, the operator, who can be a doctor or the patient himself, disposes the active substance or a solution comprising the active substance under the eyelid of the eye to be treated, and then places the main electrode **2** on the eyelid and the return electrode 3 on the adjacent tissues of the eyeball to be treated. The electrodes are next connected to the generator and the circuit assembly is powered according to a defined current and a defined application time. The electrodes are then removed.

In the case of the second and fourth embodiments of the main electrode **2** described above, the operator soaks the absorbent foam layer with the active substance or a solution comprising the active substance. The operator positions the main electrode on the eyelid of the eyeball to be treated and places the return electrode on the tissues surrounding the eyeball to be treated. Next the operator performs the same operations as described previously.

Many useful modifications can be made to the device. For example, the return electrode **3** disposed on the tissues adjacent to the eyeball can be connected to the main electrode **2** by means of a non-conductive film **50** and disposed on the temple of the patient to be treated as illustrated in FIGS. 27a and 27b. There can be two main electrodes **2** (one for each eye), disposed on a mask **100** (such as a sleeping mask used in air transport for example), the return electrode **3** associated with the generator (component **43**) can be placed on the forehead of the patient, and connected to the main electrodes by means of electric leads **31**, as illustrated, for example, in FIG. 28a. The mask **100** can be held on the head of the patient, for example, by elastic means such as, for example, a headband. In a variant illustrated

in FIG. 28b of the arrangement described previously, a return electrode and the generator (component **43**) can be placed inside the elastic headband for holding the mask 100 comprising the two main electrodes **2**, so that the return electrode is on one of the temples (or else both in a variant disposition where there are two return electrodes). Here, the elastic holding means also serves as an electric lead, which is implemented in the form of conductive tracks for example.

FIG. 28c illustrates a pair of spectacles **120** comprising two main electrodes **2** situated in place of the lenses of the pair of spectacles **120**. A generator **4** is installed on the frame of the pair of spectacles **120** and connected, on the one hand, to the two electrodes **2** and, on the other hand, to the return electrodes **30** that are situated beneath the main electrodes **2** so as to be in contact (whilst being insulated from these main electrodes) with the skin of the top of the cheeks of the patient or situated on the ends of the side pieces of the pair of spectacles **120** so as to be in contact with the skin situated behind the ears of the patient.

FIG. 29 illustrates another variant implementation in which the two main electrodes **2** are mounted on a cap **110**, as is the component **43** comprising the generator and the return electrode.

In the last four variants illustrated in FIGS. 28a, 28b, 28c and 29, the return electrode can be placed on the tissues of the face surrounding the eyeball such as the temples, the forehead, the cheeks, etc., in general terms. Furthermore, the generator can be associated therewith or not. It is possible, in variants, to place at a distance the generator connected to the main **2** and return **3** electrodes by means of electrical wires. In the contrary case, the connecting wires are integrated with the device in the form of conductive tracks for example. These four embodiments are well adapted for treating degenerative pathologies such as, for example, diabetic retinopathy, macular degeneration related to age, and pigmentary retinitis. For these pathologies, the probability that both eyes are affected is high (of the order of 50% of cases). In this case, it is necessary to treat both eyes at the same time or over one and the same time interval alternately.

EXEMPLIFICATION

The invention will now be described further in detail with respect to specific embodiments by way of examples, it being understood that these are intended to be

illustrative only and the invention is not limited to the materials, amounts, procedures and process parameters, etc. recited therein. All parts and percentages recited are by weight unless otherwise specified.

Example 1. PRINT manufactured particle performance by *in vitro* transport studies and *in vivo* studies.

In vitro transport study using Ussing chamber was performed with fluorescent-labeled cationic (+) and anionic (-) PEG hydrogel nanoparticles. The following batches of particles were tested. Particles: # 1, 2, and 3, and particles: # A and B.

Particle parameters:

Size / Shape: cylindrical, 200 × 200 nm

1 mg contains ~ 3.6×10^{16} particles

Size by DLS measurement:

Particle # 1 : 329 ± 6 nm

Particle # 2 : 298 ± 2 nm

Particle # 3 : 347 ± 6 nm

Zeta Potential:

Particle # 1 : + 36.7 ± 3.2 mV

Particle # 2 : + 48.4 ± 2.3 mV

Particle # 3 : - 47.8 ± 2.8 mV

In vitro study for particles # 2 and # 3.

The positively charged particle # 2 was tested in Na^+ citrate and K^+ acetate while the negatively charged particle # 3 was only tested in the citrate buffer. Samples were collected at t = 60 min.

pH variations, fluorescent microscopy, and fluorescent intensity measurements utilizing the fluorimeter were used to determine/analyze flux.

Results/Discussions:

No passive flux.

Only active at + 4 mA for particle # 2 and - 4 mA for particle # 3.

Fluorescent microscopy images suggest that particle # 2 penetrates into and all the way through the scleral tissue from the donor to the receptor end of the tissue. Images suggest that particle # 3 penetrates the tissue but aggregates inside and very

little was seen at the receptor end of the tissue. No fluorescence was detected in the solution of both receptor chambers when samples were measured using the fluorimeter. Further studies indicate that the particles aggregate on the return electrode.

In vivo studies were performed using rabbits. Each animal was dosed with a volume of 0.5 mL of particle suspension per eye. Samples were harvested immediately after dosing.

(a) Three white New Zealand rabbits were used for *in vivo* study of Particle # 1: Animal 1 was treated with 1 mg/mL particle suspension dosed as eye drops. Animal 2 was treated with 1 mg/mL particle suspension applied passively using the EyeGate® II applicator without current; 0 mA for 10 min (0 mA•min dose). Animal 3 was treated with 4 mg/mL particle suspension applied via anodic iontophoresis using the EyeGate® II applicator; +4 mA for 10 min (40 mA•min dose).

Results:

Particle # 1 was observed by fluorescent microscopy to penetrate into conjunctiva (FIGS. 6-9).

(b) Two white New Zealand rabbits were used for *in vivo* study of Particle # 2: Animal 1 was treated with 1 mg/mL particle suspension applied passively using the EyeGate® II applicator without current; 0 mA for 10 min (0 mA•min dose). Animal 2 was treated with 4 mg/mL particle suspension applied via anodic iontophoresis using the EyeGate® II applicator; +4 mA for 10 min (40 mA•min dose).

(c) Two white New Zealand rabbits were used for *in vivo* study of Particle # 3: Animal 1 was treated with 1 mg/mL particle suspension applied passively using the EyeGate® II applicator without current; 0 mA for 10 min (0 mA•min dose). Animal 2 was treated with 4 mg/mL particle suspension applied via cathodic iontophoresis using the EyeGate® II applicator; -4 mA for 10 min (40 mA•min dose).

Results/Discussion:

Particles penetrate the conjuctiva and can even be spotted in the lamina propria, which is the layer of tissue in between the conjuctiva and sclera. It appears as if some of the particles are aggregating. The large bright areas are aggregated particles (FIGS. 10-13).

The eye tissue in itself fluoresces at a wavelength similar to the wavelength that FITC fluoresces. Cy-5 dye and/or an active agent can be encapsulated in the next batch of particles to improve the image quality and take advantage of HPLC methods to determine distribution.

In vitro studies for fluorescent-labeled crosslinked PEG nanoparticles Batch # B under passive and active flux conditions. Particles were dispersed at 1 mg/mL in water. One EyeGate® II Device was used. It has a 3-mL Cup containing 10 mM NaCl.

Passive Flux was at 0 mA for 10 min. Active Flux was at +4 mA for 10 min.

Particle size, zeta potential, and fluorescent microscopy were analyzed.

Particle parameters:

Size / Shape: 200 × 200 nm, cylindrical

Size measurements: 209 ± 7 nm x 136 ± 1 nm (SEM); 342.6 ± 5 nm (DLS)

Zeta Potential: +19.8 ± 2.5 mV

Results/discussions:

(+) nanoparticles are iontophoretically mobile and a substantial number of particles is capable of being delivered using an unmodified EyeGate® II applicator. Count rate during particle measurements was low in both passive and active samples but was much lower for the active sample. Due to the interaction of the charged particles with the return electrode little or no particles are found in the receptor chamber solution.

Example 2. PRINT manufactured particle performance by *in vivo* studies.

In vivo studies were performed using rabbits.

PRINT® Nanoparticles

Fluorescently-labeled positively charged PRINT® nanoparticles were fabricated by Liquidia Technologies (Research Triangle Park, NC, USA) and supplied as dry powder. The particles were made of amine-modified crosslinked

polyethylene glycol (PEG) diacrylate polymer, and were labeled with fluorescein isothiocyanate (FITC). They featured a cylindrical shape and uniform size distribution. Scanning electron microscopy images of PRINT® nanoparticles are shown in FIG. 19. Additional exemplary illustrations of PRINT® nanoparticles are available for review on Liquidia's corporate website.

The particles were primarily characterized upon fabrication by their physical dimensions, 210 ± 10 nm (dia.) and 170 ± 7 nm (length), obtained by scanning electron microscopy (SEM) in a dry state, and by their hydrodynamic diameter (376 ± 17 nm) and zeta potential (+31 ± 0.8 mV) determined by differential light scattering (DLS) and electrophoretic light scattering (ELS) techniques, respectively, in a wet state (aqueous suspension). FITC was chemically conjugated to the PEG through a UV-active double bond. Examination of labeled particles with similar compositions by confocal microscopy (high powered, low detection limit systems) confirmed the lack of any residual FITC (unreacted) and ensured that the fluorescence is limited to the particles.

Prior to iontophoresis, the nanoparticles were dispersed in purified water (injection grade, WFI). The dispersion was sonicated for 30 min and then vortexed for 30 min to ensure the uniformity of the dispersion before use. Aliquots of the suspended nanoparticles diluted to 0.1 and 0.05 mg/mL were used for size distribution and zeta potential analysis, respectively. Nanoparticles were analyzed by DLS and ELS techniques using a Zetasizer Nano ZS90 instrument (Malvern Instruments, Westborough, MA) in the laboratories of EyeGate Pharma.

Animals

Female New Zealand White rabbits weighing 2.5 to 3.0 kg (Millbrook Breeding Labs, Amherst, MA) were used. Animals were acclimated for at least 3 days before the study was initiated. The experiments were performed under identical environmental conditions. All procedures were performed with adherence to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with approval of the Institutional Animal Care and Use Committee (IACUC).

Iontophoretic Delivery System

The iontophoretic delivery system consisted of an annular shaped device (EyeGate Pharmaceuticals, Waltham, MA, USA), a 1.5" × 2" Tricot rectangular snap return electrode (Pepin Manufacturing Inc., Lake City, MN, USA), a Yokagawa GS610 low-voltage DC generator (Yokogawa Corp., Newnan, GA, USA), and electrical wires and connectors. Two variations of the applicator, hereinafter referred to as Type I and Type II, were utilized in this study. A schematic simplified diagram of the ocular applicator is shown in FIG. 20. The applicators were similar in their basic design but Type II contained an additional layer of agarose hydrogel overlaying the device electrode in the reservoir section. The agarose hydrogel was prepared from a 3% pregel solution of agarose (Agarose for electrophoresis, Sigma-Aldrich, St. Louis, MO, USA) containing 1M NaCl. The reservoir of Type I applicator could hold approximately 500 μL of the nanoparticle dispersion, while the reservoir of Type II applicator could hold about 250 μL of the nanoparticle dispersion. The applicators and the return electrodes were disposable and discarded after each treatment.

Iontophoretic Treatment Protocol

The setup for iontophoretic delivery of nanoparticles in New Zealand rabbit eyes is shown in FIG. 21. Iontophoretic dosing was performed on animals anesthetized by intramuscular injection of xylazine (5 mg/kg; IVX Animal Health Inc., St. Joseph, MO) and ketamine (35 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA). Once the animal was anesthetized, the return electrode was placed on the animal's left ear and connected to the generator. The ocular applicator was then loaded with the corresponding nanoparticle suspension and visually inspected to ensure complete hydration of the foam insert inside the reservoir. A drop of topical anesthetic (0.5% proparacaine hydrochloride; Bausch and Lomb; Rochester, NY) was applied to each eye. The loaded ocular applicator was first placed on the animal's right eye and connected to the generator. After applying the corresponding iontophoretic dose the applicator was removed from the right eye and the process was repeated on the left eye (and right ear) with a new device and return electrode. The iontophoretic dose represents the cumulative electrical charge carried over time ('x' mA for 'y' min).

