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(54) Title: USE OF LIPID AND HYDROGEL VEHICLES FOR TREATMENT AND DRUG DELIVERY

(57) Abstract: The present invention relates to compositions and methods for the administration of lipid-based vehicles to treat various disorders, including bladder inflammation, infection, dysfunction, and cancer. In various aspects, the compositions and methods of the invention are useful for prolonged delivery of drugs, e.g., antibiotics, pain treatments, and anticancer agents, to the bladder, genitourinary tract, gastrointestinal system, pulmonary system, and other organs or body systems. In particular, the present invention relates to liposome- or hydrogel-based delivery of vanilloid compounds, such as resiniferatoxin, capsaicin, or tinyatoxin, and toxins, such as botulinum toxin, for the treatment of bladder conditions, including pain, inflammation, incontinence, and voiding dysfunction.



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**USE OF LIPID AND HYDROGEL VEHICLES
FOR TREATMENT AND DRUG DELIVERY**

[0001] This application is a continuation-in-part of U.S. Application Serial No. 10/218,797 filed August 13, 2002, which is a continuation-in-part of U.S. Application Serial No. 60/311,868 filed August 13, 2001, each of which is incorporated by reference herein in their entireties.

1. FIELD OF THE INVENTION

[0002] The present invention relates to compositions and methods for the instillation of lipid (e.g., micelles, microemulsions, macroemulsions, and liposomes) and/or hydrogel vehicles to prevent, manage, ameliorate, and/or treat various disorders, particularly disorders involving the bladder. The vehicles of the present invention are administered either alone or as delivery vehicles for one or more therapeutic agents used in prevention, management, amelioration, and/or treatment of the disorder. The methods and compositions of the invention serve to improve the duration of vehicle attachment to the target area, thus providing a long-term therapeutic effect or intravesical delivery platform for the one or more therapeutic agents. The vehicles of the present invention can be further modified to comprise one or more adhesives, e.g., antibodies or mucoadhesives, that further improve the duration or specificity of vehicle attachment to the target area. Specifically, the present invention relates to liposome-based and/or hydrogel-based delivery of one or more therapeutic agents for the prevention, management, amelioration, and/or treatment of bladder pain, bladder inflammation, incontinence, voiding dysfunction, and/or involuntary muscle contractions of the urinary system.

2. BACKGROUND OF THE INVENTION

Disorders

[0003] Neuropathic pain is thought to occur because of a sensitization in the peripheral and central nervous systems after an initial injury to the peripheral system (see N. Attal, 2000, *Clin. J. Pain* 16(3 Suppl):S118-30). Direct injury to the peripheral nerves as well as many systemic diseases including AIDS/HIV, Herpes Zoster, syphilis,

diabetes, and various autoimmune diseases, can induce this disorder. Such pain is also associated with conditions of the bladder, including interstitial cystitis. Neuropathic pain is typically experienced as burning, shooting, and unrelenting in its intensity, and can sometimes be more debilitating than the initial injury or the disease process which induced it. Unfortunately, the few remedies that have been reported to alleviate this condition are effective in only a small percentage of patients.

[0004] Interstitial cystitis (IC) is characterized by bladder pain, irritative voiding symptoms, and sterile urine (see R. Doggweiler-Wiygul et al., 2000, *Curr. Rev. Pain* 4(2):137-41). In IC, the bladder wall shows inflammatory infiltration with mucosal ulceration and scarring that causes smooth muscle contraction, diminished urinary capacity, hematuria, and frequent, painful urination. Although the pathogenesis of IC is uncertain, it seems likely that a dysfunctional epithelium results in the transepithelial migration of solutes, such as potassium, which depolarizes sensory nerves, and produces the symptoms (C.L. Parsons et al., 1991, *J. Urol.* 145:732; C.L. Parsons et al., 1994, *J. Urol.* 73:504; G. Hohlbrugger, 1999, *Br. J. Urol.* 83(suppl. 2):22; C.L. Parsons et al., 1998, *J. Urol.* 159:1862). Previous reports have shown that IC patients have defects in the glycosaminoglycan (GAG) layers of the uroepithelium (C.L. Parsons et al., 1991, *J. Urol.* 145:732; C.L. Parsons et al., 1994, *J. Urol.* 73:504; G. Hohlbrugger, 1999, *Br. J. Urol.* 83(2):22). Thus, therapies that restore the mucosal lining or surface GAG layer, e.g., administration of heparine, hyaluronic acid, or pentosanpolysulfate, can reduce the leakage of irritant and result in palliation of IC symptoms (see, e.g., C.L. Parsons et al., 1994, *Br. J. Urol.* 73:504; J.I. Bade et al., 1997, *Br. J. Urol.* 79:168; J.C. Nickel et al., 1998, *J. Urol.* 160:612).

[0005] Dystonias are neurological movement disorders characterized by involuntary muscle contractions that force certain parts of the body into abnormal, sometime painful, movements or postures (see S.B. Bressman, 2000, *Clin. Neuropharmacol.* 23(5):239-51). Dystonia disorders cause uncontrolled movement and prolonged muscle contraction, which can result in spasms, twisting body motions, tremor, or abnormal posture. These movements may involve the entire body, or only an isolated area, such as the arms and legs, trunk, neck, eyelids, face, bladder sphincter, or vocal cords. Dystonias result from environmental or disease-related damage to the

basal ganglia, birth injury, (particularly due to lack of oxygen), certain infections, reactions to certain drugs, heavy-metal or carbon monoxide poisoning, trauma, or stroke can cause dystonic symptoms. Dystonias can also be symptoms of other diseases, some of which may be hereditary.

[0006] Urinary detrusor-sphincter dyssynergia (UDSD; also called detrusor-external sphincter dyssynergia and urethral dyssynergia) is a specific type of neurological movement disorder (see H. Madersbacher, 1990, *Paraplegia* 28(4):217-29; J.T. Andersen et al., 1976, *J. Urol.* 116(4):493-5). UDSD is characterized by involuntary urinary sphincter spasms occurring simultaneously with bladder contractions. The lack of coordination between detrusor contraction and urethral relaxation causes urinary obstruction (i.e., partial or complete block of urination). As a result of UDSD, the bladder cannot empty completely. This creates a buildup of urinary pressure, and can lead to severe urinary tract damage and life-threatening consequences. UDSD results from lesions of the corticospinal tract, which are caused by spinal cord injury, multiple sclerosis, or related conditions.

[0007] Another neurological movement disorder is hyperactive (also called contracted; spastic) neurogenic bladder (see M.H. Beers and R. Berkow (eds), 1999, *The Merck Manual of Diagnosis and Therapy*, Section 17:Genitourinary Disorders, Chapter 216: Myoneurogenic Disorders). In hyperactive bladder, the bladder contracts more frequently than normal, due to instability and inappropriate contraction of detrusor muscles (see, e.g., C.F. Jabs et al., 2001, *Int. Urogynecol. J. Pelvic Floor Dysfunct.* 12(1):58-68; S.K. Swami and P. Abrams, 1996, *Urol. Clin. North Am.* 23(3):417-25). Hyperactive bladders can empty spontaneously and result in urinary incontinence (urge incontinence). In addition, the uncoordinated contraction between the bladder and bladder outlet (vesical neck or external urinary sphincter) can result in vesicoureteral reflux with concomitant renal damage. Hyperactive bladder is usually due to brain or suprasacral spinal cord damage. The most common cause is spinal cord injury from transverse myelitis or traumatic cord transection. Hyperactive bladder can also be caused by conditions such as anxiety, aging, infections (e.g., syphilis), diabetes mellitus, brain and spinal cord tumors, stroke, ruptured intervertebral disk, and

demyelinating and degenerative diseases (e.g., multiple sclerosis and amyotrophic lateral sclerosis).

Capsaicin

[0008] Capsaicin is a homovanillic acid derivative (8-methyl-N-vanillyl-6-nonenamid). It is the active component of the red pepper of the genus *Capsicum*, and has been used in humans for topical treatment of cluster headache, herpes zoster, and vasomotor rhinitis (see P. Holzer, 1994, *Pharmacol. Rev.* 43:143; Sicuteri et al., 1988, *Med. Sci. Res.* 16:1079; Watson et al., 1988, *Pain* 33:333; Marabini et al., 1988, *Regul. Pept.* 22:1). *In vitro* capsaicin modulates cellular growth, collagenase synthesis, and prostaglandin secretion from rheumatoid arthritis synoviocytes (see Matucci-Cerinic et al., 1990, *Ann. Rheum. Dis.* 49:598). Capsaicin has also been shown to be immunomodulatory as indicated by its ability to modulate lymphocyte proliferation, antibody production, and neutrophil chemotaxis (see Nilsson et al., 1988, *J. Immunopharmac.* 10:747; Nilsson et al., 1991, *J. Immunopharmac.* 13:21; and Eglezos et al., 1990, *J. Neuroimmunol.* 26:131). These effects play an important role in the use of capsaicin for treatment of arthritis. In addition, capsaicin induces mitochondrial swelling, inhibits NADH oxidase, induces apoptosis of transformed cells, stimulates adenylate cyclase, activates protein kinase C, inhibits superoxide anion generation and alters the redox state of the cell.

[0009] The various effects of capsaicin are mediated through a specific cellular receptor referred to as a vanilloid receptor. This receptor is shared by resiniferatoxin, an alkaloid derived from plants of the genus *Euphorbia*. Resiniferatoxin is a structural homologue of capsaicin, and has been shown to mimic many of the actions of capsaicin. Resiniferatoxin is also structurally similar to phorbol esters (phorbol myristate acetate), which interact with distinct binding sites and activate protein kinase C (see Szallasi, et al., 1989, *Neurosci.* 30:515; and Szallasi and Blumberg, 1989, *Neurosci.* 30:515). Unlike resiniferatoxin, capsaicin has no homology to phorbol myristate acetate. However, capsaicin can activate protein kinase C, suggesting that such activation is not due entirely to the phorbol ester-like moiety on resiniferatoxin.

[0010] Capsaicin has been used as an experimental tool because of its selective action on the small diameter afferent nerve fibers, or C fibers, which mediate pain. From studies in animals, capsaicin appears to trigger C fiber membrane depolarization by opening cation selective channels for calcium and sodium. Although detailed mechanisms of action are not yet known, capsaicin mediated effects include: (i) activation of nociceptors in peripheral tissues; (ii) eventual desensitization of peripheral nociceptors to one or more stimulus modalities; (iii) cellular degeneration of sensitive unmyelinated C fiber afferents; (iv) activation of neuronal proteases; (v) blockage of axonal transport; and (vi) the decrease of the absolute number of C fibers without affecting the number of myelinated fibers.

[0011] Because of the ability of capsaicin to desensitize nociceptors in peripheral tissues, its potential analgesic effects have been assessed in various clinical trials. U.S. Pat. No. 5,431,914, issued July 11, 1995, suggests that a topical preparation containing a concentration of capsaicin of about 0.01% to about 0.1% could be used to treat internal organ pathologies. U.S. Pat. No. 5,665,378, issued Sep. 9, 1997, discusses a transdermal therapeutic formulation comprising capsaicin, a non-steroidal anti-inflammatant, and pamadorm (a diuretic agent) where the composition is said to contain from about 0.001-5% by weight capsaicin and to be useful in treating the pain and discomfort associated with menstrual cramps, bloating, and/or muscular pain such as muscular back pain. Several studies have assessed intravesical capsaicin as a treatment for urge incontinence in patients with spinal detrusor hyperreflexia or bladder hypersensitivity disorders (see F. Cruz, 1998, *Int. Urogynecol. J. Pelvic Floor Dysfunct.* 9:214-220).

[0012] However, since capsaicin application itself frequently causes burning pain and hyperalgesia apart from the neuropathic pain being treated, patient compliance has been poor and the drop out rates during clinical trials have exceeded fifty percent. The spontaneous burning pain and heat hyperalgesia are believed to be due to intense activation and temporary sensitization of the peripheral nociceptors at the site of capsaicin application (primary hyperalgesia). Mechanical hyperalgesia evident in areas surrounding the site of topical application appears to originate from central sensitization of dorsal horn neurons involved in pain transmission (secondary hyperalgesia).

Because of these side effects, the maximal capsaicin concentration used in previous human studies has usually been limited to 0.075%.

Botulinum Toxins

[0013] Botulinum toxins are zinc endopeptidases produced by the anaerobic bacterium *Clostridium botulinum*. Previously known as a cause of a serious and often fatal paralysis acquired through ingestion of contaminated food, botulinum neurotoxins are presently used in both therapeutic and cosmetic applications (see N. Mahant et al., 2000, *J. Clin. Neurosci.* 7(5):389-94; A. Carruthers and J. Carruthers, 2001, *Semin. Cutan. Med. Surg.* 20(2):71-84). In particular, these toxins are used in the treatment of conditions involving involuntary muscle spasms, frown lines, and facial wrinkles.