Passive dosing was performed on one animal by placing the ocular applicator loaded with the corresponding nanoparticle suspension on each eye for the same amount of time as iontophoretic delivery but without the application of any current. A new device and return electrode were used for each treatment.

Animals were euthanized immediately after dosing of the left eye, or 24 hours post-treatment by intravenous overdose of Euthasol® (sodium pentobarbital; Virbac Inc., Fort Worth, TX). Ocular tissues were immediately harvested and processed.

Iontophoresis of PRINT® Nanoparticles

New Zealand White rabbits were treated with a single transscleral anodal iontophoretic dose (20 mA•min at +4 mA for ~ 5 min) of PRINT® nanoparticles (1 mg/mL dispersion in WFI) into each eye (Table 1) using Type I (Study # 1) or Type II applicator (Study # 2). Control animals received passive dosing by placing the nanoparticle-containing applicator (Type II) on each eye for 5 min without the application of any current (Study # 2). Animals were sacrificed immediately after dosing of the second eye and ocular tissues were processed for fluorescence microscopic imaging.

Table 1. Experimental Conditions for Transscleral Iontophoresis of Nanoparticles in New Zealand White Rabbits [a]

Study No.	Nanoparticles	Dispersion Conc. (mg/mL)	Dispersant	Device	Current (mA)	Time (min)	No. Animals
1	PRINT®, FITC-PEG	1	WFI[a]	Type I	+4	5	1
2	PRINT®, FITC-PEG	1	WFI	Type II	+4	5	1
					0	**5**	**1**

[a]WFI: Water for injection

[b]Maximum current applied in iontophoresis with ascending profile

pH Measurements

The pH of nanoparticle suspension remaining in the applicator reservoir after dosing was measured by using a Hydrion Insta-Chek mechanical pH pencil (Micro Essential Laboratory, Inc., Brooklyn, NY, USA).

Fluorescence Microscopy and Imaging

Enucleated eyes were hemisected, lens and vitreous humor were removed, and the remaining eye tissue was placed in a Peel-A-Way® Embedding Mold, 22 mm wide × 30 mm long × 20 mm deep (Polysciences Inc., Warrington, PA, USA) filled with Tissue-Tek® 4583 O.C.T.™ cutting compound (Sakura Finetek Europe B.V., Netherlands). The tissue was oriented such that the cut surface would be face down in the block allowing for a cross section of the eye to be cut. The tissue embedded in the O.C.T.™ was then placed in liquid nitrogen and snap frozen until ~3/4 of the O.C.T.™ was frozen solid. The block was then placed on dry ice to allow the block to finish solidifying while the second half of the eye was frozen in liquid nitrogen. Frozen eyes were stored at -20°C until sectioning.

Just prior to sectioning, blocks were transferred to a Leica Jung CM3000 Cryostat (Leica Microsystems, Bannockburn, IL) and allowed to equilibrate to the temperature within the cutting chamber. All blocks were cut at a temperature of approximately -20°C. Sections were about 10 microns thick and 2 serial sections were placed on each slide. Ten sections (100 microns of tissue) were discarded between each pair picked up on slides. Slides used were VWR® Superfrost® Plus micro slides (VWR International, West Chester, PA, USA). Slides were placed in a 25 count slide box in the Cryostat until sectioning was finished. The slide box was then stored at -20°C until imaged. All eye halves from this experiment were sectioned and then examined. Slides were individually removed from the slide box stored at -20°C and quickly warmed to room temperature. A small amount of ProLong® Gold antifade reagent with DAPI (Invitrogen, Carlsbad, CA, USA) was placed on the sections and a coverslip placed over the sections and sealed with nail polish. The sections were then examined using a Leica DMRXA upright epifluorescent microscope and representative images taken with a Hamamatsu C4742-95 CCD camera (Bridgewater, NJ, USA) using Metamorph® software (Molecular Devices, Ver 7.1.0.0). Composite images and modifications for

presentation were made using Adobe® Photoshop® CS2 (Ver 9.0.2) and Microsoft® Office PowerPoint® 2003 (Ver 5.1.2600).

Characterization of Nanoparticles

Particle characteristics of FITC-labeled PEG PRINT® nanoparticles including hydrodynamic diameter, polydispersity index, and zeta potential are summarized in Table 2.

Table 2. Nanoparticles Characterization Data

Study No.	Nanoparticles	Hydrodynamic Diameter (nm)	PdI [b]	Zeta Potential [c] (mV)
1 & 2	PRINT®, FITC-PEG [a]	328	0.074	+29

[a] Dry particle dimensions: 210 ± 10 nm (dia.) x 170 ± 7 nm (length)

[b] PdI: Polydispersity Index

[c] Samples prepared in WFI (water for injection)

Iontophoresis of PRINT® Nanoparticles

FITC-labeled positively charged PEG PRINT® nanoparticles (1 mg/mL dispersion) were dosed into the New Zealand White rabbits' eyes by using annular shaped ocular applicators, identified as Type I and Type II. The animals received a single anodal iontophoretic dose of 20 mA•min (+4 mA for ~ 5 min) on each eye (studies # 1 and 2, Table 1) and euthanized immediately following the treatment of both eyes. Nanoparticles were mainly detected in the conjunctival layer of the eyes that received an iontophoretic treatment (FIGS. 4a and 4a). These nanoparticles appeared as masses of particles deposited at various depths across the conjunctiva. In comparison, only a limited number of particles (or small colonies of particles) were observed in the eyes treated under passive (no current applied) condition (FIG. 4c). Both iontophoretic applicators (Type I and Type II) were suitable for nanoparticle delivery, however, based on fluorescent intensity, there were substantially larger numbers of nanoparticles delivered by Type II applicators as compared to Type I.

During iontophoresis the pH of nanoparticle suspension in the applicators remained unchanged at about 6-7 for Type II applicator and decreased to about 2-3 for Type I applicator.

EQUIVALENTS

Although the present invention has been described in considerable detail with reference to certain preferred versions thereof, other versions are possible. Therefore, the spirit and scope of the appended claims should not be limited to the description of the preferred versions contained herein. All references are herein incorporated by reference in their entireties.

CLAIMS

What is claimed is:

1. A method of delivering an active agent to the eye of a subject, comprising applying the active agent to the eye of the subject, wherein the active agent is molded alone or with one or more excipients into a nanoparticle; and applying an electric current such that the nanoparticle is iontophoresed into the subject's eye, thereby causing the active agent to be delivered to the eye of the subject.

2. The method of Claim 1, wherein the electric current is between about -10 mA and about +10 mA.

3. The method of Claim 1, wherein the iontophoresis is performed for a duration of 10 minutes.

4. The method of Claim 1, wherein the molded nanoparticle is prepared as a suspension at a concentration between about 0.01 mg/mL and about 100 mg/mL.

5. The method of Claim 1, wherein the molded nanoparticle comprises a charged nanoparticle having a zeta potential between about -60mV and about +60mV.

6. The method of Claim 1, wherein the electric current is between about -10 mA to about +10 mA.

7. A molded nanoparticle comprising an active agent and one or more excipients, wherein the active agent is useful for treating one or more ocular conditions, and wherein the molded nanoparticle is capable of releasing the active agent in a desired tissue of a subject.

8. The molded nanoparticle of Claim 7, wherein the molded nanoparticle is delivered to the desired tissue by iontophoresis.

9. A system for treating an ocular condition, comprising:

a) a device comprising a first reservoir configured and dimensioned to be positioned adjacent to an eye of a subject and deliver a controlled electric current to the eye; and

b) a plurality of charged nanoparticles within the first reservoir wherein each nanoparticle comprises a predetermined three dimensional shape and an active agent,

whereby delivering the controlled electric current to the eye drives the plurality of nanoparticles from the first reservoir into tissue of the eye.

10. The system of Claim 9, wherein the charged nanoparticle further comprises a biodegradable polymer or a polyelectrolyte.

11. The system of Claim 10, wherein the biodegradable polymer is selected from the group consisting of: chitosan, polylacticcoglycolic acid, polyvinylalcohol, crosslinked cationic polyethyleneglycol and crosslinked anionic polyethyleneglycol.

12. The system of Claim 10, wherein the biodegradable polymer comprises a synthetic polyelectrolyte and a polar polymer selected from the group consisting of: poly(acrylic acid), poly(styrene sulfonate), carboxymethylcellulose, poly(vinyl alcohol), poly(ethylene oxide), poly(vinyl pyrrolidone), dextran, and the like, reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, branched polyethylene glycols, combinations thereof, poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone), and combinations thereof.

13. The system of Claim 10, wherein the biodegradable polymer comprises poly(lactic-co-glycolic acid) and polylactic acid.

14. The system of Claim 10, wherein the biodegradable polymer comprises a compound selected from the group consisting of poly(vinyl pyrrolidinone), reactive oligomeric poly(vinyl pyrrolidinone), poly(ethylene glycol), protected polyvinyl alcohol, poly(dimethyl amino ethyl methacrylate), hydroxyethyl acrylate, hydroxyethyl methacrylate, branched polyethylene glycols, and combinations thereof.

15. The system of Claim 10, wherein the biodegradable polymer comprises a compound selected from the group consisting of poly(beta-aminoesters), poly(lactic-co-glycolic acid), polylactic acid, poly(caprolactone).

16. The system of Claim 10, wherein the biodegradable polymer comprises one or more monomeric subunits selected from the group consisting of: butadienes, styrenes, propene, acrylates, methacrylates, vinyl ketones, vinyl esters, vinyl acetates, vinyl chlorides, vinyl fluorides, vinyl ethers, vinyl pyrrolidone, acrylonitrile, methacrylnitrile, acrylamide, methacrylamide allyl acetates, fumarates, maleates, ethylenes, propylenes, tetrafluoroethylene, ethers, isobutylene, fumaronitrile, vinyl alcohols, acrylic acids, amides, carbohydrates, esters, urethanes, siloxanes, formaldehyde, phenol, urea, melamine, isoprene, isocyanates, expoxides, bisphenol A, chlorsianes, dihalides, dienes, alkyl olefins, ketones, aldehydes, vinylidene chloride, anhydrides, saccharide, acetylenes, naphthalenes, pyridines, lactams, lactones, acetals, thiiranes, episulfide, peptides, derivatives thereof, and combinations thereof.

17. The system of Claim 10, wherein the biodegradable polymer comprises one or more monomeric subunits selected from the group consisting of: cationically charged [2-(acryloyloxy)ethyl]trimethyl ammonium chloride, 2-aminoethyl methacrylate hydrochloride, anionically charged monomers, and combinations thereof.

18. The system of Claim 10, wherein the polyelectrolyte is selected from the group consisting of: crosslinked cationic poly allylamine hydrochloric acid and anionic polystyrene sodium sulfonate.

19. The system of Claim 9, wherein the nanoparticle further comprises a surfactant.

20. The system of Claim 9, wherein the active agent is selected from the group consisting of an anti-inflammatory agent, an anti-viral agent, an anti-neovascularization agent, and an antibiotic agent.

21. The system of Claim 9, wherein the nanoparticle further comprises a composition selected from the group consisting of: targeting ligands (activating and/or inactivating), enzyme inhibitors, allosteric modulators, oligonucleotides, bioprocess activators, cell-targeting peptides, cell-penetrating peptides, integrin receptor peptides, hormones, antibodies, vitamins, viruses, polysaccharides, cyclodextrins, liposomes, proteins, fluorescent tags, imaging agents, and combinations thereof.

22. The system of Claim 9, wherein the nanoparticle has a hydraulic diameter between about 1 nm and about 1000 nm.

23. The system of Claim 9, wherein the nanoparticle has a hydraulic diameter between about 200 nm and about 400 nm.

24. The system of Claim 9, wherein the nanoparticle has a hydraulic diameter between about 50 nm and about 400 nm.

25. The system of Claim 9, wherein the nanoparticle has a zeta potential between about -60 mV and about +60 mV.

26. The system of Claim 9, wherein the predetermined three dimensional shape is selected from the group consisting of cylinder, trapezoid, bar, cone, boomerang, and arrow.

27. A method for delivering an active agent to the eye of a subject, comprising:

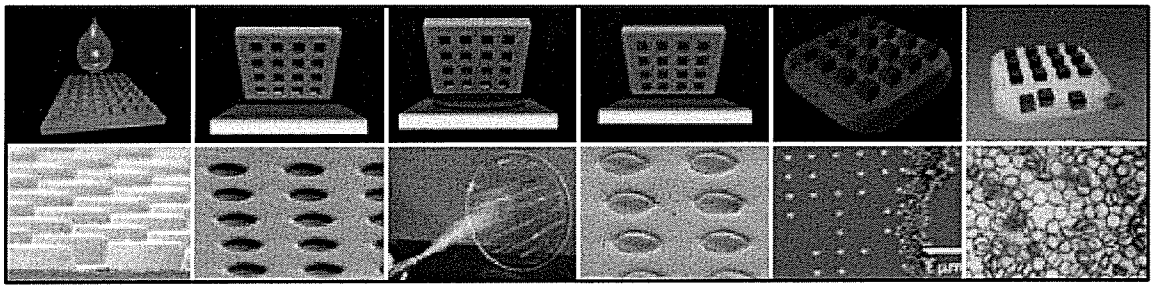
a) positioning a delivery device comprising a reservoir adjacent to an eye of a subject, wherein the delivery device is configured to deliver a controlled electric current to the eye, wherein the reservoir is configured and dimensioned to house a plurality of charged nanoparticles, and wherein the charged nanoparticles comprise a predetermined three dimensional shape and an active agent;

b) applying a controlled current to the eye through the device; and

c) iontophoretically delivering the charged nanoparticles into tissue of the eye.

28. The method of claim 27, wherein the electric current is applied at between about -10 mA and about +10 mA.

29. The method of claim 27, wherein the charged nanoparticles are iontophoretically delivered to the eye for between one minute and about ten minutes.



STEP 1: FORM FLUOROCUR™ MOLD

STEP 2: APPLY MATERIAL TO BE MOLDED

STEP 3: SOLIDIFY PARTICLES & REMOVE MOLD

STEP 4: HARVEST PARTICLES INTO FINAL FORM

FIG. 1

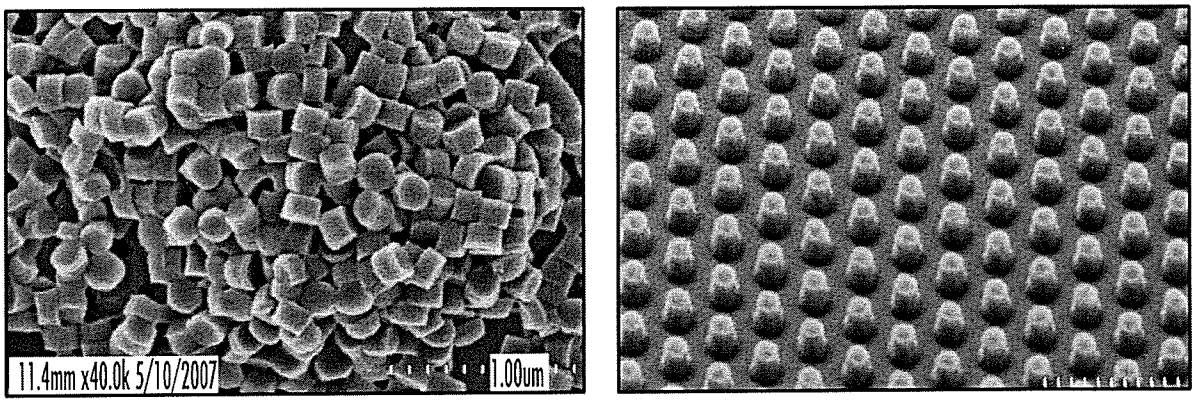


FIG. 2

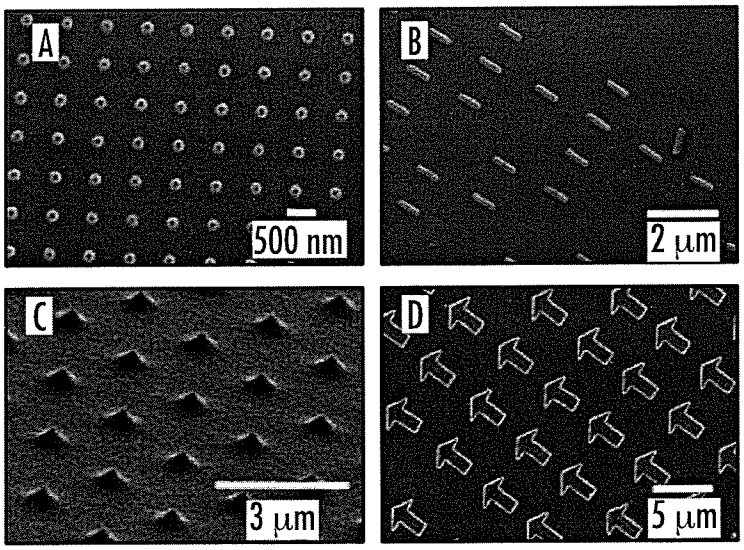


FIG. 3

FIG. 4

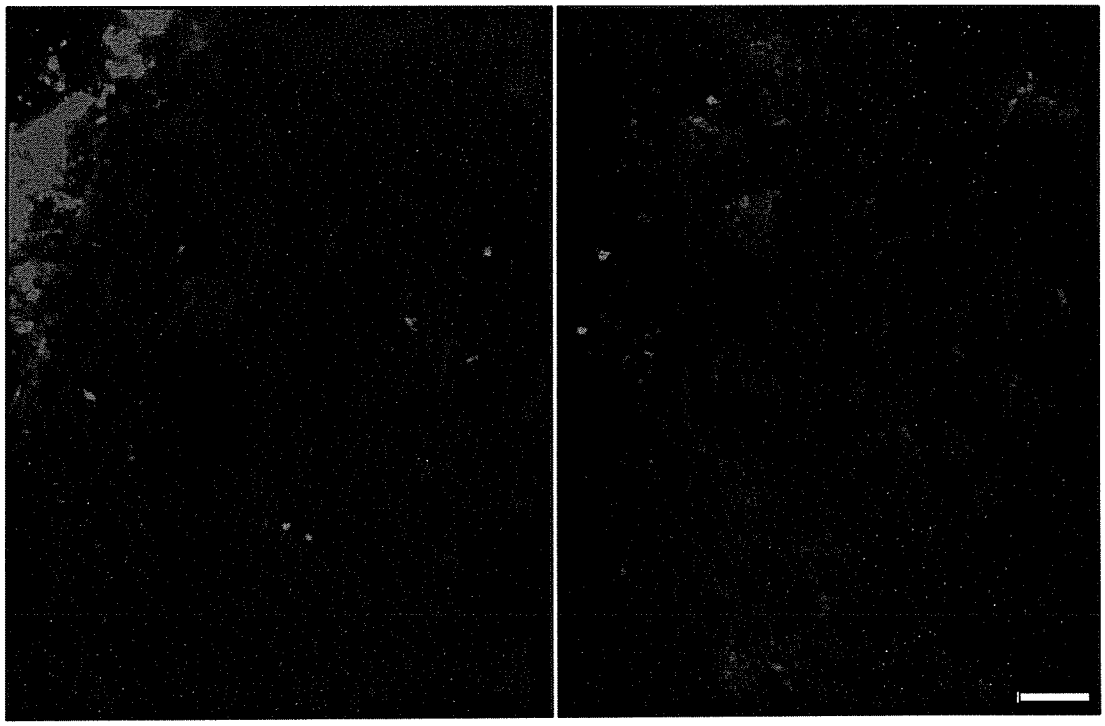
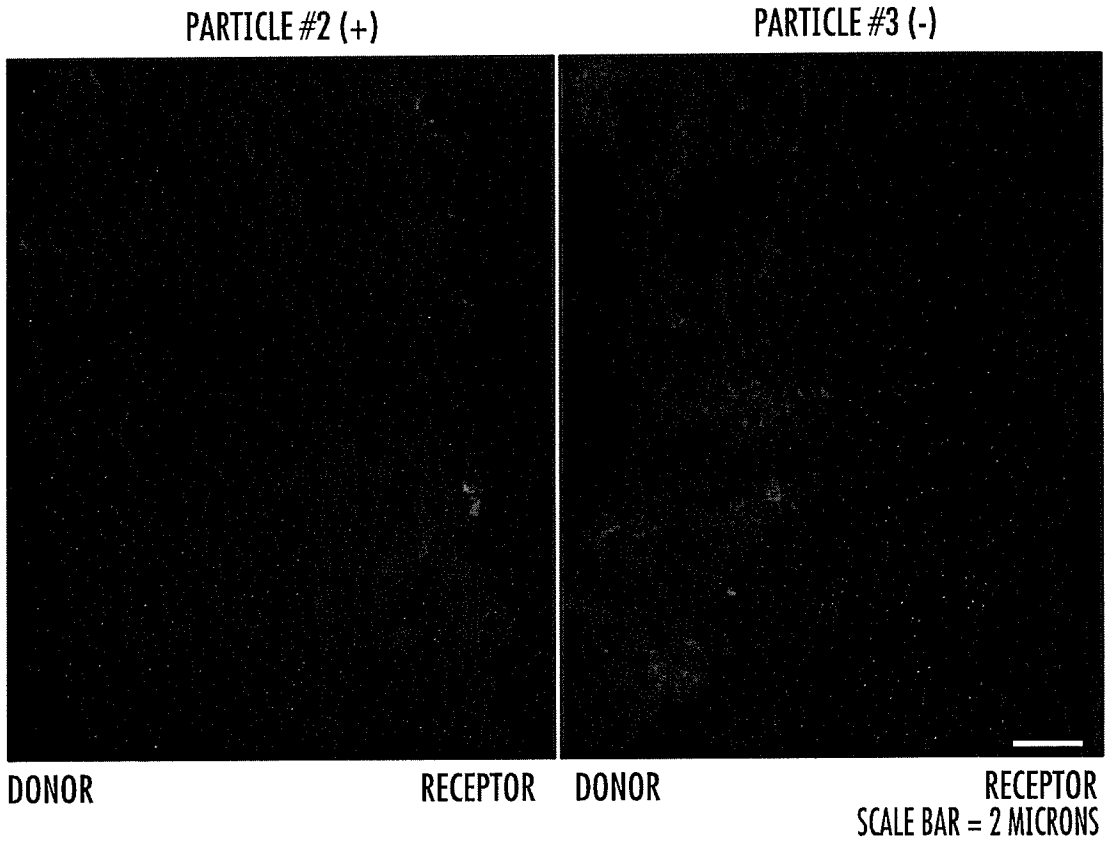

PARTICLE #2 (+)
PARTICLE #3 (-)
DONOR
RECEPTOR
DONOR
RECEPTOR
SCALE BAR = 2 MICRONS

FIG. 5


PARTICLE #2 (+)
PARTICLE #3 (-)
DONOR
RECEPTOR
DONOR
RECEPTOR
SCALE BAR = 2 MICRONS

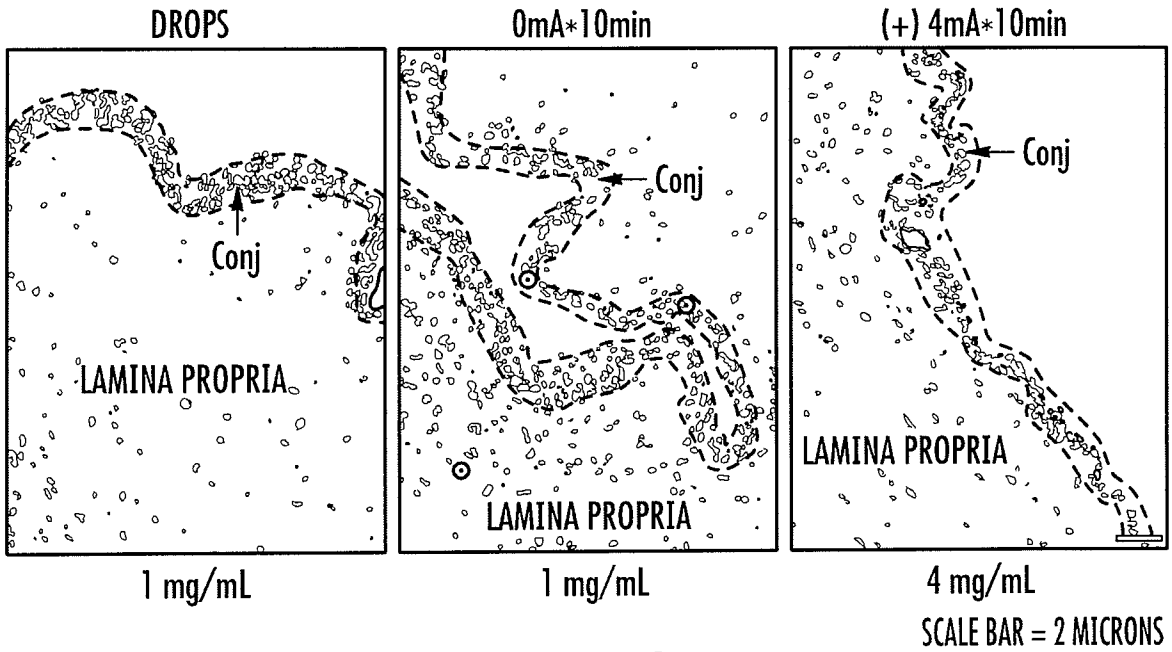

DROPS
0mA*10min
(+) 4mA*10min
Conj
LAMINA PROPRIA
Conj
LAMINA PROPRIA
Conj
LAMINA PROPRIA
1 mg/mL
1 mg/mL
4 mg/mL
SCALE BAR = 2 MICRONS