[0014] There are seven known serotypes of botulinum toxins (designated A-G). The serotypes differ in their cellular targets, potency, and duration of action, but all exert their paralytic effect by inhibiting acetylcholine release at the neuromuscular junction (see M.F. Brin, 1997, *Muscle Nerve* 20(suppl 6):S146-S168). Each serotype acts by cleaving one or more proteins involved in vesicle transport and membrane fusion. For example, botulinum toxin A is internalized by endocytosis at the axon terminal, where it is fully activated by disulfide reduction reactions, and it targets SNAP-25 (see M.F. Brin, 1997, *Muscle Nerve* 20(suppl 6):S146-S168). The extent of botulinum toxin-mediated paralysis depends on the dose, volume, and serotype employed. Botulinum toxin A causes reversible denervation atrophy that is typically terminated by axon sprouting within 2-6 months (see M.F. Brin, 1997, *Muscle Nerve* 20(suppl 6):S146-S168).

[0015] A major drawback of current botulinum toxin therapies is the development of antitoxin antibodies in patients. Antitoxin antibodies result in resistance to botulinum toxin, and the reduction or elimination of its therapeutic effect. It has been estimated that the prevalence of neutralizing antibodies among patients receiving chronic treatment at the higher doses for torticollis or spasticity is probably at least 3% (see M.F. Brin, 1997, *Muscle Nerve* 20(suppl 6):S146-S168). Patients with botulinum toxin A resistance may benefit from injections with other serotypes, including botulinum toxin B, C, and F. However, differences in the duration of the effects of the other serotypes can

be significant, and cause dramatic reductions in treatment efficacy (see M.F. Brin, 1997, *Muscle Nerve* 20(suppl 6):S146-S168).

Liposomes

[0016] Liposomes are self-assembling structures comprising concentric amphipathic lipid (e.g., phospholipid) bilayers separated by aqueous compartments (see, e.g., K. Reimer et al., 1997, *Dermatology* 195(suppl. 2):93; M. Schafer-Korting et al., 1989, *Dermatology* 21:1271). In liposomes, the amphipathic lipid molecules comprise a polar headgroup region covalently linked to one or two non-polar acyl chains. The energetically unfavorable contact between the hydrophobic acyl chains and the aqueous solution surrounding the lipid molecules causes the polar headgroups and acyl chains to rearrange. The polar headgroups become oriented toward the aqueous solution, while the acyl chains orient towards the interior part of the bilayer. The lipid bilayer structure thereby comprises two opposing monolayers, wherein the acyl chains are shielded from contact with the surrounding medium.

[0017] Liposomes are excellent vehicles for drug delivery and gene therapy (K. Reimer et al., 1997, *Dermatology* 195(suppl. 2):93; T. Tsuruta et al., 1997, *J. Urol.* 157:1652; F. Szoka, 2000, *Mol. Therapy* 1:S2; G. Gregoriadis, 1976, *New Eng. J. Med.* 295:704). Previous studies have demonstrated that submucosal injection of liposomal doxorubicin into bladder wall provides an effective and safe treatment for bladder cancer with pelvic lymph node metastasis (I. Tsuruta et al., 1997, *J. Urol.* 157:1652). In a liposome-drug delivery system, an active ingredient, such as a drug, is encapsulated or entrapped in the liposome and then administered to the patient to be treated. Alternatively, if the active ingredient is lipophilic, it may be associated with the lipid bilayer. Active ingredients encapsulated by liposomes reduce toxicity, increase efficacy, or both. Notably, liposomes are thought to interact with cells by stable absorption, endocytosis, lipid transfer, and fusion (R.B. Egerdie et al., 1989, *J. Urol.* 142:390). In this way, liposomes comprise molecular films, which fuse with cells and provide optimal conditions for wound healing (K. Reimer et al., 1997, *Dermatology* 195(suppl. 2):93; M. Schafer-Korting et al., 1989, *J. Am. Acad. Dermatol.* 21:1271). Generally, liposomes have low antigenicity and can be used to encapsulate and deliver components that

cause undesirable immune responses in patients (see A. Natsume et al., 2000, *Jpn. J. Cancer Res.* 91:363-367).

Hydrogels

[0018] Hydrogels are an improvement over the class of biodegradable polymeric materials that have been used for drug delivery systems. Hydrogels generally have excellent biocompatibility and can swell by taking up large amounts of fluid without dissolving thus allowing protracted release of the fluid (and pharmaceutical agents in the fluid) in an area of interest.

[0019] Drug release from hydrogels can be affected by several parameters, such as pore size and degradability of the hydrogel and size, hydrophobicity, and concentration of the drug. Typically, the release mechanism from a biodegradable hydrogel is diffusion-controlled at an initial stage and then a combination of diffusion and degradation at a later stage.

3. SUMMARY OF THE INVENTION

[0020] The present invention encompasses improved methods of prevention, management, amelioration, and/or treatment for pain, pain-intensive disorders, muscle contraction disorders, cancer, infections, and related disorders, especially those disorders of the bladder, genitourinary tract, gastrointestinal tract, pulmonary system, by providing compositions and methods for the intravesical administration of lipid and/or hydrogel vehicles either alone or comprising one or more therapeutic agents. The methods and compositions of the invention serve to improve the duration of vehicle attachment to the target area, thus providing a long-term therapeutic effect or intravesical delivery platform for the one or more therapeutic agents. In embodiments where hydrogel vehicles are used, the hydrogel must be able to gelate in the conditions of the local environment of the target area. In specific embodiments, the hydrogel can gelate in the bladder in the presence of urine.

[0021] In one embodiment, the invention encompasses compositions comprising lipid and/or hydrogel vehicles that further comprise one or more adhesives, e.g., antibodies or mucoadhesives, for use in prevention, management, amelioration, and/or

treatment of a disorder or as a delivery vehicle for one or more therapeutic agents. Such adhesives serve to further improve the duration or specificity of vehicle attachment to the target area, thus providing an even longer-term or more specific therapeutic effect or intravesical delivery platform for the one or more therapeutic agents. These adhesives can act to target the vehicles to specific areas (e.g., mucoadhesives to target to mucus membranes) or specific cell types and/or receptors (e.g. antibodies).

[0022] In embodiments where the vehicles of the invention are used as a delivery vehicle for one or more therapeutic agents, the compositions provide for longer-term intravesical administration of the one or more therapeutic agents than administration of the therapeutic agent alone, in the absence of the disclosed vehicles. Therapeutic agents administered include, but are not limited to, vaniloids (e.g., capsaicin, resiniferatoxin, tinyatoxin), bioactive agents (e.g., nucleic acids or polypeptides), small molecule drugs (e.g., pain therapeutics, anticancer treatments, or antibiotics), and toxins (e.g., botulinum toxin). In a specific embodiment, vehicles of the invention can be used simultaneously to deliver one or more therapeutic agents as well as ameliorate irritation caused by irritating therapeutic agents (e.g., vaniloids such as capsaicin, resiniferatoxin, tinyatoxin). In another specific embodiment, vehicles of the invention can be used simultaneously to deliver one or more therapeutic agents as well as reduce or prevent antibody-mediated resistance to antigenic therapeutic agents (e.g., botulinum toxin). In embodiments where the therapeutic agent is soluble in water, the delivery vehicle is preferably a hydrogel delivery vehicle. In embodiments where the therapeutic agent is insoluble in water, the delivery vehicle is preferably a lipid delivery vehicle, more preferably, liposomes.

[0023] The invention also encompasses pharmaceutical compositions for administration to patients to prevent, manage, ameliorate, and/or treat disorders, particularly disorders of the bladder, genitourinary tract, gastrointestinal tract, or pulmonary system, according to the methods of the invention.

[0024] Other applications and advantages afforded by the present invention will be apparent from the detailed description and exemplification hereinbelow.

4. BRIEF DESCRIPTION OF THE DRAWINGS

[0025] The appended drawings of the figures are presented to further describe the invention and to assist in its understanding through clarification of its various aspects.

[0026] FIG. 1 shows the temperature sensitive gel formation of the hydrogel in presence of urine contents. Polymer was dissolved in human urine and the solution turn to gel in 37°C water bath. Left tube in the panel is the tube kept at room temperature, middle and the right tube were at 37°C. Tube inversion is used show formation of gel by lack of any flow due to gravity.

[0027] FIG. 2 shows cumulative urine output of the rats instilled with free FITC solution (□) and FITC entrapped in a hydrogel (□) formed by a thermosensitive polymer. Difference in the cumulative amount of urine excreted for 24h after instillation was not significant (*p> 0.05). (n=6).

[0028] FIG. 3 shows the semi-logarithmic plot of fluorescence intensity of the urine measured at various time points after instillation of free FITC and FITC entrapped in hydrogel. Urine was diluted several folds before measuring the fluorescence of FITC at 512nm in the Perkin Elmer spectrofluorimeter with excitation at 498nm (mean±SEM; n=6) *p<0.01.

[0029] FIGS. 4A-4D show the effect of intravesically delivered misoprostol in rats treated with cyclophosphamide-induced cystitis. When the bladder was continuously filled, multiple contractions were elicited and the number of micturitions (indexed by peaks in the cystometric measurement (CMG)) evoked over a 60 min infusion period was used to describe micturition frequency. (A) Cyclophosphamide administration markedly increased micturition frequency representing saline treated rats. Rats treated with (B) misoprostol in saline or (C) hydrogel alone showed a decreased micturation frequency as compared to saline-treated rats. (D) Cyclophosphamide induced increased micturition frequency was dramatically reduced in the rats instilled with misoprostol entrapped in hydrogel. Scale on vertical axis is intravesical pressure in cm of water (cm/w) and time is on the horizontal axis.

[0030] FIGS. 5A-5D show haemtoxylin and eosin staining cyclophosphamide treated rat bladders in representative cross sections. (A) saline instilled rats showed severe lesions in the epithelium, extensive submucosal edema, and multiple bleeding vessels in subepithelium, (B) rats instilled with misoprostol alone and (C) rats instilled with hydrogel alone showed edema, moderate vascular ectasia and vascular congestion compared to saline treated rats, (D) rats instilled with misoprostol entrapped in hydrogel prior to cyclophosphamide treatment showed only slightly enlarged subepithelial layer due to edema. Lumen side and bleeding spots are marked by white arrow in all sections and magnification is 10x.

[0031] FIGS. 6A-6G show representative CMG measurements from various groups of treated rats. (A) rats treated with normal saline showed periodic micturition events under urethane anesthesia. Rats treated with (B) 30% ethanol alone or (C) liposomes alone show the dissimilar effects of ethanol and liposomes on bladder afferents by decrease in bladder contraction frequency. Rats treated with hydrogel in the (D) absence and (E) presence of capsaicin, respectively showed a decrease in bladder contraction frequency in presence of capsaicin. Rats treated with (F) liposomes and capsaicin or (G) 30% ethanol and capsaicin showed a complete blockade of micturition reflex. Raised plateau of bladder contraction pressure reflects urinary retention.

[0032] FIG. 7 shows bladder contraction frequency 48 h after administration of capsaicin in various vehicles. All capsaicin treated groups showed significant difference from saline treated group ($p < 0.05$). Comparison of hydrogel treated group in absence of capsaicin with saline treatment; ethanol and liposome in presence of capsaicin; and liposome in presence and absence of capsaicin, respectively, was not significant ($p > 0.05$).

[0033] FIGS. 8A-8H show gross bladder morphology of previously fixed rat bladder 48 h after administration of capsaicin in various vehicles. Bladders were from rats that were (A) untreated, or treated with (B) saline, (C) 30% ethanol in normal saline, (D) 30% ethanol in normal saline with 1mM capsaicin, (E) liposomes, (F) liposomes with

1mM capsaicin, (G) hydrogel, (H) hydrogel with 1mM capsaicin. Photographs were taken through dissecting microscope at 1x magnification.

[0034] FIGS. 9A-9F show the localization of calcitonin gene related peptide (CGRP) in nerve fibers in rat bladder 48 h after administration of capsaicin in various vehicles. Primary rabbit polyclonal antibody bound to the CGRP containing nerve fibers and is visualized with fluorescently labeled secondary goat antibody. CGRP-containing fibers are visible as white lines against the dull tissue auto-fluorescence (marked by horizontal white arrows in the sections visible). Bladders were from rats that were (A) untreated or treated with (B) 30% ethanol in normal saline with 1mM capsaicin, (C) liposomes alone, (D) liposomes with 1 mM capsaicin, (E) hydrogel alone, or (F) hydrogel with 1mM capsaicin. Magnification was 10 X in all panels.

[0035] FIGS. 10A-10F show haemtoxylin and eosin staining of rat bladder cross sections 48h after administration of capsaicin in various vehicles. Lumen side of bladder is facing upwards (marked by horizontal white arrow in all panels). Bladders were from rats that were treated with (A) 30% ethanol in normal saline alone, (B) 30% ethanol in normal saline with 1 mM capsaicin, (C) liposomes alone, (D) liposomes with 1 mM capsaicin, (E) hydrogel alone, or (F) hydrogel with 1 mM capsaicin. Bladders of rats treated with 30% ethanol either with or without capsaicin showed partial to complete urothelial denudation and vascular congestion. Bladders of rats treated with liposomes or hydrogel either in the absence or presence of capsaicin showed a normal and intact urothelium. Magnification was 10 X in all panels.