FIG. 6

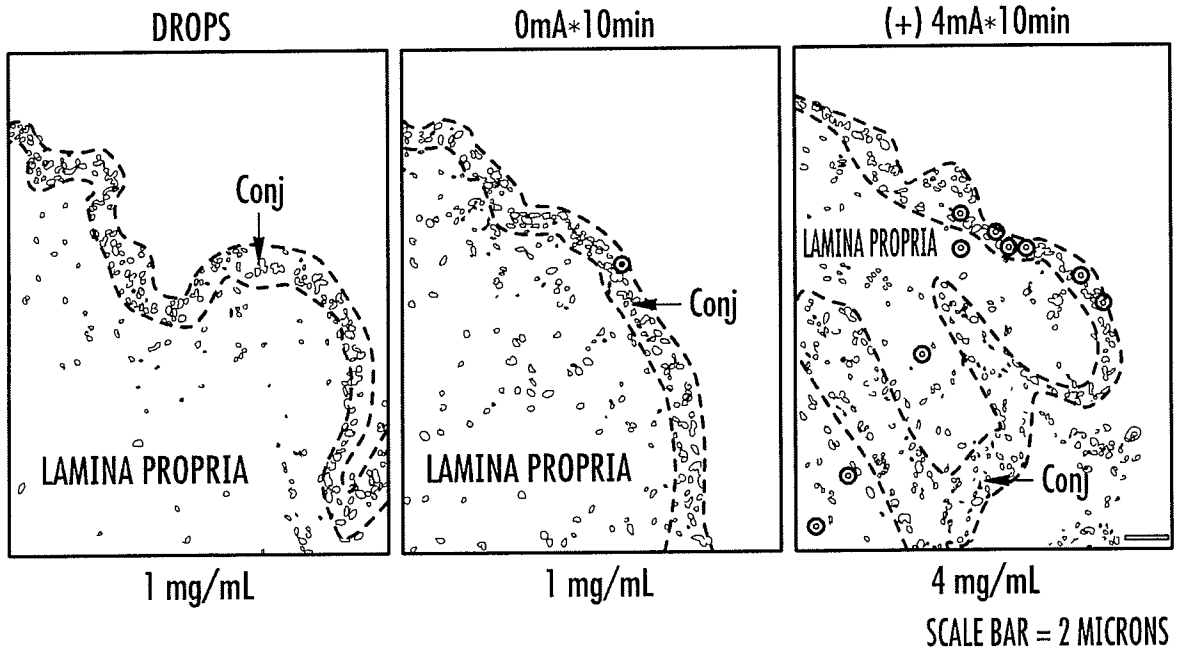
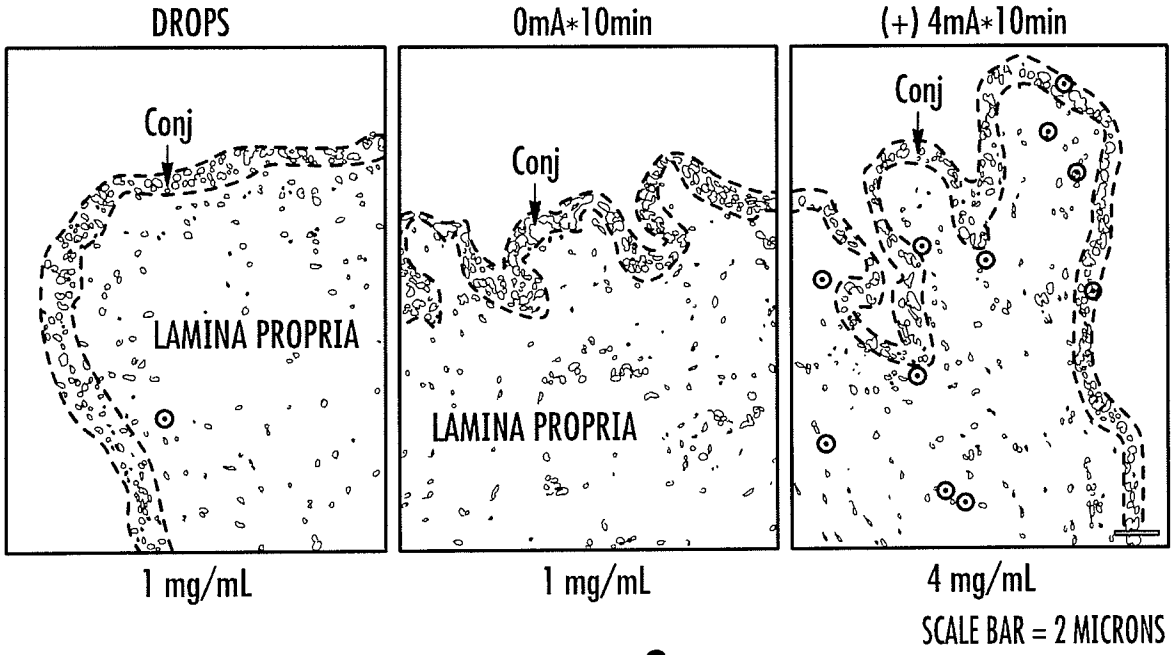

DROPS
0mA*10min
(+) 4mA*10min
Conj
LAMINA PROPRIA
Conj
LAMINA PROPRIA
LAMINA PROPRIA
Conj
1 mg/mL
1 mg/mL
4 mg/mL
SCALE BAR = 2 MICRONS

FIG. 7


DROPS
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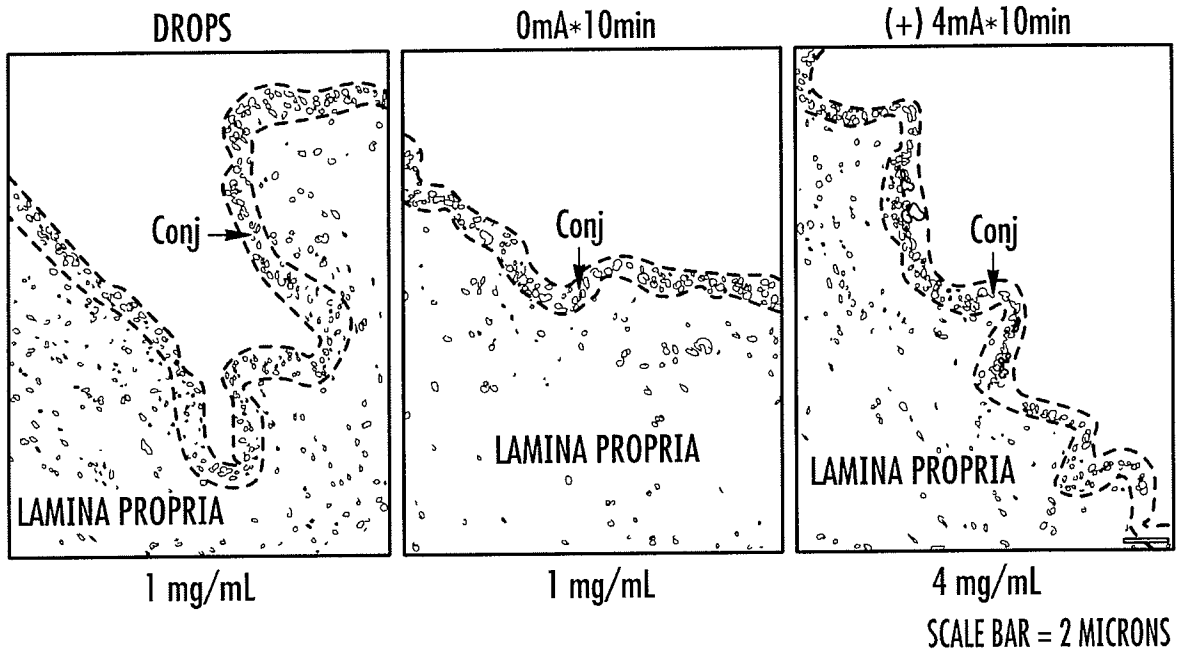
(+) 4mA*10min
Conj
Conj
Conj
LAMINA PROPRIA
LAMINA PROPRIA
1 mg/mL
1 mg/mL
4 mg/mL
SCALE BAR = 2 MICRONS

FIG. 8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(+) 4mA*10min
Conj
Conj
Conj
LAMINA PROPRIA
LAMINA PROPRIA
LAMINA PROPRIA
1 mg/mL
1 mg/mL
4 mg/mL
SCALE BAR = 2 MICRONS

FIG. 9

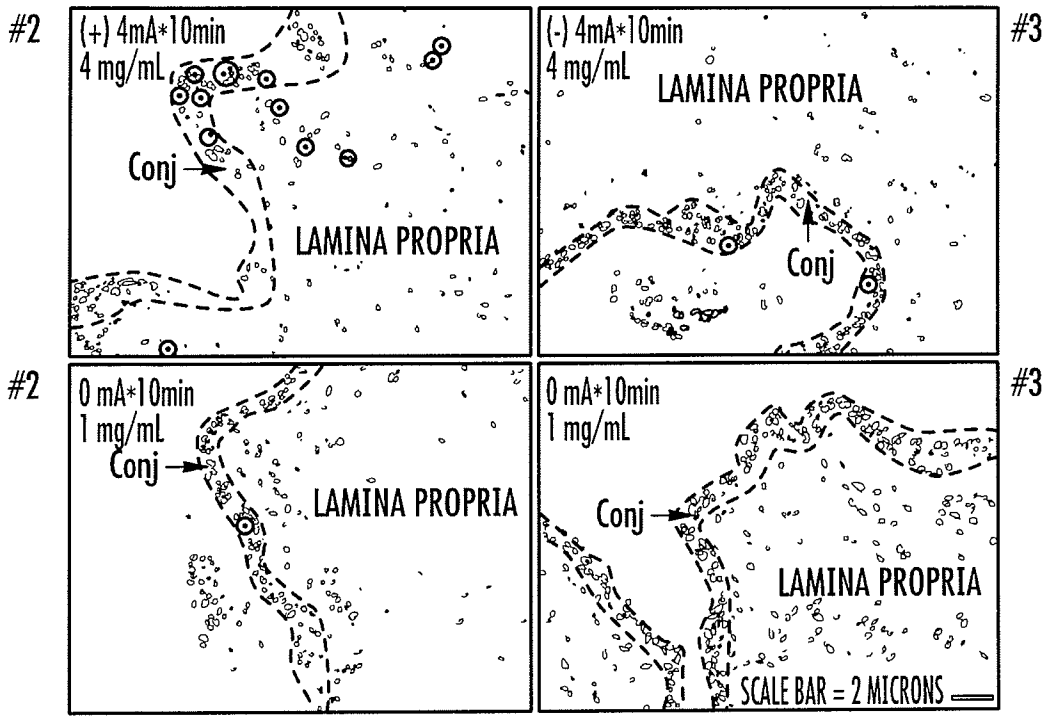
#2
(+) 4mA*10min
4 mg/mL
Conj
LAMINA PROPRIA
#3
(-) 4mA*10min
4 mg/mL
LAMINA PROPRIA
Conj
#2
0 mA*10min
1 mg/mL
Conj
LAMINA PROPRIA
#3
0 mA*10min
1 mg/mL
Conj
LAMINA PROPRIA
SCALE BAR = 2 MICRONS

FIG. 10

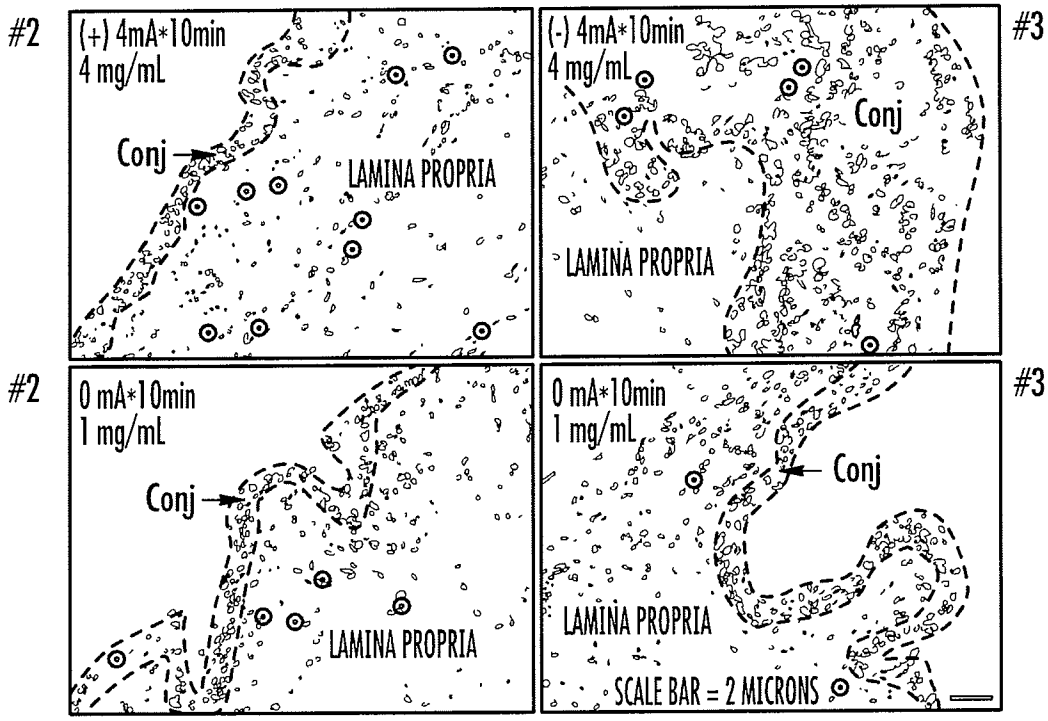
#2
(+) 4mA*10min
4 mg/mL
Conj
LAMINA PROPRIA
#3
(-) 4mA*10min
4 mg/mL
Conj
LAMINA PROPRIA
#2
0 mA*10min
1 mg/mL
Conj
LAMINA PROPRIA
#3
0 mA*10min
1 mg/mL
Conj
LAMINA PROPRIA
SCALE BAR = 2 MICRONS

FIG. 11

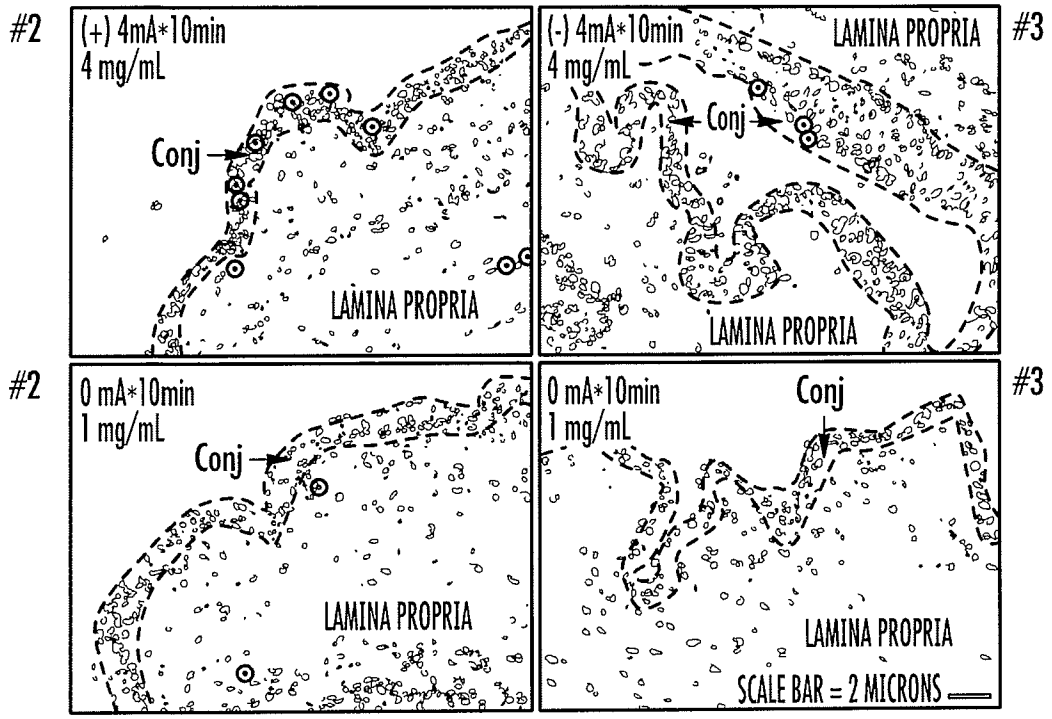
#2
(+) 4mA*10min
4 mg/mL
Conj
LAMINA PROPRIA
(-) 4mA*10min
4 mg/mL
LAMINA PROPRIA
#3
Conj
LAMINA PROPRIA
#2
0 mA*10min
1 mg/mL
Conj
LAMINA PROPRIA
0 mA*10min
1 mg/mL
Conj
#3
LAMINA PROPRIA
SCALE BAR = 2 MICRONS

FIG. 12

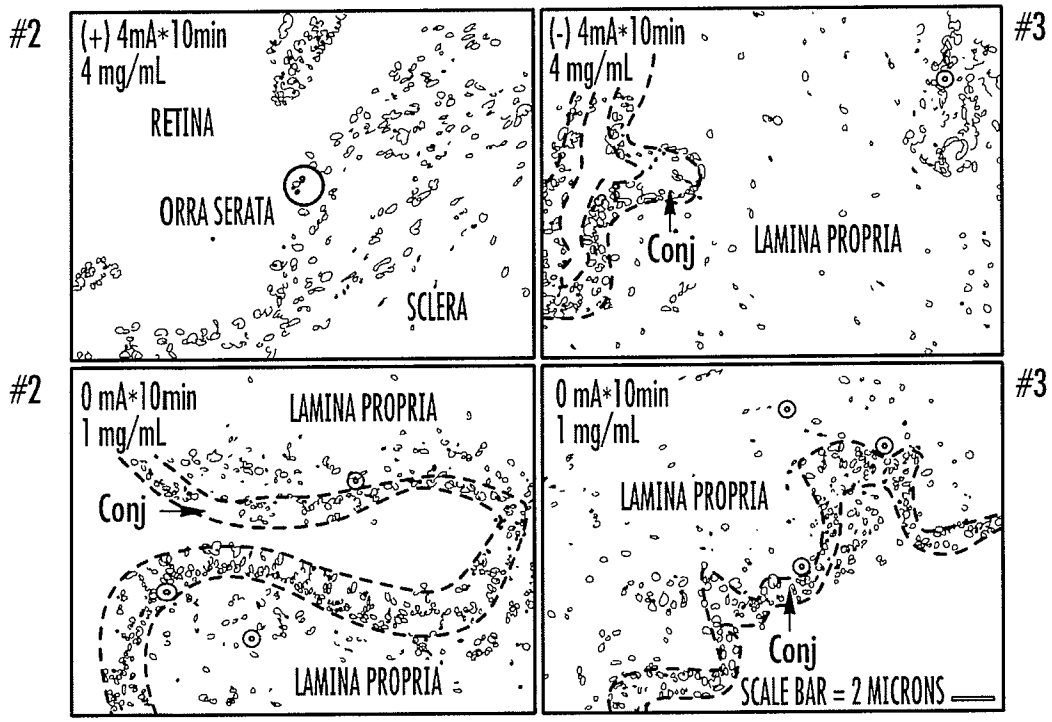
#2
(+) 4mA*10min
4 mg/mL
RETINA
ORRA SERATA
SCLERA
(-) 4mA*10min
4 mg/mL
#3
Conj
LAMINA PROPRIA
#2
0 mA*10min
1 mg/mL
LAMINA PROPRIA
Conj
LAMINA PROPRIA
0 mA*10min
1 mg/mL
#3
LAMINA PROPRIA
Conj
SCALE BAR = 2 MICRONS