5. DETAILED DESCRIPTION OF THE INVENTION

[0036] The present invention relates to compositions and methods for the instillation of lipid (e.g., micelles, microemulsions, macroemulsions, and liposomes) and/or hydrogel vehicles to treat various disorders, particularly disorders involving the bladder. The vehicles of the present invention are useful for administration either alone or as delivery vehicles for one or more therapeutic agents used in treatment of the disorder. The methods and compositions of the invention serve to improve the duration or specificity of vehicle attachment to the target area, thus providing a longer-term or more specific therapeutic effect or intravesical delivery platform for the one or more

therapeutic agent. In particular embodiments, the compositions and methods of the invention relate to prevention, management, amelioration, and/or treatment for disorders affecting urinary system components, e.g., bladder, urethra, kidneys, ureters, and/or sphincter muscles. In other particular embodiments, the compositions and methods of the invention relate to prevention, management, amelioration, and/or treatment for disorders involving pain (e.g., neuropathic pain, IC), aberrant muscle contractions (e.g., IC, hyperactive bladder, UDSD, dystonia, dyssynergia, spasticity), cancer (e.g., of the bladder), and infection (e.g., urinary tract infection). In preferred embodiments, the compositions and methods of the invention prevent, manage, ameliorate, and/or treat bladder related disorders such as those disorders involving bladder irritation and irritation-induced bladder dysfunction.

[0037] In one embodiment, the invention encompasses compositions comprising lipid and/or hydrogel vehicles that further comprise one or more adhesives, e.g., antibodies or mucoadhesives, for use in prevention, management, amelioration, and/or treatment of a disorder or as a delivery vehicle for one or more therapeutic agents. Such adhesives serve to further improve the duration or specificity of vehicle attachment to the target area, thus providing an even longer-term or more specific therapeutic effect or intravesical delivery platform for the one or more therapeutic agents. These adhesives can act to target the vehicles to specific areas (e.g., mucoadhesives to target to mucus membranes) or specific cell types and/or receptors (e.g. antibodies).

5.1 LIPID VEHICLES

[0038] In one embodiment, lipid vehicles of the invention may be administered to a patient to prevent, manage, ameliorate, and/or treat a disorder. In another embodiment, lipid vehicles of the invention that comprise a therapeutic agent may be administered to a patient to prevent, manage, ameliorate, and/or treat a disorder.

[0039] The lipid vehicles for use in the methods of the present invention encompass micelles, microemulsions, macroemulsions, liposomes, and similar carriers. The term micelles refers to colloidal aggregates of amphipathic (surfactant) molecules that are formed at a well-defined concentration known as the critical micelle concentration. Micelles are oriented with the nonpolar portions at the interior and the

polar portions at the exterior surface, exposed to water. The typical number of aggregated molecules in a micelle (aggregation number) is 50 to 100. As described herein, microemulsions are essentially swollen micelles, although not all micellar solution can be swollen to form microemulsion. Microemulsions are thermodynamically stable, are formed spontaneously, and contain particles that are extremely small. Droplet diameters in microemulsions typically range from 10-100 nm. In contrast, the term macroemulsions refers to droplets with diameters greater than 100 nm. As described herein, liposomes are closed lipid vesicles comprising lipid bilayers that encircle aqueous interiors. Liposomes typically have diameters of 25 nm to 1 μ m (see, e.g., D.O. Shah (ed), 1998, *Micelles, Microemulsions, and Monolayers: Science and Technology*, Marcel Dekker; A.S. Janoff (ed), 1998, *Liposomes: Rational Design*, Marcel Dekker).

[0040] In one preferred embodiment, the lipid vehicle for use in the methods of the present invention is a liposome. The principal lipid of a liposome for use in the methods of the invention is, preferably, phosphatidylcholine, but can include various natural (e.g., tissue derived L- α -phosphatidyl: egg yolk, heart, brain, liver, soybean) and/or synthetic (e.g., saturated and unsaturated 1,2-diacyl-*SN*-glycero-3-phosphocholines, 1-acyl-2-acyl-*SN*-glycero-3-phosphocholines, 1,2-diheptanoyl-*SN*-glycero-3-phosphocholine) derivatives of the same. Such lipids can be used alone, or in combination with a helper lipid. Preferred helper lipids are non-ionic or uncharged at physiological pH. Particularly preferred non-ionic lipids include, but are not limited to, cholesterol and DOPE (1,2-dioleoylglyceryl phosphatidylethanolamine), with cholesterol being most preferred. The molar ratio of a phospholipid to helper lipid can range from about 3:1 to about 1:1, more preferably from about 1.5:1 to about 1:1, and most preferably, the molar ratio is about 1:1.

[0041] A liposome used for the preparation of a vehicle of the invention is, in simplest form, composed of two lipid layers. The lipid layer may be a monolayer, or may be multilamellar and include multiple layers. Constituents of the liposome may include, for example, phosphatidylcholine, cholesterol, phosphatidylethanolamine, etc. Phosphatidic acid, which imparts an electric charge, may also be added. Exemplary

amounts of these constituents used for the production of the liposome include, for instance, 0.3 to 1 mol, preferably 0.4 to 0.6 mol of cholesterol; 0.01 to 0.2 mol, preferably 0.02 to 0.1 mol of phosphatidylethanolamine; 0.0-0.4 mol, preferably 0-0.15 mol of phosphatidic acid per 1 mol of phosphatidylcholine.

[0042] Liposomes of the present invention can be constructed by well-known techniques (see, e.g., G. Gregoriadis (ed.), 1993, *Liposome Technology* Vols. 1-3, CRC Press, Boca Raton, FL). Lipids are typically dissolved in chloroform and spread in a thin film over the surface of a tube or flask by rotary evaporation. If liposomes comprised of a mixture of lipids are desired, the individual components are mixed in the original chloroform solution. After the organic solvent has been eliminated, a phase consisting of water optionally containing buffer and/or electrolyte is added and the vessel agitated to suspend the lipid. Optionally, the suspension is then subjected to ultrasound, either in an ultrasonic bath or with a probe sonicator, until the particles are reduced in size and the suspension is of the desired clarity. For transfection, the aqueous phase is typically distilled water and the suspension is sonicated until nearly clear, which requires several minutes depending upon conditions, kind, and quality of the sonicator. Commonly, lipid concentrations are 1 mg/ml of aqueous phase, but could be higher or lower by about a factor of ten.

[0043] Liposomes according to the invention optionally have one or more amphiphiles. The exact composition of the liposomes will depend on the particular circumstances for which they are to be used. Those of ordinary skill in the art will find it a routine matter to determine a suitable composition. The liposomes of the present invention comprise at least one compound of the present invention. In a preferred embodiment, the liposomes of the present invention consist essentially of a single type of phospholipid. In another preferred embodiment, the liposomes comprise mixtures of phospholipids. In yet another preferred embodiment, the liposomes of the present invention comprise one or more phospholipids in a mixture with one or more natural or synthetic lipids, e.g., cholesterol or DOPE.

[0044] Liposomes can be produced in accordance with established methods. For example, a mixture of the above-mentioned lipids, from which the solvents have been

removed, can be emulsified by the use of a homogenizer, lyophilized, and melted to obtain multilamellar liposomes. Alternatively, unilamellar liposomes can be produced by the reverse phase evaporation method (Szoka and Papahadjopoulos, 1978, *Proc. Natl. Acad. Sci. USA* **75**:4194-4198). Unilamellar vesicles can also be prepared by sonication or extrusion. Sonication is generally performed with a bath-type sonifier, such as a Branson tip sonifier (G. Heinemann Ultrashall und Labortechnik, Schwabisch Gmund, Germany) at a controlled temperature as determined by the melting point of the lipid. Extrusion may be carried out by biomembrane extruders, such as the Lipex Biomembrane Extruder (Northern Lipids Inc, Vancouver, British Columbia, Canada). Defined pore size in the extrusion filters may generate unilamellar liposomal vesicles of specific sizes. The liposomes can also be formed by extrusion through an asymmetric ceramic filter, such as a Ceraflow Microfilter (commercially available from the Norton Company, Worcester, MA).

[0045] Following liposome preparation, the liposomes that have not been sized during formation may be sized by extrusion to achieve a desired size range and relatively narrow distribution of liposome sizes. A size range of about 0.2-0.4 microns will allow the liposome suspension to be sterilized by filtration through a conventional filter (e.g., a 0.22 micron filter). The filter sterilization method can be carried out on a high throughput basis.

[0046] Several techniques are available for sizing liposomes to a desired size, including, ultrasonication, high-speed homogenization, and pressure filtration (M.J. Hope et al., 1985, *Biochimica et Biophysica Acta* **812**:55; U.S. Patent Nos. 4,529,561 and 4,737,323). Sonicating a liposome suspension either by bath or probe sonication produces a progressive size reduction down to small unilamellar vesicles less than about 0.05 microns in size. Multilamellar vesicles can be recirculated through a standard emulsion homogenizer until selected liposome sizes, typically between about 0.1 and 0.5 microns. The size of the liposomal vesicles may be determined by quasi-elastic light scattering (QELS) (see Bloomfield, 1981, *Ann. Rev. Biophys. Bioeng.* **10**:421-450). Average liposome diameter may be reduced by sonication of formed liposomes. Intermittent sonication cycles may be alternated with QELS assessment to guide efficient liposome synthesis.

[0047] Liposomes can be extruded through a small-pore polycarbonate membrane or an asymmetric ceramic membrane to yield a well-defined size distribution. Typically, a suspension is cycled through the membrane one or more times until the desired liposome size distribution is achieved. The liposomes may be extruded through successively smaller-pore membranes, to achieve a gradual reduction in liposome size. For use in the present invention, liposomes have a size of about 0.05 microns to about 0.5 microns. More preferred are liposomes having a size of about 0.05 to about 0.2 microns.

[0048] Various conditions can be used to trigger the liposome to release its payload or active agent, including pH, ionic strength, controlled release and antibody attachment. Research related to pH-sensitive liposomes has focused principally on anionic liposomes comprised largely of phosphatidylethanolamine (PE) bilayers (see, Huang et al., 1989, *Biochemistry* **28**:9508-9514; Duzgunes et al., 1990, "pH-Sensitive Liposomes" *Membrane Fusion* J. Wilschut and D. Hoekstra (eds.), Marcel-Decker Inc., New York, NY pp. 713-730; Yatvin et al., 1980, *Science*, 210, 1253-1255). More recently, pH-sensitive cationic liposomes have been developed to mediate transfer of DNA into cells. For instance, researchers have described a series of amphiphiles with headgroups containing imidazole, methylimidazole, or aminopyridine moieties (see, Budker et al., 1996, *Nature Biotech.* **14**:760-764). Also described are lipid molecules within liposome assemblies that are capable of structural reorganization upon a change in pH (see, e.g., U.S. Patent No. 6,200,599 to Nantz et al.).

5.2 HYDROGELS

[0049] Hydrogels of the invention may be administered to a patient to prevent, manage, ameliorate, and/or treat a disorder. In another embodiment, hydrogels of the invention that comprise a therapeutic agent may be administered to a patient to prevent, manage, ameliorate, and/or treat a disorder.

[0050] Hydrogels for use in the present invention are preferably thermosensitive biodegradable hydrogels. Thermosensitive hydrogels have many advantages over traditional hydrogels because they can exhibit sol-gel transition at both room temperature and elevated temperatures. These changes corresponding to swelling and

de-swelling of the polymer can be controlled to release encapsulated compounds in response to temperature changes. In a preferred embodiment, the thermosensitive hydrogel is a triblock copolymer comprising polyethylene glycol- poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

[0051] The amount of PLGA is at least 3000 block length, at least 3500 block length, at least 4000 block length, at least 4500 block length, at least 5000 block length, at least 5500 block length, at least 6000 block length, or at least 6500 block length.

[0052] Any method known in the art can be used to determine the contribution of PLGA in the hydrogel. In one embodiment, proton NMR is used to determine the contribution of PLGA in the hydrogel.

[0053] In preferred embodiments, the hydrogel for use in the methods of the invention is substantially liquid at room temperature and substantially solid at body temperature. In more preferred embodiments, the hydrogel for use in the methods of the invention can become substantially solid in the bodily fluid surrounding the hydrogel when administered to the desired area of the patient in need. In a specific preferred embodiments, the hydrogel can become substantially solid at body temperature in urine. In a another specific preferred embodiments, the hydrogel can become substantially solid at body temperature in blood. In a another specific preferred embodiments, the hydrogel can become substantially solid at body temperature in mucus.

[0054] As used herein, "substantially liquid" refers to a hydrogel solution that is at least 60% liquid, 70% liquid, 80% liquid, 90% liquid, 95% liquid, 99% liquid, or greater amount liquid. As used herein, "substantially solid" refers to a hydrogel solution that is at least 60% solid, 70% solid, 80% solid, 90% solid, 95% solid, 99% solid, or greater amount solid.

[0055] Any method known in the art can be used to make a thermosensitive hydrogel for use in the present invention. In a preferred embodiment, the thermosensitive triblock co-polymer, (polyethylene glycol- poly[lactic acid-co-glycolic acid]-polyethylene glycol) PEG-PLGA-PEG is synthesized according to a protocol modified from procedures previously described (Jeong et al, 1999, Colloids and Surface

B: Biointerfaces 16:185, Jeong et al., 2000, J. Biomed. Mater. Res. 50:171, and Kim et al., 2001, Pharm. Res. 50:440).

[0056] PEG-PLGA-PEG is a triblock copolymer made in two stages. First, a diblock polymer is made. Lactic acid, glycolic acid and polyethylene glycol are mixed in a particular ratio in toluene using stannous 2-ethyl hexonate as a catalyst (e.g., glycolic acid and lactic acid in the ratio of 1:3 and 3.5 gram of polyethylene glycol (mw 750)). The mixture is refluxed at 150°C for 4 hour. Toluene is removed using rotary evaporator and the diblock polymer is purified by fractional precipitation using methylene chloride and heptane and dried overnight under vacuum. The molecular weight of diblock copolymers is controlled at around 3,000~4,500.

[0057] Molecular weight of polymer can be determined by any known method. In one embodiment, the molecular weight is determined by the process of gel permeation chromatography (GPC). The molecular weight of the hydrogel can be controlled by determining the optimum ratio of reactants and duration of reaction in the first step by optimization experiments.

[0058] Second, a triblock polymer is made. A particular amount of previously synthesized diblock copolymer was used for the synthesis of the triblock copolymer in a coupling reaction using hexamethylene diisocyanate (HMDI) as a catalyst in toluene at 40-50°C for 15-20 hour followed by reflux at 120°C for 5 hour. Toluene is removed from the synthesized triblock copolymer and the copolymer is purified by fractional precipitation from methylene chloride using heptane. Finally, the product is dried for about 36 h under reduced pressure up to constant weight.

[0059] Any method known in the art can be used to confirm the triblock nature of the copolymer. In one embodiment, ¹H NMR is used.

[0060] In preferred embodiments, the hydrophobicity of the hydrogel is increased as compared to hydrogels synthesized by an unmodified procedure. The hydrogel's hydrophobicity is related to the amount of PLGA in the hydrogel thus increased PLGA content correlates with increased hydrophobicity. The reaction conditions in the first step of hydrogel synthesis determines the length of the PLGA block in the final product.

[0061] The hydrogels may be made such that no therapeutic agent is contained within the hydrogel. Alternatively, the hydrogels may be made such that they comprise a therapeutic agent. In such embodiments, the one or more therapeutic agents is mixed with the liquid hydrogel before administration to a patient. The therapeutic agent mixed with the hydrogel should not substantially interfere with the hydrogel's ability to polymerize at body temperature in the environment where it is administered. Thus the amount of the therapeutic agent that can be added to the hydrogel can be determined empirically taking into account such factors as the therapeutic agent's pH, what it is dissolved in, concentration, etc.

[0062] From the detailed description herein, it will be clear to those skilled in the art that the vehicles of the present invention are useful for both *in vitro* and *in vivo* applications requiring delivery of one or more therapeutic agents (e.g., vaniloids, bioactive agents, small molecule drugs, toxins) into target areas or cells.

5.3 THERAPEUTIC AGENTS

[0063] The vehicles of the present invention can be used to deliver a broad range of therapeutic agents including, but not limited to, vaniloids, bioactive agents, small molecules, and toxins. As used herein, the term "therapeutic agent" refers to a molecule that has a desired biological effect in the prevention, management, amelioration (symptom reduction), and/or treatment of a disorder. Therapeutic agents include, but are not limited to, proteinaceous molecules, including, but not limited to, peptides, polypeptides, proteins, post-translationally modified proteins, antibodies etc., or small molecules (less than 1000 daltons), inorganic or organic compounds; or nucleic acid molecules including, but not limited to, double-stranded or single-stranded DNA, or double-stranded or single-stranded RNA (e.g., antisense, RNAi, etc.), as well as triple helix nucleic acid molecules. Agents can be derived from any known organism (including, but not limited to, animals, plants, bacteria, fungi, and protista, or viruses) or from a library of synthetic molecules.

[0064] Therapeutic agents may have a number of different properties including, but not limited to, antimicrobials, antibiotics, antimycobacterial, antifungals, antivirals, antineoplastic agents, agents affecting the immune response, blood calcium regulators,

agents useful in glucose regulation, anticoagulants, antithrombotics, antihyperlipidemic agents, cardiac drugs, thyromimetic and antithyroid drugs, adrenergics, antihypertensive agents, cholinergics, anticholinergics, antispasmodics, antiulcer agents, skeletal and smooth muscle relaxants, prostaglandins, general inhibitors of the allergic response, antihistamines, local anesthetics, analgesics, narcotic antagonists, antitussives, sedative-hypnotic agents, anticonvulsants, antipsychotics, anti-anxiety agents, antidepressant agents, anorexigenics, non-steroidal anti-inflammatory agents, steroidal anti-inflammatory agents, antioxidants, vaso-active agents, bone-active agents, antiarthritics, antiparasitic agents, hormones, hormone antagonists, neurotransmitter antagonists, vitamins, narcotics, and diagnostic agents (e.g., imaging agents).

[0065] In one embodiment, a therapeutic agent is a vanilloid, such as resiniferatoxin, capsaicin, tinyatoxin, and related compounds.

[0066] In another embodiment, a therapeutic agent is a botulinum toxin, such as botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, and botulinum toxin G.

[0067] In another embodiment, a therapeutic agent is an anticancer agent, such as abiraterone, acivicin, aclacinomycin, aclarubicin, acodazole hydrochloride, acronine, actinomycin D, acylfulvene, adecypenol, adozelesin, adriamycin, aldesleukin, alkylative agents, ALL-TK antagonists, altretamine, ambomycin, ambamustine, ametantrone acetate, amidox, amifostine, aminoglutethimide, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, andrographolide, angiogenesis inhibitors, antagonist D, antagonist G, antarelix, anthracycline, anthramycin, antisense RNA (e.g., of oncogenes), anti-dorsalizing morphogenetic protein-1, antiandrogens, antiestrogens, antineoplaston, aphidicolin glycinate, apoptosis gene modulators, apoptosis regulators, apurinic acid, ara-CDP-DL-PTBA, arginine deaminase, asulacrine, atamestane, atrimustine, asparaginase, asperlin, axinastatin 1, axinastatin 2, axinastatin 3, azacitidine, azasetron, azetepa, azatoxin, azatyrosine, azotomycin, baccatin III derivatives, balanol, batimastat, BCR/ABL antagonists, benzochlorins, benzodepa, benzoylstaurosporine, beta lactam derivatives, beta-alethine, betaclamycin B, betulinic

acid, bFGF inhibitor, bicalutamide, bisantrene, bisaziridinylspermine, bisnafide, bistratene A, bizelesin, bleomycin, breflate, brequinar sodium, broprimine, budotitane, busulfan, buthionine sulfoximine, cactinomycin, calcipotriol, calphostin C, calusterone, camptothecin derivatives, canarypox IL-2, capecitabine, caracemide, carbetimer, carboplatin, carboxamide-amino-triazole, carboxyamidotriazole, CaRest M3, carmustine, CARN 700, cartilage derived inhibitor, carubicin hydrochloride, carzelesin, casein kinase inhibitors (ICOS), castanospermine, cecropin B, cedefingol, cetorelix, chlorambucil, chloroquinoxaline sulfonamide, cicaprost, cirolemycin, cis-porphyrin, cisplatin, cladribine, clomifene analogues, clotrimazole, collismycin A, collismycin B, combretastatin A4, combretastatin analogue, conagenin, crambescidin 816, crisnatol, cryptophycin 8, cryptophycin A derivatives, curacin A, cyclopentantraquinones, cycloplatam, cyclophosphamide, cypemycin, cystine arabinoside, cytarabine ocfosfate, cytolytic factor, cytostatin, dacarbazine, dacliximab, dactinomycin, daunorubicin hydrochloride, decarbazine, decitabine, dehydroidemnin B, deslorelin, dexamethasone, dexifosfamide, dexrazoxane, dexormaplatin, dexverapamil, dezaguanine, dezaguanine mesylate, diaziquone, didemnin B, didox, diethylnorspermine, dihydro-5-azacytidine, dihydrotaxol, dioxamycin, diphenyl spiromustine, diphtheria toxin, docetaxel, docosanol, dolasetron, doxifluridine, doxorubicin, droloxifene, dromostanolone propionate, dronabinol, duazomycin, duocarmycin SA, ebselen, ecomustine, edatrexate, edelfosine, edrecolomab, eflornithine hydrochloride, elemene, elsamitucin, emitefur, enloplatin, enpromate, epipropidine, epirubicin, epristeride, erbulozole, esorubicin hydrochloride, estramustine, etanidazole, etoposide, etoposide phosphate, etoprine, exemestane, fadrozole, fazarabine, fenretinide, filgrastim, finasteride, flavopiridol, flezelastine, floxuridine, fluasterone, fludarabine phosphate, fluorodaunorubicin hydrochloride, fluorouracil, flurocitabine, fosquidone, forfenimex, formestane, fostriecin, fotemustine, gadolinium texaphyrin, gallium nitrate, galocitabine, ganirelix, gelatinase inhibitors, gemcitabine, glutathione inhibitors, hepsulfam, herceptin, heregulin, hexamethylene bisacetamide, hydroxyurea, hypericin, ibandronic acid, idarubicin, idoxifene, idramantone, ifosfamide, ilmofofosine, ilomastat, imidazoacridones, imiquimod, immunostimulant peptides, insulin-like growth factor-1 receptor inhibitor, interferon agonists, interferons, interleukins

(including interleukin 2), interferon alpha-2a, interferon alpha-2b, interferon alpha-n1, interferon alpha-n3, interferon beta-1a, interferon gamma-1b, iobenguane, iododoxorubicin, ipomeanol, iproplatin, irinotecan hydrochloride, iroplact, irsogladine, isobengazole, isohomohalicondrin B, itasetron, jasplakinolide, kahalalide F, lamellarin-N triacetate, lanreotide, leinamycin, lenograstim, lentinan sulfate, leptolstatin, letrozole, leukemia inhibiting factor, leukocyte alpha interferon, leuprolide, leuprorelin, levamisole, liarozole, linear polyamine analogue, lipophilic disaccharide peptide, lipophilic platinum compounds, lissoclinamide 7, lobaplatin, lombricine, lometrexol, lomustine, lonidamine, losoxantrone, lovastatin, loxoribine, lurtotecan, lutetium texaphyrin, lysofylline, lytic peptides, maitansine, mannostatin A, marimastat, masoproc, maspin, matrilysin inhibitors, matrix metalloproteinase inhibitors, maytansine, mechlorethamine hydrochloride, megestrol acetate, melengestrol acetate, melphalan, menogaril, merbarone, mercaptopurine, meterelin, methioninase, methotrexate, metoclopramide, metoprine, meturedopa, MIF inhibitor, mifepristone, miltefosine, mirimostim, mitindomide, mitocarcin, mitocromin, mitogillin, mitoguazone, mitolactol, mitomalcin, mitomycin, mitonafide, mitosper, mitotane, mitotoxin fibroblast growth factor-saporin, mitoxantrone, mofarotene, molgramostim, monoclonal antibody, human chorionic gonadotrophin, monophosphoryl lipid A+myobacterium cell wall sk, mopidamol, multiple drug resistance gene inhibitor, multiple tumor suppressor 1-based therapy, mustard anticancer agent, mycaperoxide B, mycobacterial cell wall extract, mycophenolic acid, myriaporone, N-acetyldinaline, N-substituted benzamides, nafarelin, nagrestip, naloxone+pentazocine, napavin, naphterpin, nartograstim, nedaplatin, nemorubicin, neridronic acid, neutral endopeptidase, nilutamide, nisamycin, nitric oxide modulators, nitroxide antioxidant, nitrosoureas (such as carmustine and lomustin), nitrullyn, nocodazole, nogalamycin, nucleoside analogs (such as purine analogs, and pyrimidine analogs), O6-benzylguanine, octreotide, okicenone, oligonucleotides, onapristone, ondansetron, ondansetron, oracin, ormaplatin, oral cytokine inducer, ormaplatin, osaterone, oxaliplatin, oxaunomycin, oxisuran, palauamine, palmitoylrhizoxin, pamidronic acid, panaxytriol, panomifene, parabactin, paclitaxel, pazelliptine, pegaspargase, peldesine, peliomycin, pentosan polysulfate sodium, pentamustine, pentostatin, pentrozole, peplomycin sulfate, perflubron, perfosfamide, perillyl alcohol,