FIG. 13

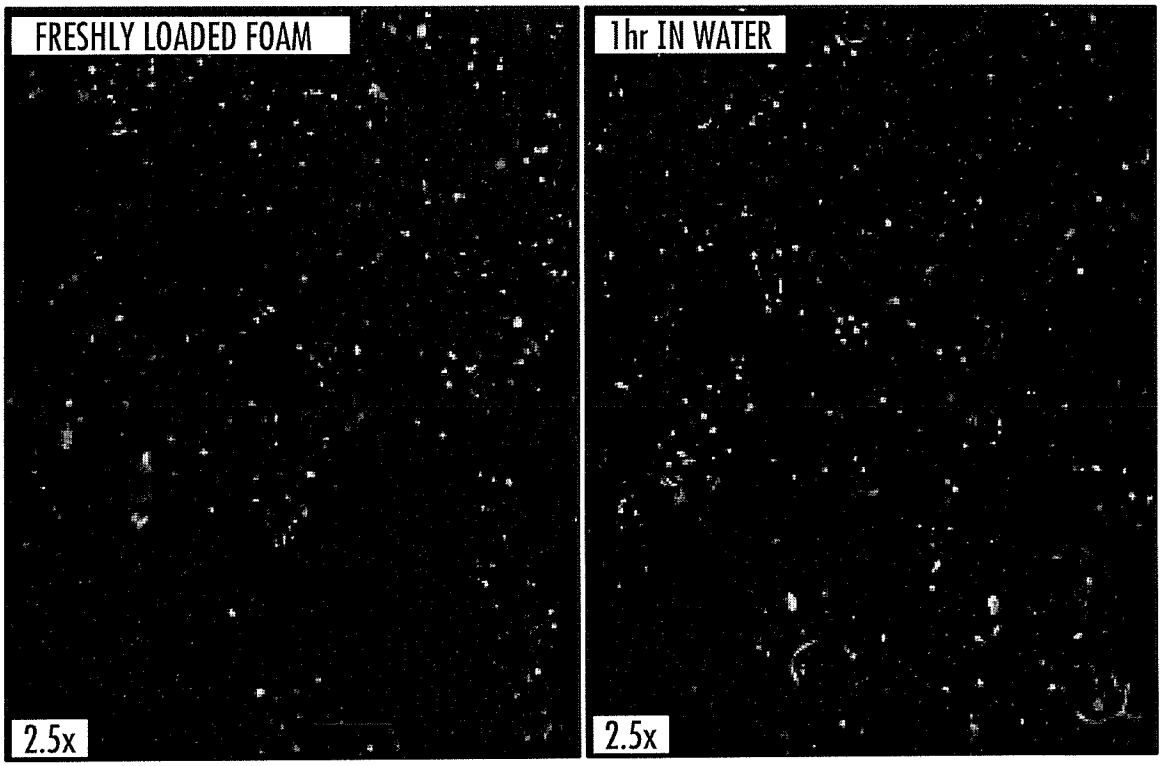

FIG. 14

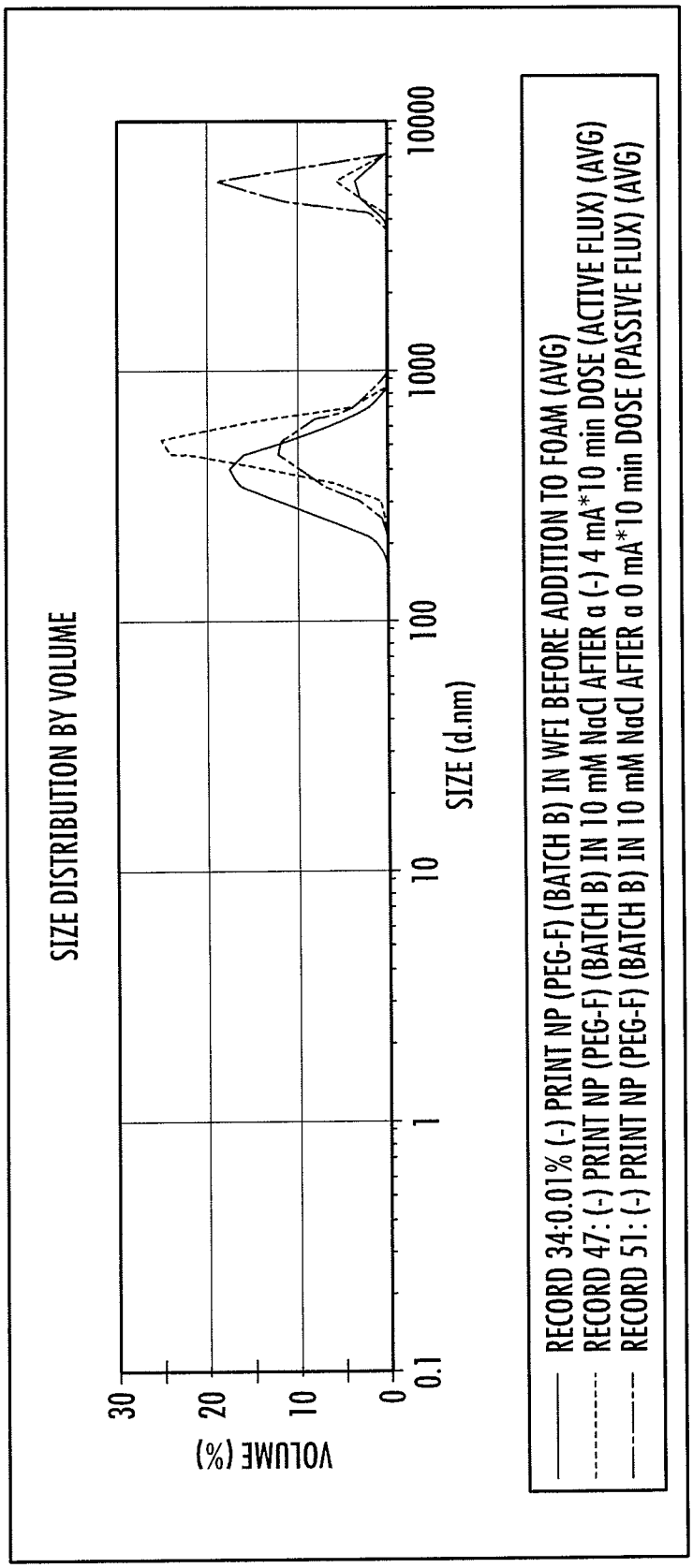
SIZE DISTRIBUTION BY VOLUME
VOLUME (%)
0
10
20
30
0.1
1
10
100
1000
10000
SIZE (d.nm)
RECORD 34:0.01% (-) PRINT NP (PEG-F) (BATCH B) IN WFI BEFORE ADDITION TO FOAM (AVG)
RECORD 47: (-) PRINT NP (PEG-F) (BATCH B) IN 10 mM NaCl AFTER α (-) 4 mA*10 min DOSE (ACTIVE FLUX) (AVG)
RECORD 51: (-) PRINT NP (PEG-F) (BATCH B) IN 10 mM NaCl AFTER α 0 mA*10 min DOSE (PASSIVE FLUX) (AVG)