phenazinomycin, phenylacetate, phosphatase inhibitors, picibanil, pilocarpine hydrochloride, piposulfan, pirarubicin, piritrexim, piroxantrone hydrochloride, placetin A, placetin B, plasminogen activator inhibitor, platinum-triamine complex, plicamycin, plomestane, porfimer sodium, procarbazine, porfimer sodium, porfiromycin, prednimustine, prednisone, procarbazine hydrochloride, propyl bis-acridone, prostaglandin J2, proteasome inhibitors, protein A-based immune modulator, protein kinase C inhibitor, protein kinase C inhibitors, microalgal, protein tyrosine phosphatase inhibitors, purine nucleoside phosphorylase inhibitors, purpurins, puromycin, pyrazofurin, pyrazoloacridine, pyridoxylated hemoglobin polyoxyethylene conjugate, radioisotopes (such as I¹²⁵, palladium, iridium), raf antagonists, raltitrexed, ramosetron, ras farnesyl protein transferase inhibitors, ras inhibitors, ras-GAP inhibitor, retelliptine demethylated, rhenium etidronate, rhizoxin, riboprime, ribozymes, ricin A, RII retinamide, rogletimide, rohitukine, romurtide, roquinimex, rubiginone B1, ruboxyl, safinol, saintopin, SarCNU, sarcophytol A, sargramostim, Sdi 1 mimetics, semustine, senescence derived inhibitor 1, sense oligonucleotides, signal transduction inhibitors, signal transduction modulators, simtrazene, single chain antigen binding protein, sizofiran, sobuzoxane, sodium borocaptate, sodium phenylacetate, solverol, somatomedin binding protein, sonermin, sparfosic acid, sparsomycin, spicamycin D, , spirogermanium hydrochloride, spiromustine, spiroplatin, splenopentin, spongistatin 1, squalamine, stem cell inhibitor, stem-cell division inhibitors, stipiamide, streptonigrin, streptozocin, stromelysin inhibitors, sulfinosine, sulofenur, superactive vasoactive intestinal peptide antagonist, suradista, suramin, swainsonine, synthetic glycosaminoglycans, tallimustine, talisomycin, tamoxifen, tauromustine, taxol, tazarotene, tecogalan, tegafur, telomerase inhibitors, tellurapyrylium, teloxantrone hydrochloride, temoporfin, temozolomide, teniposide, teroxirone, testolactone, tetrachlorodecaoxide, tetrazomine, thaliblastine, thalidomide, thiamiprine, thiocoraline, thioguanine, thiotepa, thrombopoietin, thrombopoietin mimetic, thymalfasin, thymopoietin receptor agonist, thymotrnan, thyroid stimulating hormone, tiazofurin, tin ethyl etiopurpurin, tirapazamine, titanocene bichloride, topsentin, topotecan, toremifene, totipotent stem cell factor, translation inhibitors, trestolone acetate, tretinoin, triacetyluridine, tricyribine phosphate, trimetrexate, trimetrexate glucuronate, triptorelin,

tropisetron, tubulozole hydrochloride, turosteride, tyrosine kinase inhibitors, tyrphostins, UBC inhibitors, ubenimex, uracil mustard, uredepa, urokinase receptor antagonists, vapreotide, variolin B, velaresol, veramine, verdins, verteporfin, vinblastine, vincristine sulfate, vindesine, vindesine sulfate, vinepidine, vinglycinate sulfate, vinleurosine sulfate, vinorelbine tartrate, vinrosidine sulfate, vinoxaltine, vinzolidine sulfate, vitaxin, vorozole, zanoterone, zeniplatin, zilascorb, zinostatin, zorubicin hydrochloride, 20-epi-1,25 dihydroxyvitamin D3, and 5-ethynyluracil.

[0068] In another embodiment, a therapeutic agent is an inhibitor of a kinase. Such kinases include, but are not limited to, ABL, ACK, AFK, AKT (*e.g.*, AKT-1, AKT-2, and AKT-3), ALK, AMP-PK, ATM, Auroral, Aurora2, bARK1, bArk2, BLK, BMX, BTK, CAK, CaM kinase, CDC2, CDK, CK, COT, CTD, DNA-PK, EGF-R, Eph receptor kinases (*e.g.*, EphA2, EphA4), ErbB-1, ErbB-2, ErbB-3, ErbB-4, ERK (*e.g.*, ERK1, ERK2, ERK3, ERK4, ERK5, ERK6, ERK7), ERT-PK, FAK, FGR (*e.g.*, FGF1R, FGF2R), FLT (*e.g.*, FLT-1, FLT-2, FLT-3, FLT-4), FRK, FYN, GSK (*e.g.*, GSK1, GSK2, GSK3-alpha, GSK3-beta, GSK4, GSK5), G-protein coupled receptor kinases (GRKs), HCK, HER2, HKII, JAK (*e.g.*, JAK1, JAK2, JAK3, JAK4), JNK (*e.g.*, JNK1, JNK2, JNK3), KDR, KIT, IGF-1 receptor, IKK-1, IKK-2, INSR (insulin receptor), IRAK1, IRAK2, IRK, ITK, LCK, LOK, LYN, MAPK, MAPKAPK-1, MAPKAPK-2, MEK, MET, MFPK, MHCK, MLCK, MLK3, NEU, NIK, PDGF receptor alpha, PDGF receptor beta, PHK, PI-3 kinase, PKA, PKB, PKC, PKG, PRK1, PYK2, p38 kinases, p135tyk2, p34cdc2, p42cdc2, p42mapk, p44mpk, RAF, RET, RIP, RIP-2, RK, RON, RS kinase, SRC, SYK, S6K, TAK1, TEC, TIE1, TIE2, TRKA, TXK, TYK2, UL13, VEGFR1, VEGFR2, YES, YRK, ZAP-70, and all subtypes of these kinases (see *e.g.*, Hardie and Hanks (1995) *The Protein Kinase Facts Book*, I and II, Academic Press, San Diego, Calif.).

[0069] In another embodiment, a therapeutic agent is an antibiotic, such as gentamycin, ciprofloxacin, cefoxitin, macrolides, lincosamines (*e.g.*, lincomycin, erythromycin, dirithromycin, clindamycin, clarithromycin, and azithromycin); penicillins (*e.g.*, ampicillin, amoxicillin, piperacillin, mezlocillin, carbenicillin indanyl, bacampicillin, ampicillin, amoxicillin, penicillin G, penicillin V, piperacillin plus tazobactam, and ticarcillin plus clavulanic acid), beta-lactamase inhibitors (*e.g.*, amoxicillin-clavulanic acid, ampicillin-sulbactam, benzylpenicillin, cloxacillin,

dicloxacillin, methicillin, oxacillin); aminoglycosides (e.g., amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, and tobramycin); and tetracyclines (e.g., tetracycline, oxytetracycline, minocycline, methacycline, doxycycline, and demedocycline).

[0070] In another embodiment, a therapeutic agent is an antifungal agent, such as miconazole, terconazole, econazole, isoconazole, butaconazole, clotrimazole, itraconazole, nystatin, naftifine and amphotericin B.

[0071] In another embodiment, a therapeutic agent is a nucleic acid (see Section 5.3.1). In certain specific embodiments, the nucleic acid therapeutic agent is an antisense molecule or RNA interference molecule.

[0072] In another embodiment, a therapeutic agent is a polypeptide (see Section 5.3.2). In certain specific embodiments, the polypeptide therapeutic agent is an antibody.

[0073] In another embodiment, a therapeutic agent is a local anesthetic, such as dibucaine and chlorpromazine.

[0074] In another embodiment, a therapeutic agent is a beta-adrenergic blocker, such as propranolol, timolol and labetolol.

[0075] In another embodiment, a therapeutic agent is an antihypertensive agent such as clonidine and hydralazine.

[0076] In another embodiment, a therapeutic agent is an anti-depressant, such as imipramine, amitriptyline and doxepin.

[0077] In another embodiment, a therapeutic agent is an anticonversant, such as phenytoin.

[0078] In another embodiment, a therapeutic agent is an antihistamine, such as diphenhydramine, chlorpheniramine and promethazine.

[0079] Those of skill in the art will know of other agents suitable for use with the formulations and methods of the present invention.

5.3.1 NUCLEIC ACIDS AS THERAPEUTIC AGENTS

[0080] Therapeutic agents for use in the methods of the invention may be nucleic acids. As used herein, the terms nucleic acid and polynucleotide are synonymous, and refer to purine- and pyrimidine-containing polymers of any length, either polyribonucleotides or polydeoxyribonucleotides or mixed polyribo-polydeoxyribonucleotides. Such therapeutic agents may be isolated from natural sources or, more preferably, may be made recombinantly. In one embodiment, the nucleic acid therapeutic agents may be made through the well-known technique of solid phase synthesis. Equipment for such synthesis is available from several vendors, including PE Applied Biosystems (Foster City, CA). In another embodiment, the nucleic acid therapeutic agents may be made through recombinant DNA methods. In practicing the present invention, many conventional techniques in molecular biology, microbiology, and recombinant DNA may be employed. Such techniques are well known and are explained fully in, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York; F.M. Ausubel et al. (eds), 1995, *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc., New York, NY; D.N. Glover (ed), 1985, *DNA Cloning: A Practical Approach, Volumes I and II*; M.L. Gait (ed), 1984, *Oligonucleotide Synthesis*; Hames and Higgins (eds), 1985, *Nucleic Acid Hybridization*; Hames and Higgins (eds), 1984, *Transcription and Translation*; Perbal, 1984, *A Practical Guide to Molecular Cloning: The Series*, Methods in Enzymology, Academic Press, Inc.; J. H. Miller and M. P. Calos (eds), 1987, *Gene Transfer Vectors for Mammalian Cells*, Cold Spring Harbor Laboratory; Wu and Grossman (eds), *Methods in Enzymology*, Vol. 154; Wu (ed), *Methods in Enzymology*, Vol. 155.

[0081] Nucleic acids of all types may be therapeutic agents for use in the methods of the invention. Nucleic acids may be single- or double-stranded molecules, i.e., DNA, RNA, or DNA-DNA, DNA-RNA or RNA-RNA hybrids, or protein nucleic acids (PNAs) formed by conjugating bases to an amino acid backbone. Nucleic acids may also be oligonucleotides such as antisense oligonucleotides, RNA interference (RNAi) molecules, chimeric DNA-RNA polymers, and ribozymes, as well as modified versions

of these nucleic acids wherein the modification may be in the base, the sugar moiety, the phosphate linkage, or in any combination thereof.

[0082] The nucleic acid therapeutic agents may comprise modified nucleic acids. In some embodiments, at least one of the phosphodiester bonds of a nucleic acid therapeutic agent has been modified such as with phosphorothioate bonds, methyl phosphonate bonds, or short chain alkyl or cycloalkyl structures. Persons of ordinary skill in the art will be able to select other linkages for use in the practice of the invention. In other embodiments, at least one of the bases of a nucleic acid therapeutic agent has been modified. Non-naturally occurring purines and pyrimidines may be used. In certain other embodiments, nucleic acids with modifications on the furanosyl portions of the nucleotide subunits may also be used as nucleic acid therapeutic agents. Examples of such modifications are 2'-O-alkyl- and 2'-halogen-substituted nucleotides. Some non-limiting examples of modifications at the 2' position of sugar moieties which are useful in the present invention include OH, SH, SCH₃, F, OCH₃, OCN, O(CH₂)_n NH₂ and O(CH₂)_n CH₃, where n is from 1 to about 10. Such oligonucleotides are functionally interchangeable with natural oligonucleotides or synthesized oligonucleotides, which have one or more differences from the natural structure.

[0083] The nucleic acid therapeutic agents may cause the expression of a polypeptide of interest, e.g., through gene therapy (see Section 5.3.1.1). The polypeptide of interest to be expressed may be endogenous in which the target cell or cells are deficient in some manner such as through mutation or underexpression (e.g., tumor suppressor genes such as p53, p110Rb, and p72) or exogenous (e.g., cytotoxins such as diphtheria toxin, Pseudomonas exotoxin A, pertussis toxin, and the pertussis adenylate cyclase). Alternatively, the nucleic acid therapeutic agents may inhibit the expression of a polypeptide of interest through, e.g., antisense oligonucleotides or RNAi molecules (see Sections 5.3.1.2 and 5.3.1.3, respectively).

[0084] Various assays may be used to test the ability of nucleic acid therapeutic agent to cause the expression of (e.g., gene therapy) or inhibit the expression of (e.g., antisense oligonucleotides or RNAi) a polypeptide of interest. For example, mRNA levels can be assessed by Northern blot analysis (Sambrook et al., 1989; Ausubel et al.,

1992; J.C. Alwine et al. 1977, *Proc. Natl. Acad. Sci. USA* **74**:5350-5354; I.M. Bird, 1998, *Methods Mol. Biol.* **105**:325-36), quantitative or semi-quantitative RT-PCR analysis (see, e.g., W.M. Freeman et al., 1999, *Biotechniques* **26**:112-122; Ren et al., 1998, *Mol. Brain Res.* **59**:256-63; J.M. Cale et al., 1998, *Methods Mol. Biol.* **105**:351-71), or *in situ* hybridization (reviewed by A.K. Raap, 1998, *Mutat. Res.* **400**:287-298) can be used to measure RNA levels. Alternatively, polypeptide levels can be measured, e.g., by western blot analysis, indirect immunofluorescence, or immunoprecipitation techniques (see, e.g., J.M. Walker, 1998, *Protein Protocols on CD-ROM*, Humana Press, Totowa, NJ).

5.3.1.1 GENE THERAPY

[0085] Nucleic acid therapeutic agents that encode a polypeptide of interest may be delivered by the vehicles of the invention. Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[0086] For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488; Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926-932; Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191; May, 1993, *TIBTECH* 11:155; and Friedman, 1991, *Therapy for Genetic Diseases*, Friedman, Ed., Oxford University Press, pages 105-121. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990).

[0087] In one embodiment, a nucleic acid therapeutic agent is a nucleic acid molecule that encodes a polypeptide of interest, wherein expression of that polypeptide of interest in an affected cell or tissue type is desired, said nucleic acid being part of an expression vector that expresses the nucleic acid in a suitable host. In particular, such nucleic acids have promoters, preferably heterologous promoters, said promoter being inducible or constitutive, and, optionally, tissue-specific. Alternatively, nucleic acid molecules are used in which the nucleic acid therapeutic agent is flanked by regions

that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the nucleic acid therapeutic agent. (Koller and Smithies, 1989, *PNAS* 86:8932; Zijlstra et al., 1989, *Nature* 342:435).