FIG. 15

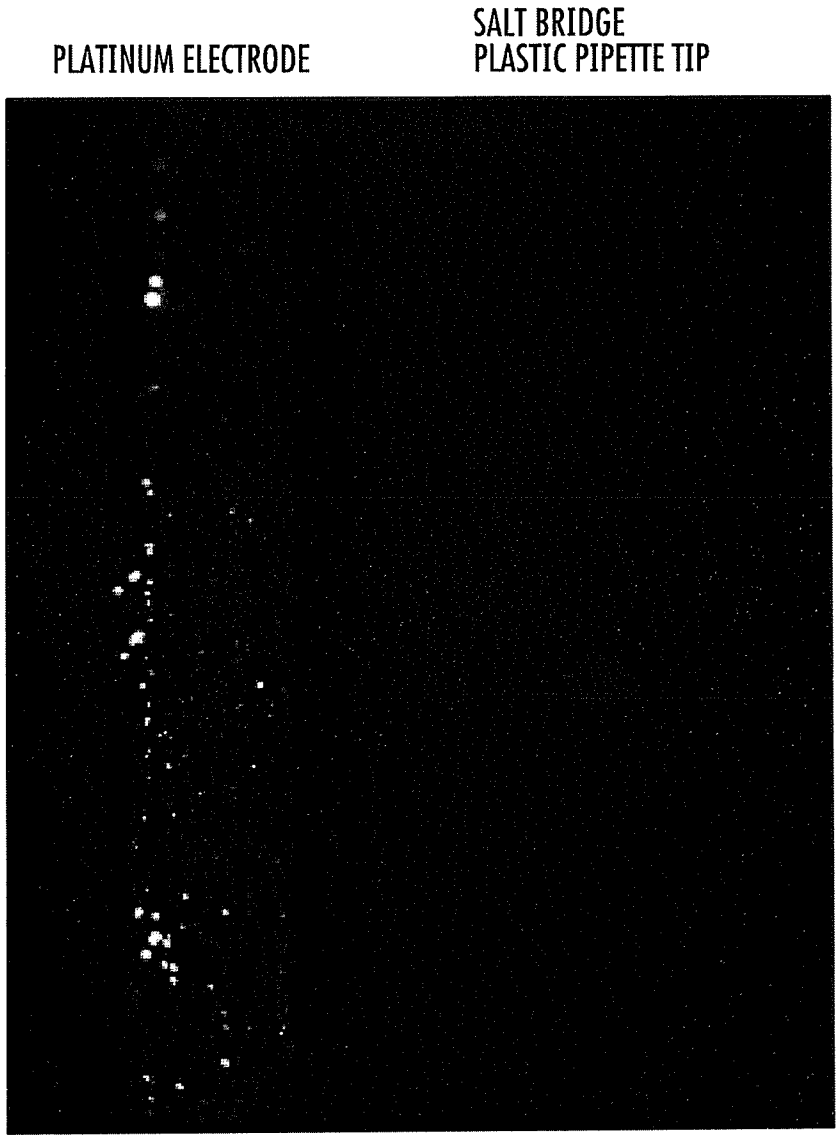

FIG. 16

FIG. 17A

FIG. 17B

FIG. 17C

FIG. 18A

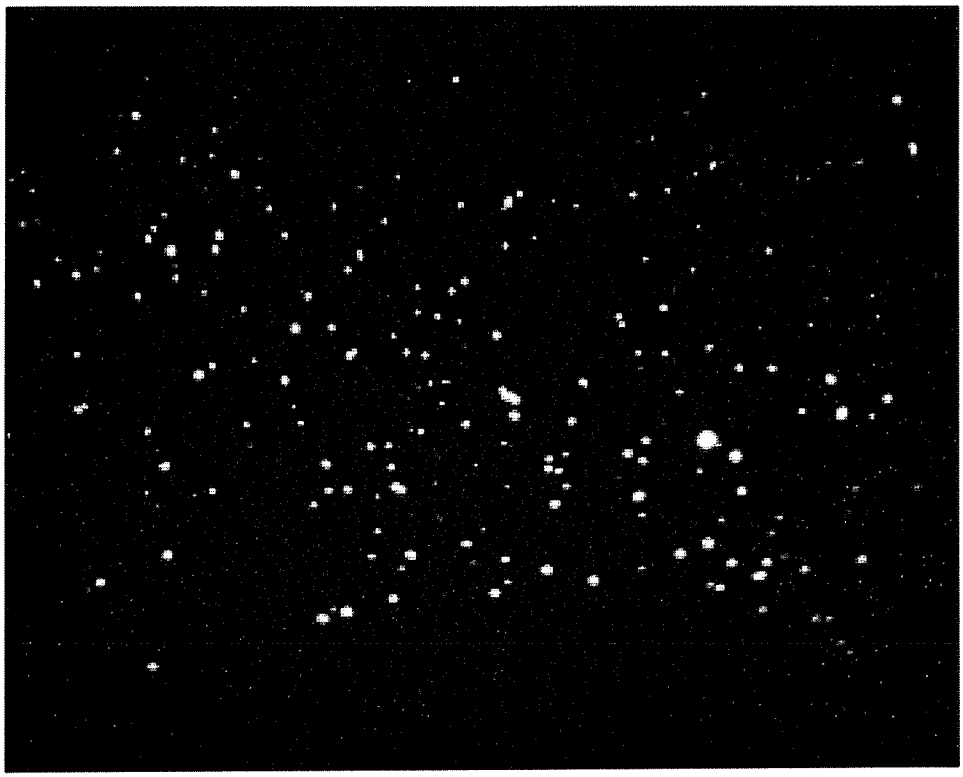

FIG. 18B

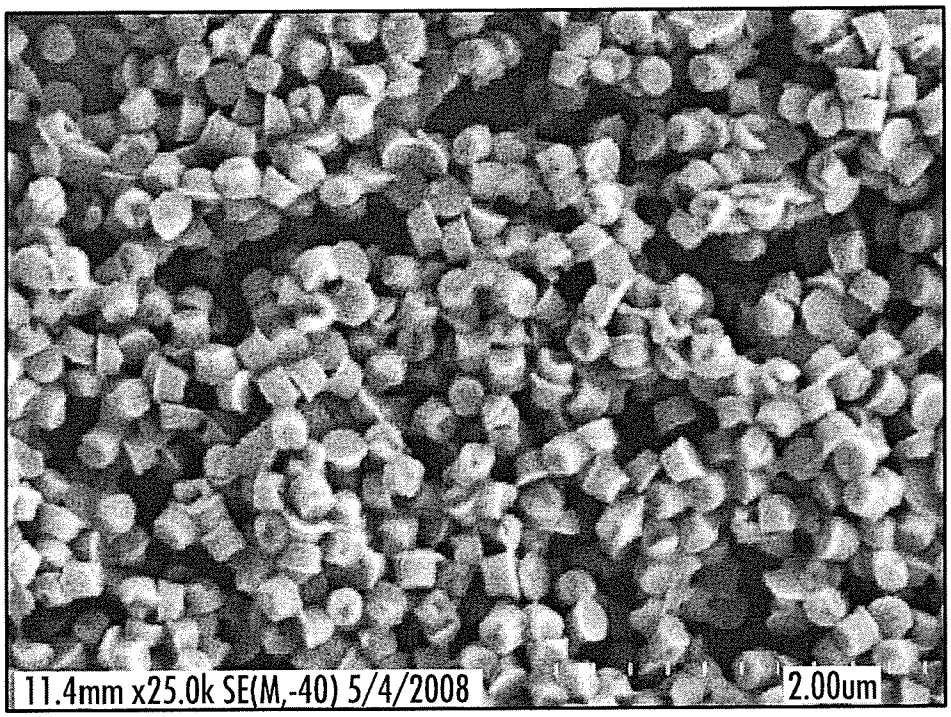

FIG. 19

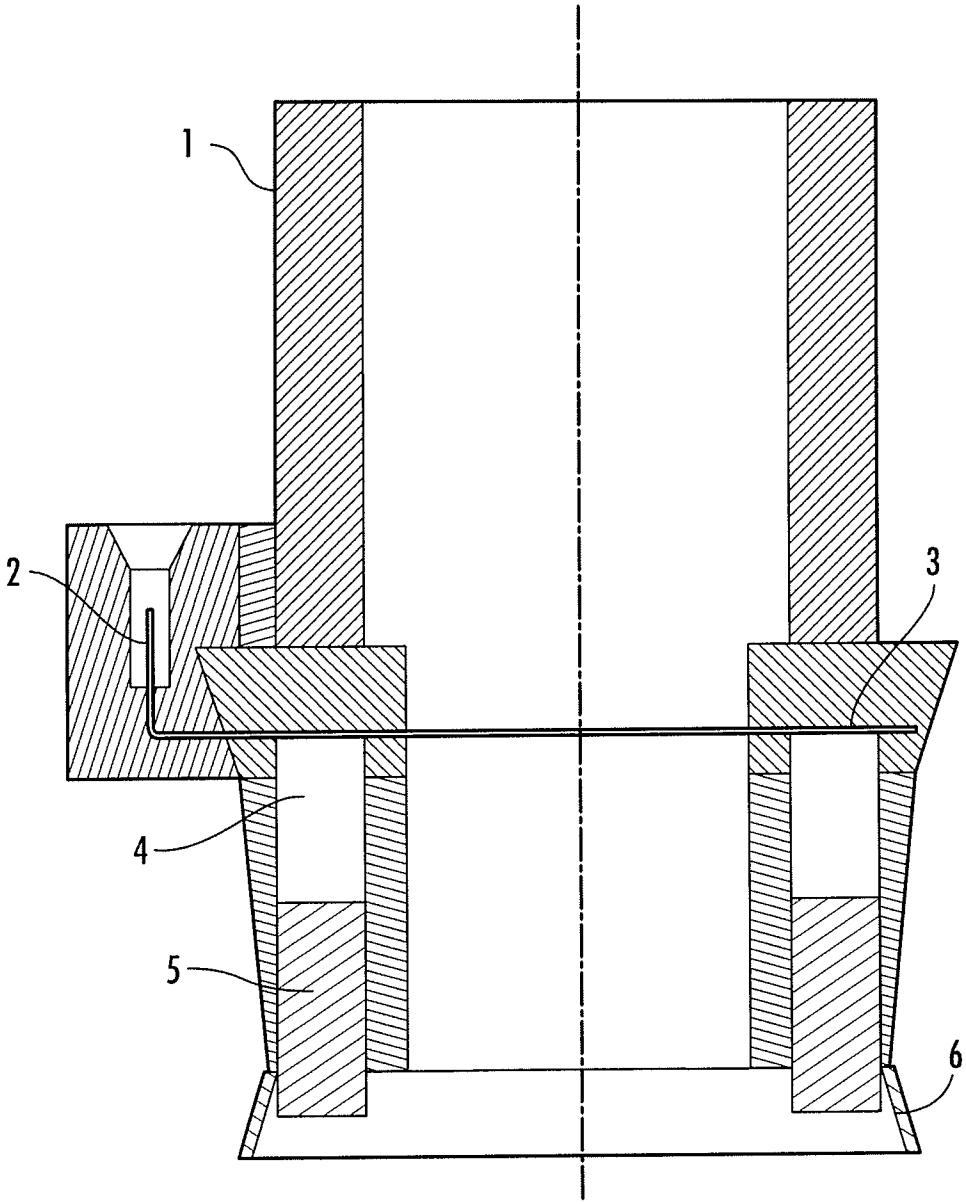

FIG. 20

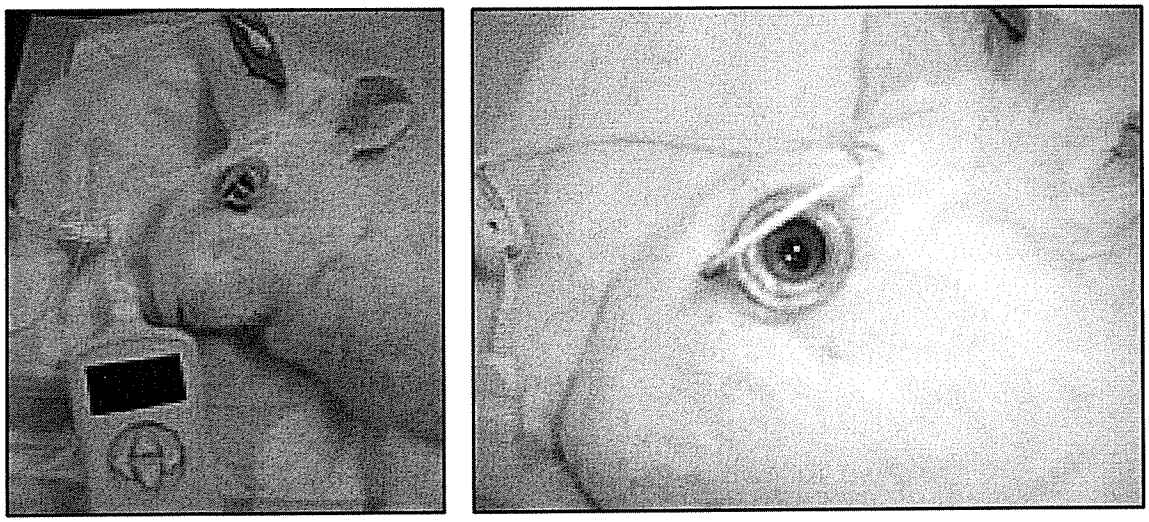

FIG. 21

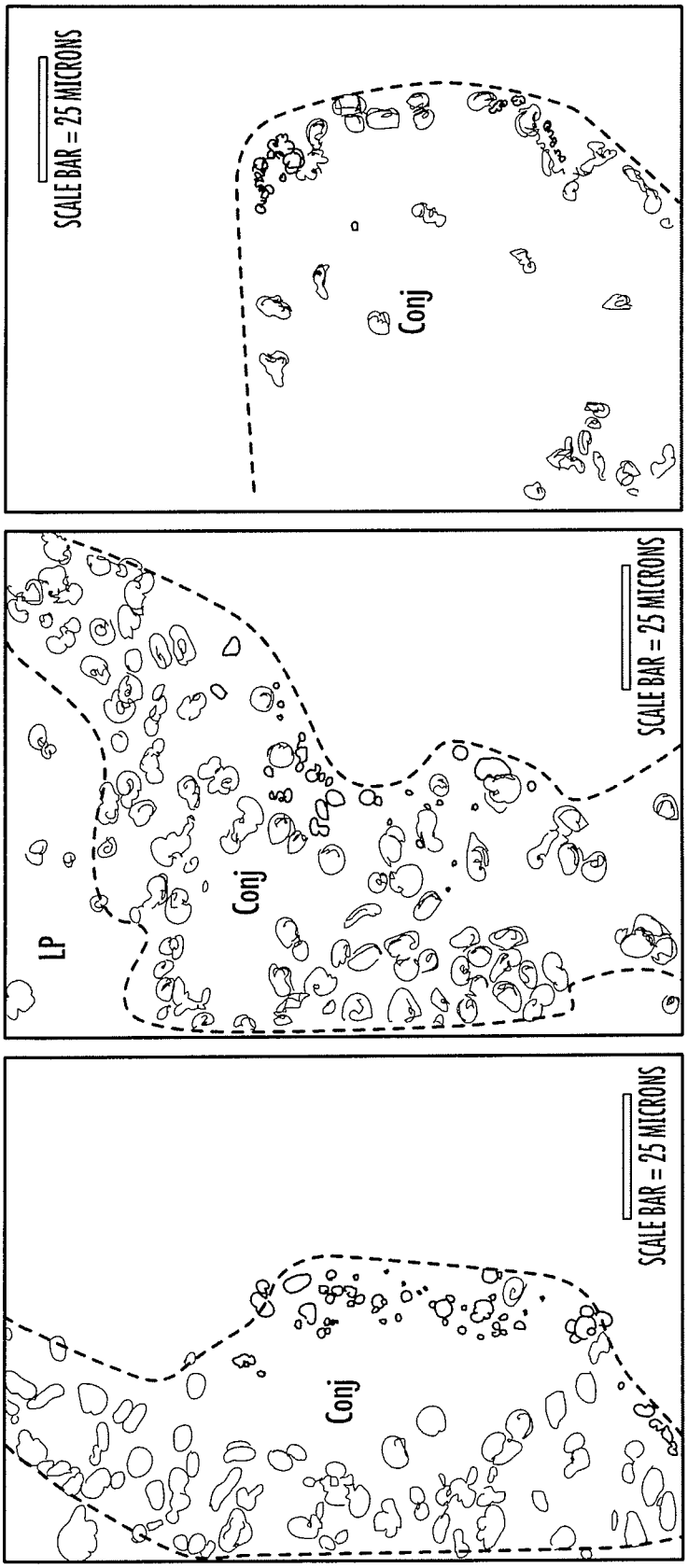
SCALE BAR = 25 MICRONS
Conj
LP
Conj
SCALE BAR = 25 MICRONS
Conj
SCALE BAR = 25 MICRONS

FIG. 22A

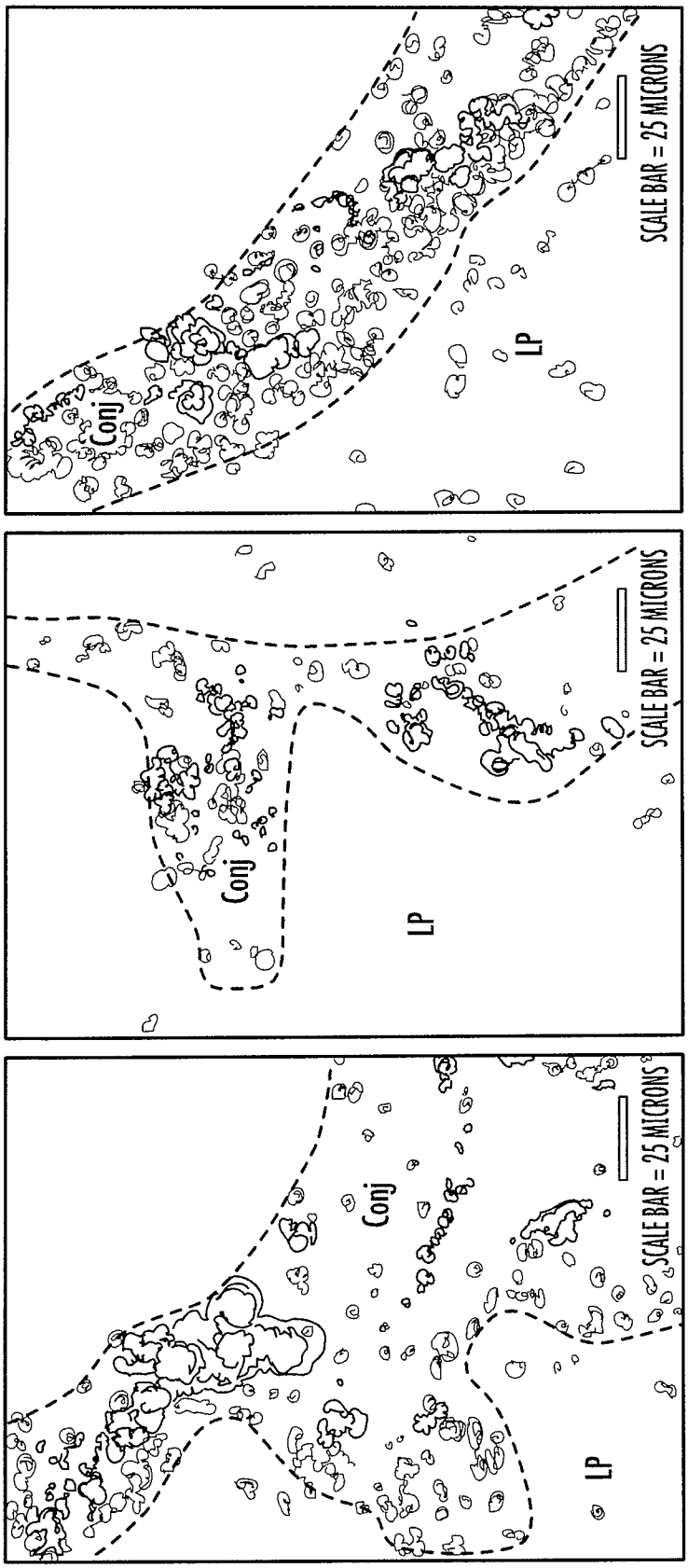
Conj
LP
SCALE BAR = 25 MICRONS
Conj
LP
SCALE BAR = 25 MICRONS
Conj
LP
SCALE BAR = 25 MICRONS

FIG. 22B

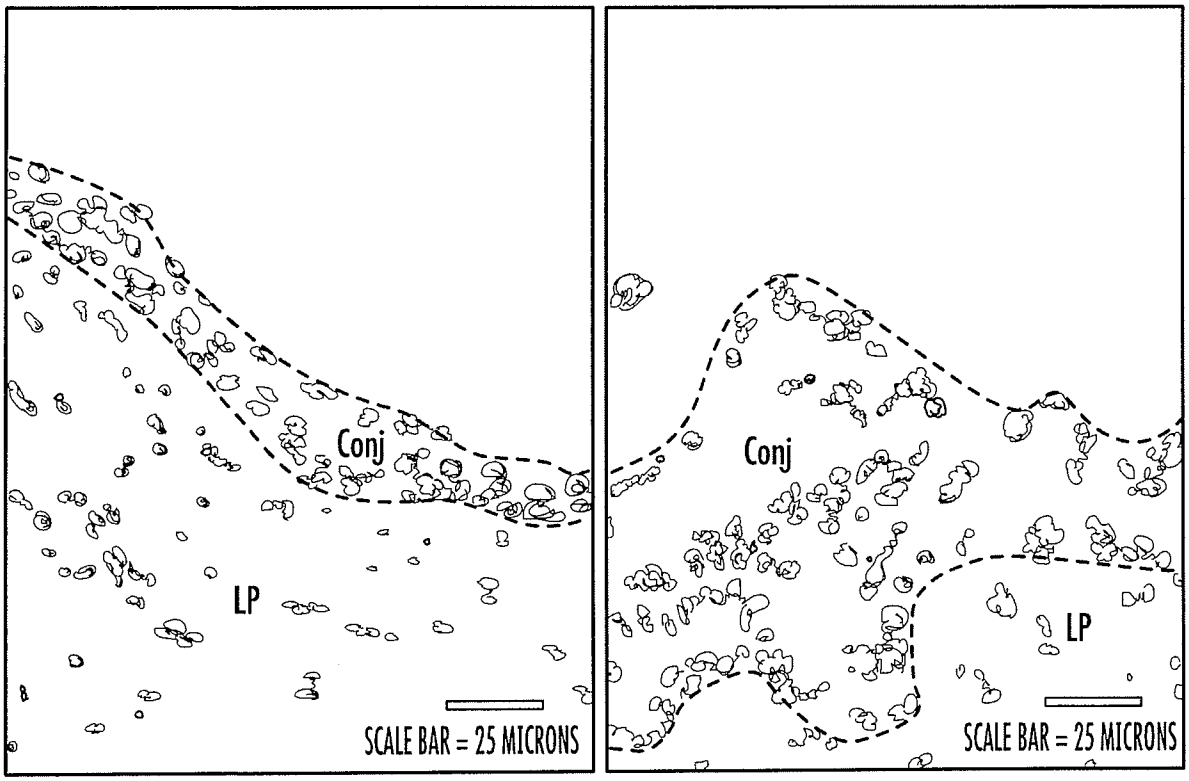

FIG. 22C

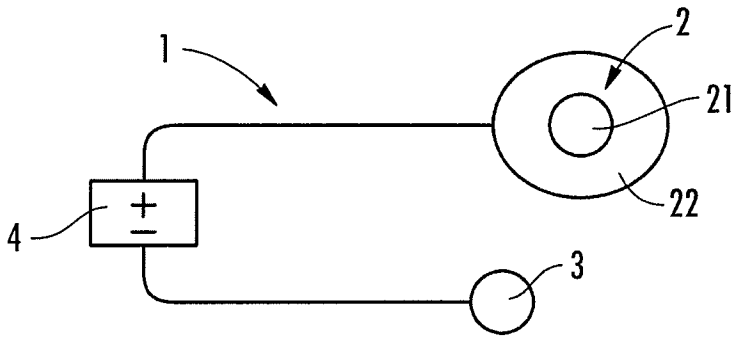

FIG. 23

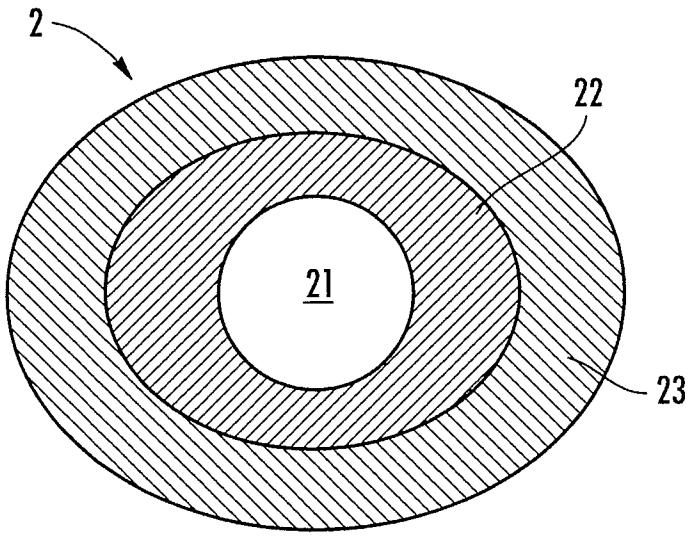

FIG. 24

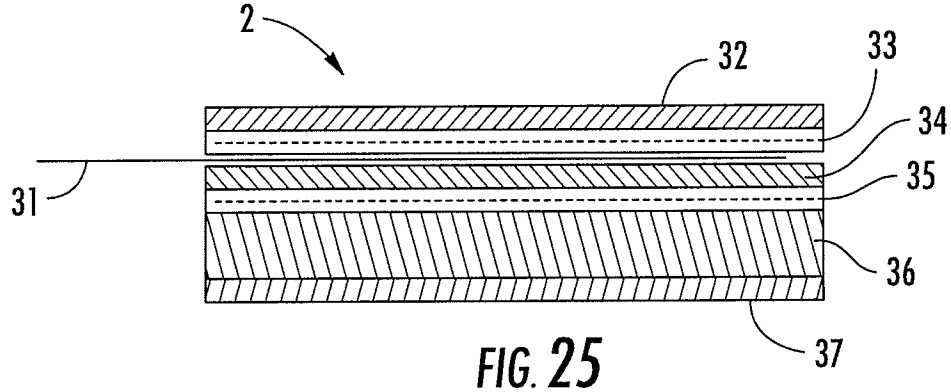

FIG. 25

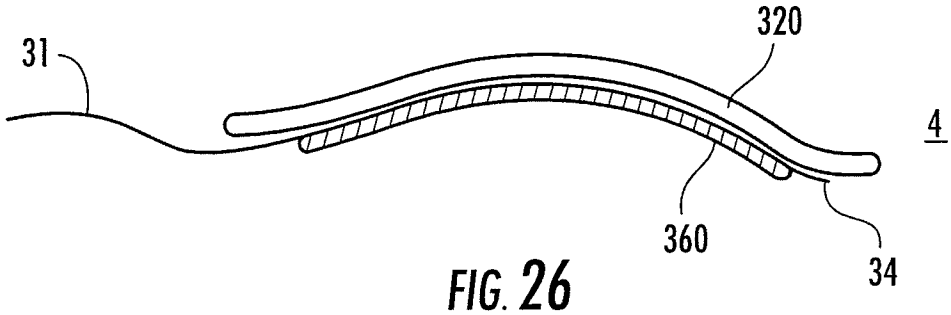

FIG. 26

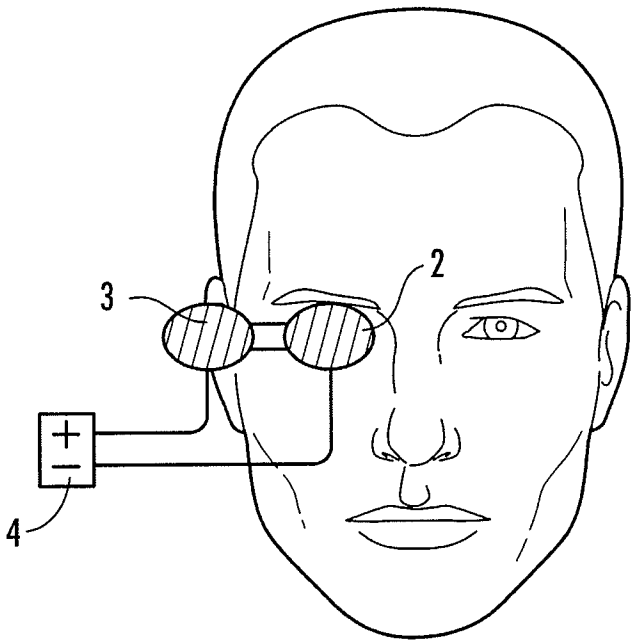

FIG. 27A

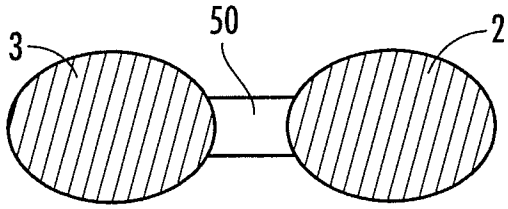

FIG. 27B

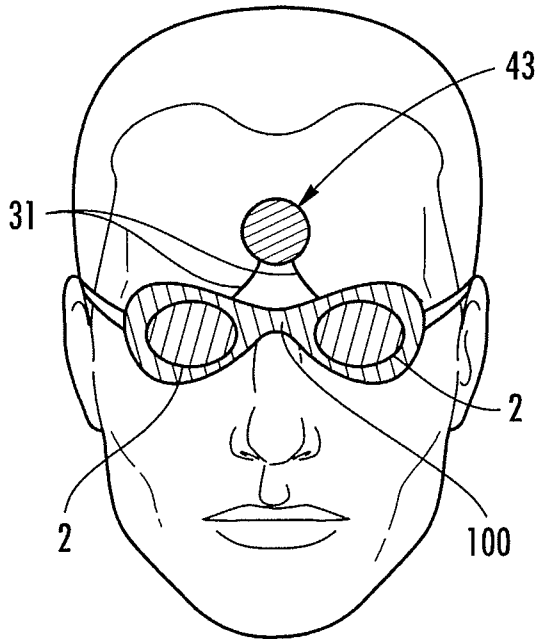

FIG. 28A

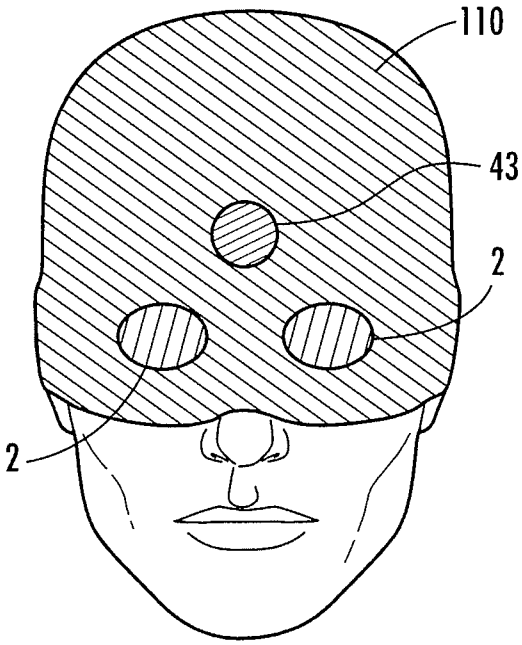

FIG. 29

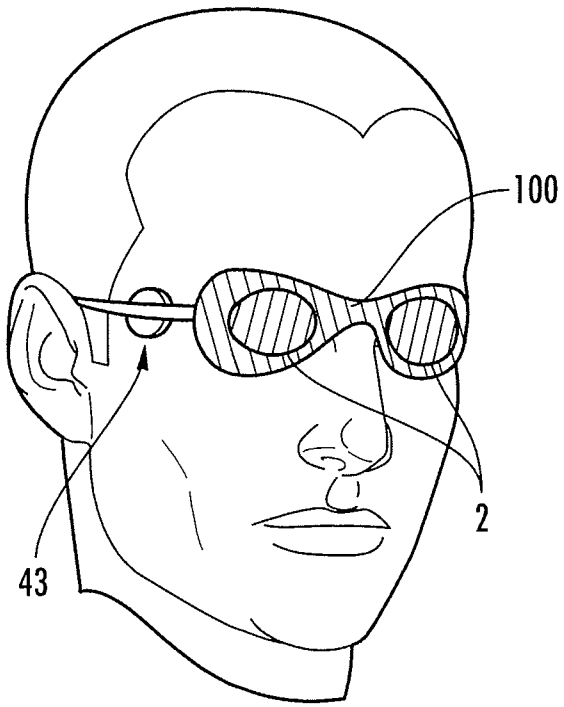

FIG. 28B

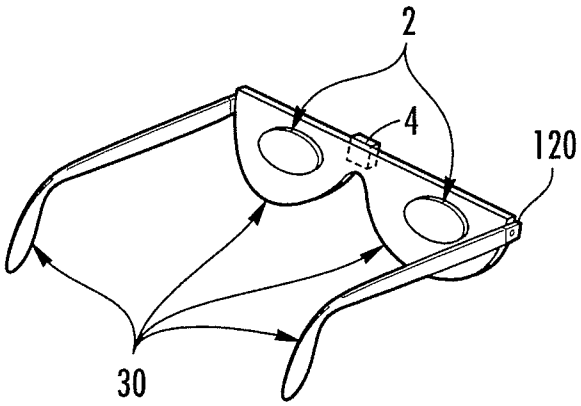

FIG. 28C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/050482

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61N 01/30 (2009.01)
USPC - 604/20
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - A61N 01/30 (2009.01)
USPC - 604/20, 604/22

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)
PatBase

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2007/0123814 A1 (ROY) 31 May 2007 (31.05.2007) entire document	1-10, 20, 25-29
Y		11-19, 21-24
Y	US 2003/0065305 A1 (HIGUCHI et al) 03 April 2003 (03.04.2003) entire document	18
Y	US 6,835,718 B2 (KOSAK) 28 December 2004 (28.12.2004) entire document	11-17, 19, 21-24

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

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
01 September 2009	15 SEP 2009

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774