[0088] In a specific embodiment, viral vectors that contain the nucleic acid therapeutic agents are used. For example, a retroviral vector can be used (see Miller et al., 1993, *Meth. Enzymol.* 217:581). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the nucleic acid into a subject. More detail about retroviral vectors can be found in Boesen et al., 1994, *Biotherapy* 6:291-302, which describes the use of a retroviral vector to deliver the *mdr 1* gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., 1994, *J. Clin. Invest.* 93:644-651; Klein et al., 1994, *Blood* 83:1467-1473; Salmons and Gunzberg, 1993, *Human Gene Therapy* 4:129-141; and Grossman and Wilson, 1993, *Curr. Opin. in Genetics Devel.* 3:110-114.

[0089] A number of viruses have been used as gene transfer vectors, including polyoma, *i.e.*, SV40 (Madzak et al., 1992, *J. Gen. Virol.*, **73**:1533-1536), adenovirus (Berkner, 1992, *Curr. Top. Microbiol. Immunol.* **158**:39-6; Berkner et al., 1988, *Bio Techniques*, **6**:616-629; Gorziglia et al., 1992, *J. Virol.*, **66**:4407-4412; Quantin et al., 1992, *Proc. Natl. Acad. Sci. USA*, **89**:2581-2584; Rosenfeld et al., 1992, *Cell*, **68**:143-155; Wilkinson et al., 1992, *Nucl. Acids Res.*, **20**:2233-2239; Stratford-Perricaudet et al., 1990, *Hum. Gene Ther.*, **1**:241-256), vaccinia virus (Mackett et al., 1992, *Biotechnology*, **24**:495- 499), adeno-associated virus (Muzyczka, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:91- 123; Ohi et al., 1990, *Gene*, **89**:279-282), herpes viruses including HSV and EBV (Margolskee, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:67-90; Johnson et al., 1992, *J. Virol.*, **66**:2952-2965; Fink et al., 1992, *Hum. Gene Ther.*, **3**:11-19; Breakfield et al., 1987, *Mol. Neurobiol.*, **1**:337-371; Fresse et al., 1990, *Biochem. Pharmacol.*, **40**:2189-2199), and retroviruses of avian (Brandyopadhyay et al., 1984, *Mol. Cell Biol.*, **4**:749-754; Petropouplos et al., 1992, *J. Virol.*, **66**:3391-3397), murine (Miller, 1992, *Curr. Top. Microbiol. Immunol.*, **158**:1-24; Miller et al., 1985, *Mol. Cell Biol.*, **5**:431-437;

Sorge et al., 1984, *Mol. Cell Biol.*, **4**:1730-1737; Mann et al., 1985, *J. Virol.*, **54**:401-407), and human origin (Page et al., 1990, *J. Virol.*, **64**:5370-5276; Buchschalcher et al., 1992, *J. Virol.*, **66**:2731-2739). Most human gene therapy protocols have been based on disabled murine retroviruses.

5.3.1.2 ANTISENSE

[0090] Therapeutic agents for use in the methods of the invention can be antisense nucleic acid molecules, *i.e.*, molecules which are complementary to all or part of a sense nucleic acid encoding a polypeptide of interest, molecules which are complementary to the coding strand of a double-stranded cDNA molecule of a polypeptide of interest, or molecules complementary to an mRNA sequence of interest. Accordingly, an antisense nucleic acid can hydrogen bond to a sense nucleic acid. The antisense nucleic acid can be complementary to an entire coding strand, or to only a portion thereof, *e.g.*, all or part of the protein coding region (or open reading frame). An antisense nucleic acid molecule can be antisense to all or part of a non-coding region of the coding strand of a nucleotide sequence encoding a polypeptide of the invention. The non-coding regions ("5' and 3' untranslated regions") are the 5' and 3' sequences which flank the coding region and are not translated into amino acids. Appropriate oligonucleotides can be designed using OLIGO software (Molecular Biology Insights, Inc., Cascade, CO; <http://www.oligo.net>).

[0091] An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense

orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest).

[0092] The antisense nucleic acid molecules are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a selected polypeptide to thereby inhibit expression, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix.

[0093] An antisense nucleic acid molecule can be an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier et al., 1987, *Nucleic Acids Res.* 15:6625). The antisense nucleic acid molecule can also comprise a 2'-*o*-methylribonucleotide (Inoue et al., 1987, *Nucleic Acids Res.* 15:6131) or a chimeric RNA-DNA analogue (Inoue et al., 1987, *FEBS Lett.* 215:327).

[0094] In one embodiment, the polypeptide of interest to be inhibited is NGF. NGF antisense nucleic acids have been described by, *e.g.*, K.A. Chang et al., 1999, *J. Mol. Neurosci.* **12**(1):69-74; C. Culmsee et al., 1999, *Neurochem. Int.* **35**(1):47-57; F. Hallbook et al., 1997, *Antisense Nucleic Acid Drug Dev.* **7**(2):89-100. Such antisense nucleic acids can be used with the vehicles of the invention for treating NGF-related diseases, including disorders of the brain (*e.g.*, Alzheimer's) (see, *e.g.*, K.A. Chang et al., 1999, *J. Mol. Neurosci.* **12**(1):69-74; R. Hellweg et al., 1998, *Int. J. Dev. Neurosci.* **16**(7-8):787-94) and bladder (*e.g.*, inflammation and dysfunction) (see, *e.g.*, M.A. Vizzard, 2000, *Exp. Neurol.* **161**(1):273-84; D. Oddiah et al., 1998, *Neuroreport.* **9**(7):1455-8; M.C. Dupont et al., 1995, *Adv. Exp. Med. Biol.* **385**:41-54).

5.3.1.3 RNA INTERFERENCE

[0095] Therapeutic agents for use in the methods of the can be an RNA interference (RNAi) molecule. RNAi is defined as the ability of double-stranded RNA (dsRNA) to suppress the expression of a gene corresponding to its own sequence.

RNAi is also called post-transcriptional gene silencing or PTGS. Since the only RNA molecules normally found in the cytoplasm of a cell are molecules of single-stranded mRNA, the cell has enzymes that recognize and cut dsRNA into fragments containing 21-25 base pairs (approximately two turns of a double helix). The antisense strand of the fragment separates enough from the sense strand so that it hybridizes with the complementary sense sequence on a molecule of endogenous cellular mRNA. This hybridization triggers cutting of the mRNA in the double-stranded region, thus destroying its ability to be translated into a polypeptide. Introducing dsRNA corresponding to a particular gene thus knocks out the cell's own expression of that gene in particular tissues and/or at a chosen time.

[0096] Double-stranded (ds) RNA can be used to interfere with gene expression in mammals (Wianny & Zernicka-Goetz, 2000, *Nature Cell Biology* 2: 70-75; incorporated herein by reference in its entirety). dsRNA is used as inhibitory RNA or RNAi of the function of a polypeptide of interest to produce a phenotype that is the same as that of a null mutant of the polypeptide of interest (Wianny & Zernicka-Goetz, 2000, *Nature Cell Biology* 2: 70-75).

5.3.2 POLYPEPTIDES AS THERAPEUTIC AGENTS

[0097] Therapeutic agents for use in the methods of the invention can be polypeptides or peptides. The terms protein and polypeptide are synonymous as used herein, and refer to polymers comprising amino acid residues linked by peptide bonds. Peptides are defined as fragments or portions of polypeptides, preferably fragments or portions having at least one functional activity (e.g., binding, antigenic, or catalytic activity) as the complete polypeptide sequence (see, e.g., by H. Lodish et al., 1999, *Molecular Cell Biology*, W. H. Freedman and Sons, NY; L. Stryer, 2001, *Biochemistry*, W. H. Freedman and Sons, NY; B. Lewin, 1999, *Genes VII*, Oxford University Press). As used herein, the term polypeptide therapeutic agent refers to therapeutic agents comprising either polypeptides, peptides, or both polypeptides and peptides. Polypeptide therapeutic agents may be endogenous polypeptides in which the target cell or cells are deficient in some manner such as through mutation or underexpression (e.g., tumor suppressor genes such as p53, p110Rb, and p72) or exogenous

polypeptides (e.g., cytotoxins such as diphtheria toxin, *Pseudomonas* exotoxin A, pertussis toxin, and the pertussis adenylate cyclase).

[0098] Such therapeutic agents may be isolated from natural sources or, more preferably, may be made recombinantly. Once a polynucleotide encoding a polypeptide therapeutic agent has been obtained, a vector for the production of the polypeptide therapeutic agent may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing polypeptide coding sequences and appropriate transcriptional and translational control signals. Thus, methods for preparing a protein by expressing a polynucleotide containing are described herein. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination.

[0099] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce a polypeptide therapeutic agent. A variety of host-expression vector systems may be utilized to express polypeptide therapeutic agents (see, e.g., U.S. Patent No. 5,807,715). Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express a polypeptide therapeutic agent of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing polypeptide therapeutic agent coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing polypeptide therapeutic agent coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, NS0, and 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of

mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant polypeptide therapeutic agent, are used for the expression of a polypeptide therapeutic agent. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for polypeptide therapeutic agents (Foecking et al., 1986, *Gene* 45:101; and Cockett et al., 1990, *BioTechnology* 8:2). In a specific embodiment, the expression of nucleotide sequences encoding a polypeptide therapeutic agent is regulated by a constitutive promoter, inducible promoter or tissue specific promoter.

[0100] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the polypeptide being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited to, the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO* 12:1791), in which the polypeptide therapeutic agent coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as *fusion* proteins with glutathione 5-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0101] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The polypeptide therapeutic agent coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and

placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0102] In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the polypeptide coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region EI or E3) will result in a recombinant virus that is viable and capable of expressing the polypeptide therapeutic agent in infected hosts (*e.g.*, see Logan & Shenk, 1984, *PNAS* 81:355-359). Specific initiation signals may also be required for efficient translation of inserted polypeptide coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see, *e.g.*, Bittner et al., 1987, *Methods in Enzymol.* 153:516-544).

[0103] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERO, BHK, HeLa, COS, MDCK, 293, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, NS0, CRL7030 and HsS78Bst cells.

[0104] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the polypeptide therapeutic agent may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the polypeptide therapeutic agent.

[0105] A number of selection systems may be used, including but not limited to, the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), glutamine synthetase, hypoxanthine guanine phosphoribosyltransferase (Szybalska & Szybalski, 1992, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:8-17) genes can be employed in tk-, gs-, hgprt- or apt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: *dhfr*, which confers resistance to methotrexate (Wigler et al., 1980, *PNAS* 77:357; O'Hare et al., 1981, *PNAS* 78:1527); *gpt*, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *PNAS* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (Wu and Wu, 1991, *Biotherapy* 3:87; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573; Mulligan, 1993, *Science* 260:926; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62: 191; May, 1993, *TIB TECH* 11:155-); and *hygro*, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990); and in Chapters 12 and

13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1, which are incorporated by reference herein in their entireties.

[0106] Once a polypeptide therapeutic agent has been produced by recombinant expression, it may be purified by any method known in the art for purification of a polypeptide, for example, by chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Further, the polypeptide therapeutic agent may be fused to heterologous polypeptide sequence known in the art to facilitate purification.

[0107] Polypeptide therapeutic agents that are antibodies may be expressed using vectors which already include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., US Patent Nos. 5,919,900; 5,747,296; 5,789,178; 5,591,639; 5,658,759; 5,849,522; 5,122,464; 5,770,359; 5,827,739; International Patent Publication Nos. WO 89/01036; WO 89/10404; Bebbington et al., 1992, *BioTechnology* 10:169). The variable domain of the antibody may be cloned into such a vector for expression of the entire heavy, the entire light chain, or both the entire heavy and light chains. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule.

5.4 ADHESIVES

[0108] The vehicles of the present invention can comprise one or more adhesives, e.g., antibodies or mucoadhesives, that further improve duration or specificity of vehicle attachment to the target area, and provide a longer-term or more specific therapeutic effect or intravesical delivery platform for the one or more therapeutic agents. These adhesives can act to target the vehicles to specific areas (e.g., mucoadhesives) or specific cell types and/or receptors (e.g. antibodies).

5.4.1 ANTIBODIES

[0109] The vehicles of the present invention can further comprise one or more

antibodies as adhesives. Antibodies for use as adhesives include, but are not limited to, monoclonal antibodies, synthetic antibodies, recombinantly produced antibodies, monospecific antibodies, multispecific antibodies (including bispecific, trispecific, or of greater multiplicity that immunospecifically bind to different epitopes of the same polypeptide of interest or that immunospecifically bind to more than one polypeptide of interest), human antibodies, humanized antibodies, chimeric antibodies, synthetic antibodies, single-chain Fvs (scFv) (e.g., including monospecific and multispecific), Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), and epitope-binding fragments of any of the above. In particular, antibodies used in the methods of the present invention include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, *i.e.*, molecules that contain at least one antigen binding site that immunospecifically binds to an antigen of interest. The immunoglobulin molecules for use in the methods of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁ and IgA₂) or subclass of immunoglobulin molecule. The antibodies used in the methods of the invention may be from any animal origin including birds and mammals (e.g., human, murine, donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken). In a most preferred embodiment, the antibody is human or has been humanized. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from mice that express antibodies from human genes.

[0110] Antibodies may be obtained from commercial sources, e.g., Jackson ImmunoResearch Laboratories, Inc., West Grove, PA; Advanced Targeting Systems, San Diego, CA; Connex GmbH (Martinsried, Germany), Covance Research Products, Cumberland, VA; Pierce Endogen, Rockford, IL; DiaSorin, Stillwater, MN; and DAKO Corporation, Carpinteria, C; produced in an animal host (e.g., rabbit, goat, mouse, or other non-human mammal) by immunization with immunogenic components; or recombinantly.

[0111] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be

produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[0112] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. Briefly, mice can be immunized with a polypeptide of interest (either the full length protein or a domain thereof) and once an immune response is detected, e.g., antibodies specific for the polypeptide of interest are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 (available from the ATCC) or NHO cells. Hybridomas are selected and cloned by limited dilution. Hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of interest. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[0113] Accordingly, monoclonal antibodies can be generated by culturing a hybridoma cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with the polypeptide of interest or a fragment thereof with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind to the polypeptide of interest.

[0114] Antibody fragments which recognize specific epitopes from polypeptides of interest may be generated by any technique known to those of skill in the art. For example, Fab and F(ab')₂ fragments may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments)

or pepsin (to produce F(ab')₂ fragments). F(ab')₂ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain. Further, the antibodies can also be generated using various phage display methods known in the art.

[0115] In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In particular, DNA sequences encoding V_H and V_L domains are amplified from animal cDNA libraries (e.g., human or murine cDNA libraries of lymphoid tissues). The DNA encoding the V_H and V_L domains are recombined together with an scFv linker by PCR and cloned into a phagemid vector (e.g., p CANTAB 6 or pComb 3 HSS). The vector is electroporated in *E. coli* and the *E. coli* is infected with helper phage. Phage used in these methods are typically filamentous phage including fd and M13 and the V_H and V_L domains are usually recombinantly fused to either the phage gene III or gene VIII. Phage expressing an antigen binding domain that binds to the polypeptide of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Examples of phage display methods that can be used to make the antibodies include those disclosed in Brinkman et al., 1995, *J. Immunol. Methods* 182:41-50; Ames et al., 1995, *J. Immunol. Methods* 184:177; Kettleborough et al., 1994, *Eur. J. Immunol.* 24:952-958; Persic et al., 1997, *Gene* 187:9; Burton et al., 1994, *Advances in Immunology* 57:191-280; International Application No. PCT/GB91/01134; International Publication Nos. WO 90/02809, WO 91/10737, WO 92/01047, WO 92/18619, WO 93/11236, WO 95/15982, WO 95/20401, and WO97/13844; and U.S. Patent Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727, 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[0116] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described below. Techniques to recombinantly produce

Fab, Fab' and F(ab')₂ fragments can also be employed using methods known in the art such as those disclosed in International Patent Publication No. WO 92/22324; Mullinax et al., 1992, *BioTechniques* 12:864; Sawai et al., 1995, *AJRI* 34:26; and Better et al., 1988, *Science* 240:1041 (said references incorporated by reference in their entireties).

[0117] To generate whole antibodies, PCR primers including V_H or V_L nucleotide sequences, a restriction site, and a flanking sequence to protect the restriction site can be used to amplify the V_H or V_L sequences in scFv clones. Utilizing cloning techniques known to those of skill in the art, the PCR amplified V_H domains can be cloned into vectors expressing a V_H constant region, e.g., the human gamma 4 constant region, and the PCR amplified V_L domains can be cloned into vectors expressing a V_L constant region, e.g., human kappa or lambda constant regions. Preferably, the vectors for expressing the V_H or V_L domains comprise an EF-1 α promoter, a secretion signal, a cloning site for the variable domain, constant domains, and a selection marker such as neomycin. The V_H and V_L domains may also be cloned into one vector expressing the necessary constant regions. The heavy chain conversion vectors and light chain conversion vectors are then co-transfected into cell lines to generate stable or transient cell lines that express full-length antibodies, e.g., IgG, using techniques known to those of skill in the art.

[0118] For some uses, including *in vivo* use of antibodies in humans, it may be preferable to use human or chimeric antibodies. Completely human antibodies are particularly desirable for therapeutic treatment of human subjects. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also U.S. Patent Nos. 4,444,887 and 4,716,111; and International Patent Publication Nos. WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[0119] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain

immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then be bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of interest. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., International Patent Publication Nos. WO 98/24893, WO 96/34096, and WO 96/33735; and U.S. Patent Nos. 5,413,923, 5,625,126, 5,633,425, 5,569,825, 5,661,016, 5,545,806, 5,814,318, and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Medarex (Princeton, NJ) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[0120] A chimeric antibody is a molecule in which different portions of the antibody are derived from different immunoglobulin molecules such as antibodies having a variable region derived from a non-human antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known

in the art. See e.g., Morrison, 1985, *Science* 229:1202; Oi et al., 1986, *BioTechniques* 4:214; Gillies et al., 1989, *J. Immunol. Methods* 125:191-202; and U.S. Patent Nos. 5,807,715, 5,585,089, 4,816,567, and 4,816,397, and Riechmann et al., 1988, *Nature* 332:323, which are incorporated herein by reference in their entirety. Chimeric antibodies comprising one or more CDRs from a non-human species and framework regions from a human immunoglobulin molecule can be produced using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; International Patent Publication No. WO 91/09967; and U.S. Patent Nos. 5,225,539, 5,530,101, and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, 1991, *Molecular Immunology* 28(4/5):489-498; Studnicka et al., 1994, *Protein Engineering* 7:805; and Roguska et al., 1994, *PNAS* 91:969), and chain shuffling (U.S. Patent No. 5,565,332).

[0121] Polyclonal antibodies to polypeptides of interest can be prepared by immunizing a suitable subject (e.g., mouse or rat) with an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme linked immunosorbent assay (ELISA) using immobilized polypeptide or peptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A chromatography to obtain the IgG fraction.

[0122] The antibodies or antibody fragments can be conjugated to the liposomes or hydrogels using conventional techniques (see, e.g., M.J. Ostro (ed.) 1987, *Liposomes: from Biophysics to Therapeutics*, Marcel Dekker, New York, NY). One preferred method of preparing liposomes and conjugating immunoglobulins to their surface is described by Y. Ishimoto et al., 1984, *J. Immunol. Met.* **75**:351-360. In accordance with this method, multilamellar liposomes composed of dipalmitoylphosphatidylcholine, cholesterol, and phosphatidylethanolamine are prepared. Purified fragments are then coupled to the phosphatidylethanolamine by the cross-linking agent N-hydroxysuccinimidyl 3-(2-pyridyldithio) propionate. The coupling of the antibody or fragment to the liposome is demonstrated by the release of a pre-trapped marker, e.g., carboxyfluorescence, from the liposomes. This release occurs upon incubation with a secondary antibody against the conjugated antibody, fragment,

or complement.

[0123] The antibodies or antibody fragments can also be coupled to a liposome or hydrogel of the invention via carbohydrate moieties. Such coupling can be used provided that the carbohydrate moiety is not in the hypervariable region or at the antibody binding sites. In this way, conjugation via the cross-linking with the carbohydrate will not affect binding, and the binding sites will still be available to bind to cell surface antigens. One preferred method for coupling antibodies or antibody fragments (other than Fv) to a liposome or hydrogel involves conjugation through the carbohydrate moieties in the constant regions. This maximizes the number of available antigen-binding sites. Methods for derivatizing sugar ring moieties to create hydrazide groups for coupling with antibody fragments (and antibodies) have been established (see J.D. Rodwell et al., 1986, *Proc. Natl. Acad. Sci. USA* **83**:2632-36). Several immunoconjugates prepared in this way are in clinical studies or pending approval for routine clinical uses.

[0124] Binding of a monoclonal antibody to the surface of a liposome or hydrogel may also be accomplished by the formation of cross-linkage between phosphatidylethanolamine and the antibody using glutaraldehyde. Alternatively, a thiolated antibody can be allowed to react with a liposome or hydrogel comprising a lipid into which a maleimide group has been incorporated. Remaining maleimide groups on the surface of the liposome or hydrogel may be further reacted with a compound containing thiolated polyalkyleneglycol moiety. Thiolation of an antibody or antibody fragment may be achieved through use of N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), which is usually used for thiolation of protein, iminothiolane, or mercaptoalkylimidate. Alternatively, a dithiol group endogenous to an antibody may be reduced to form a thiol group. The latter method is preferred for maintaining antibody function. In accordance with another method, whole antibodies are treated with an enzyme such as pepsin to form F(ab)₂ fragments, which are then reduced with dithiothreitol (DTT) to form Fab fragments, which results in the production of one to three thiol groups. The conjugation of the thiolated antibody to the maleimide group-containing liposome or hydrogel may be accomplished by reacting the components in a neutral buffer solution at pH 6.5-7.5 for 2-16 hours.

[0125] In specific embodiments, the vehicles of the present invention are conjugated to antibodies or antibody fragments directed to NGF receptor or uroplakin.

[0126] For example, many cancers are characterized by overexpression of cell surface markers such as HER2, which is expressed in breast cancer cells, or IL-13 receptor, which is expressed in gliomas (reviewed in, e.g., J. Baselga et al., 1997, *Oncology* (Huntingt) **11**(3 Suppl 2):43-8; S. Menard et al., 2000, *J. Cell. Physiol.* **182**(2):150-62; W. Debinski, 1998, *Crit. Rev. Oncog.* **9**(3-4):255-68). Certain urogenitary tract cancers are characterized by expression of the uroplakin marker (see, e.g., X. Xu et al., 2001, *Cancer* **93**(3):216-21; J.J. Lu et al., 2000, *Clin. Cancer Res.* **6**(8):3166-71; U. Kaufmann et al., 2000, *Am. J. Clin. Pathol.* **113**(5):683-7; S.M. Li et al., 1999, *J. Urol.* **162**(3 Pt 1):931-5; I. Yuasa et al., 1999, *Int. J. Urol.* **6**(6):286-92; I. Yuasa et al., 1998, *Jpn. J. Cancer Res.* **89**(9):879-82; R.L. Wu et al., 1998, *Cancer Res.* **58**(6):1291-7). In addition, neurons are characterized by the expression of NGF receptor (reviewed by, e.g., L. Tessarollo, 1998, *Cytokine Growth Factor Rev.* **9**(2):125-37; E.C. Yuen EC, et al., 1996, *Brain Dev.* **18**(5):362-8; S.B. McMahon, 1996, *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **351**(1338):431-40; G. Dechant et al., 1994, *Prog. Neurobiol.* **42**(2):347-52). Thus, targeting moieties such as anti-HER2, anti-IL-13 receptor, and anti-NGF receptor antibodies or antibody fragments can be used to deliver the vehicle to the cell of choice. The bioactive agent and/or drug is thereby delivered to the specific cell type, providing a useful and specific therapeutic treatment.

5.4.2 MUCOADHESIVES

[0127] The vehicles of the present invention can further comprise one or more mucoadhesives as adhesives. Mucoadhesives are materials that bind the mucin layer of a biological membrane. Mucadhesives should preferably possess some general physiochemical properties such as predominantly anionic hydrophilicity with numerous hydrogen bond-forming groups, suitable surface property for wetting mucus/mucosal tissue surfaces, and sufficient flexibility to penetrate the mucus network or tissue crevices (Lenaerts and Gurny, 1990, Bioadhesive Drug Delivery Systems, CRC Press, pp. 25-42 and 65-72).

[0128] In one embodiment, the vehicles of the invention comprise a

mucoadhesive to facilitate better attachment to mucosal surfaces (e.g., the bladder wall).

[0129] Such mucoadhesives may be selected from a number of known synthetic, naturally-occurring or modified naturally-occurring substances which exhibit of tackiness. Substances appropriate for use as mucoadhesives include, but are not limited to, carboxymethyl methylcellulose, hydroxypropyl methylcellulose, other cellulose derivatives, poly(methylacrylate) derivatives, tragacanth, hyaluronic acid, caraya, locust bean, synthetic and natural gums such as algin, chitosan, starches, pectins, naturally-occurring resins, polyvinyl pyrrolidone, polyvinyl alcohol, and polyacrylic acid.

5.6 THERAPEUTIC APPLICATIONS

[0130] In various aspects, the present invention encompasses novel methods of treatment that utilize the disclosed vehicles. Methods of the invention can be used to treat disorders of the urinary system, gastrointestinal tract, pulmonary system, genitourinary tract, and other organs or body systems. In one embodiment, the body system to be treated by the methods of the invention is the urinary system, e.g., kidneys, ureters, bladders, sphincter muscles, and urethras. In another embodiment, the body system to be treated by the methods of the invention is the gastrointestinal system, e.g., esophagus, stomach, large intestine, and small intestine. In another embodiment, the body system to be treated by the methods of the invention is the pulmonary system, e.g., trachea, lungs, bronchi, bronchioles, alveoli, and cilia. In another embodiment, the body system to be treated by the methods of the invention is the genitourinary tract system, e.g., the bladder, kidney, urethra, ureter, prostate, penis, testes, seminiferous tubules, epididymis, vas deferens, seminal vesicles, bulbourethral (Cowper) glands, uterus, vagina, and fallopian tubes.

[0131] Methods of the invention can be used to treat disorders involving pain (e.g., neuropathic pain), involuntary muscle contractions, cancer, and infections. In one embodiment, the disorder to be treated involves pain including, but not limited to,

[0132] In one embodiment, disorders the bladder include, but are not limited to, spastic neurogenic bladder, hypotonic neurogenic bladder, and bladder hyperactivity,

pain, irritation, inflammation, micturition pattern alteration, incontinence, infection, and cancer.

[0133] Also included are conditions relating to IC and UDSD.

[0134] In another embodiment, the disorder to be treated involves involuntary muscle contractions, including, but not limited to, tremor (voice, head, and limb tremor); palatal myoclonus; dysthyroid myopathy, hemifacial spasms; tics; strabismus (e.g., concomitant strabismus and vertical strabismus); nystagmus; eyelid entropion; myokymia; bruxism (TMJ); tardive dyskinesic syndrome, lateral rectus palsy; hyperkinesias following hypoglossal-facial anastomosis; myoclonus of spinal cord origin; voice defects (e.g., stuttering); painful rigidity; tension headaches; lumbosacral strain and back spasm (myofascial); radiculopathy with secondary muscle spasm; spasticity; IC, spastic bladder; UDSD; achalasia (esophageal); pelvirectal spasms (anismus and vaginismus); segmental dystonia, focal dystonia (e.g., blepharospasm (lid apraxia); oromandiibular distonia, facial dystonia, lingual dystonia, cervical dystonia (torticollis) and spasticity; laryngeal dystonia (spasmodic dysphonia; adductor spasmodic dysphonia, and abductor spasmodic dysphonia); task-specific dystonia (occupational cramps, such as writer's cramps); idiopathic and secondary focal distonia), and other spastic disorders,). Treatments are also provided for furrows of the face and neck, including frown lines and facial wrinkles. Treatments of involuntary contractions may be directed to any muscle groups, including those associated with control of the eyes, lips, tongue, mouth, jaw, head, neck, face, arm, hand, finger, leg, trunk, vagina, cervix, bladder, and sphincter (e.g., esophageal, cardiac, pyloric, ileocaecal, O'Beirne, anal, urethra, and bladder neck sphincters).

[0135] In another embodiment, the disorder to be treated involves cancer including, but not limited to, transitional cell carcinomas, squamous cell carcinomas, and adenocarcinomas.

[0136] In some embodiments, a therapeutic can be administered in combination with one or more other therapeutic agents useful in the treatment, prevention or management of the disorder to be treated. At least one of the therapeutic agents is administered by a vehicle of the invention. The one or more other therapeutic agents

may or may not be administered by a vehicle of the invention. The term "in combination" is not limited to the administration of therapeutic agents at exactly the same time, but rather it is meant that the therapeutic are administered to a subject in a sequence and within a time interval such that they can act together to provide an increased benefit than if they were administered otherwise. For example, each therapeutic agent may be administered at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic effect. Each therapeutic agent can be administered separately, in any appropriate form and by any suitable route.

[0137] In various embodiments, the therapeutic agents are administered less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, no more than 24 hours apart or no more than 48 hours apart. In preferred embodiments, two or more therapeutic agents are administered within the same patient visit.

[0138] The dosage and frequency of administration of the therapeutic agent(s) will typically vary according to factors specific for each patient depending on the specific therapeutic agent(s) administered, the severity and type of disorder, the route of administration, as well as age, body weight, response, and the past medical history of the patient. Suitable regimens can be selected by one skilled in the art by considering such factors and by following, for example, dosages reported in the literature and recommended in the *Physician's Desk Reference* (56th ed., 2002). In some embodiments, suitable dosages of a therapeutic agent range from about 0.1 to 20, preferably about 0.5 to about 10, and more preferably 1.0 to 5.0 milligrams of active ingredient per kilogram body weight of individual per day.

[0139] The methods of the present invention can be used to treat an animal,

preferably a mammal, more preferably a human subject. The disclosed methods comprise administering a lipid vehicle to a mammal suffering from one or more of these conditions. In one aspect, the lipid vehicle carries a biological agent (e.g., nucleic acid, peptide, polypeptide, or antibody), drug (e.g., pain therapeutics, anticancer treatments, or antibiotics), or toxin (e.g., botulinum toxin). For example, if the disease is the result of infection by a pathogen, the nucleic acid can be an antisense oligonucleotide targeted against a DNA sequence in the pathogen that is essential for development, metabolism, or reproduction of the pathogen. As another example, if the disease is related to a genetic defect (i.e., wherein certain endogenous DNA is missing or has been mutated), resulting in under- or over-expression, the nucleic acid maybe the normal DNA sequence.

[0140] Several methods of *in vivo* lipofection have been reported. In the case of whole animals, the lipid vehicle may be injected into the blood stream, directly into a tissue, into the peritoneum, instilled into the trachea, or converted to an aerosol, which the animal breathes. For example, a single intravenous injection of 100 micrograms of a mixture of DNA and DOTMA:dioleoylphosphatidylethanolamine can be used to efficiently transfect all tissues (Zhu et al., 1993, *Science* **261**:209-211). It is also possible to use a catheter to implant lipid vehicles in a blood vessel wall, which can result in successful transfection of several cell types, including endothelial and vascular smooth muscle cells. In particular, aerosol delivery of a chloramphenicol acetyltransferase (CAT) expression plasmid complexed to cationic liposomes produces high-level, lung-specific CAT gene expression *in vivo* for at least 21 days (Stribling et al., 1992, *Proc. Natl. Acad. Sci. USA* **89**:11277-11281). One representative method for aerosol delivery has been performed for as follows: 1) 6 mg plasmid DNA and 12 μ M DOTMA/DOPE liposomes were each diluted to 8 ml with water and mixed; 2) equal volumes were placed into two Acorn I nebulizers (Marquest, Englewood, CO); 3) animals were loaded into an Intox small-animal exposure chamber (Albuquerque) and an air flow rate of 4L/min is used to generate the aerosol (about 90 min were required to aerosolize this volume); 4) the animals were removed from the chamber for 1-2 hr, and the procedure was repeated.

[0141] The vehicles of the present invention that carry a bioactive agent can be delivered in any suitable manner. For agents that are soluble in aqueous solution and insoluble in organic solvents, the lipid mixture to be used for the lipid dispersion or liposomes can be coated on the inside surface of a flask or tube by evaporating the solvent from a solution of the mixture. In general, the lipid mixture should be capable of forming vesicles having single or multiple lipid bilayer walls and encapsulating an aqueous core. The aqueous phase containing the dissolved agent (e.g., physiological saline solution) can then be added to the lipid, agitated to generate a suspension, and then optionally frozen and thawed up to several times.

[0142] In particular embodiments, the vehicles of the invention can be used with or without vanilloid (e.g., capsaicin) and/or botulinum toxin (e.g., botulinum toxin D), which can then be used alone or in combination with a chemotherapeutic agent, targeting antibody, or DNA construct designed for the treatment of bladder cancer. Specifically, vehicles or vehicles comprising vanilloid and/or botulinum toxin can be used to prevent, treat, or ameliorate pain or voiding dysfunction associated with bladder cancer. Lipid-based treatments for bladder cancer that employ chemotherapeutic agents (see, e.g., J.B. Bassett et al., 1986, *J. Urol.* **135**(3):612-5; C.P. Dinney et al., 1995, *J. Interferon Cytokine Res.* **15**(6):585-92; T. Tsuruta et al., 1997, *J. Urol.* 1997 **157**(5):1652-4; H Kiyokawa et al., 1999, *J. Urol.* **161**(2):665-7), targeting antibodies (see, e.g., J. Morgan et al., 1994, *Photochem. Photobiol.* **60**(5):486-96; A. Aicher et al., 1994, *Urol. Res.* **22**(1):25-32), and DNA constructs (e.g., Y. Horiguchi et al., 2000, *Gene Ther.* **7**(10):844-51; L.A. Larchian et al., 2000, *Clin. Cancer Res.* **6**(7):2913-20; M. Cemazar et al., 2002, *Cancer Gene Ther.* **9**(4):399-406) are known in the art.

[0143] In another embodiment, vehicles of the invention may be used with or without vanilloid (e.g., capsaicin) and/or botulinum toxin (e.g., botulinum toxin D), which can then be used alone or in combination with one or more antibacterial agents. Specifically, vehicles or vehicles comprising vanilloid and/or botulinum toxin can be used to prevent, treat, or ameliorate pain or voiding dysfunction associated with a urinary system infection. Lipid-based treatments for infection are generally known in the art, including those employing tetracycline and doxycycline (L. Sangare et al., 1999, *J. Med. Microbiol.* **48**(7):689-93; L. Sangare et al., 1998, *J. Antimicrob. Chemother.*

42(6):831-4.); tobramycin (C. Beaulac et al., 1999, *J. Drug Target.* 7(1):33-41); gentamycin and ceftazidime (R.M. Schiffelers et al., 2001, *Int. J. Pharm.* 214(1-2):103-5; R.M. Schiffelers et al., 2001, *J. Pharm. Exp. Ther.* 298(1):369-75); anthracycline (N. Dos Santos et al., 2002, *Biochem. Biophys. Acta* 1561(2):188-201); ciprofloxacin (B. Wiechens et al., 1999, *Ophthalmologica* 213(2):120-8), and other anti-infectives.

5.6.1 PAIN TREATMENTS

[0144] The present invention includes but is not limited to the following embodiments:

- A method of treating pain in an organ in a mammalian subject which comprises administering to the subject a pharmaceutical composition comprising a vehicle of the invention in an amount effective to treat the condition.
- The method of the preceding embodiment, wherein the lipid vehicle is a liposome or a hydrogel.
- The method of any one of the preceding embodiments, wherein the organ is a genitourinary tract organ.
- The method of any one of the preceding embodiments, wherein the genitourinary tract organ is selected from the group consisting of a bladder, kidney, urethra, ureter, prostate, penis, testes, seminiferous tubules, epididymis, vas deferens, seminal vesicles, bulbourethral glands, uterus, vagina, and fallopian tubes.
- The method of any one of the preceding embodiments, wherein the organ is a gastrointestinal tract organ.
- The method of any one of the preceding embodiments, wherein the gastrointestinal tract organ is selected from the group consisting of esophagus, stomach, large intestine, and small intestine.

- The method of any one of the preceding embodiments, wherein the pain is associated with a condition selected from the group consisting of infection, inflammation, irritation, cancer, and spasticity.
- The method of any one of the preceding embodiments, wherein the lipid vehicle is administered using a method selected from the group consisting of intravesical instillation, intravenous, topical, nasal spray, pulmonary inhaler, and oral administration.
- The method of any one of the preceding embodiments, wherein the vehicle of the invention further comprises a vanilloid compound.
- The method of any one of the preceding embodiments, wherein the vanilloid is selected from the group consisting of capsaicin, resiniferatoxin, and tinyatoxin.
- The method of any one of the preceding embodiments, wherein the vehicle is a liposome or a hydrogel.
- The method of any one of the preceding embodiments, wherein the organ is a genitourinary tract organ.
- The method of any one of the preceding embodiments, wherein the genitourinary tract organ is selected from the group consisting of a bladder, kidney, urethra, ureter, prostate, penis, testes, seminiferous tubules, epididymis, vas deferens, seminal vesicles, bulbourethral (Cowper) glands, uterus, vagina, and fallopian tubes.
- The method of any one of the preceding embodiments, wherein the organ is a gastrointestinal tract organ.
- The method of any one of the preceding embodiments, wherein the gastrointestinal tract organ is selected from the group consisting of esophagus, stomach, large intestine, and small intestine.
- The method of any one of the preceding embodiments, wherein the organ is a pulmonary system organ.

- The method of any one of the preceding embodiments, wherein the pulmonary system organ is selected from the group consisting of trachea, lungs, bronchi, bronchioles, alveoli, and cilia.
- The method of any one of the preceding embodiments, wherein the vehicle of the invention is administered by a method selected from the group consisting of intravesical instillation, intravenous, topical, nasal spray, pulmonary inhaler, and oral administration.
- The method of any one of the preceding embodiments, wherein the carrier, excipient, or diluent comprises physiological saline.

5.6.2 MUSCLE TREATMENTS

[0145] The present invention includes but is not limited to the following embodiments:

- A method of treating involuntary muscle contraction in a mammalian subject which comprises administering to the subject a pharmaceutical composition comprising a vehicle of the invention carrying botulinum toxin in an amount effective to treat the contraction.
- The method of the preceding embodiment, wherein the vehicle is a liposome or hydrogel.
- The method of any one of the preceding embodiments, wherein the botulinum toxin is selected from the group consisting of botulinum toxins A through G.
- The method of any one of the preceding embodiments, wherein the involuntary muscle contraction affects a body part selected from the group consisting of the eye, lip, tongue, mouth, jaw, head, neck, face, arm, hand, finger, leg, trunk, vagina, cervix, and bladder.
- The method of any one of the preceding embodiments, wherein the involuntary muscle contraction affects a sphincter selected from the group consisting of

esophageal, cardiac, pyloric, ileocaecal, O'Beirne, anal, urethra, and bladder neck sphincters.

- The method of any one of the preceding embodiments, wherein the involuntary muscle contraction is associated with a condition selected from the group consisting of tremors, hemifacial spasms, tics, strabismus, nystagmus, eyelid entropion, myokymia, bruxism, tardive dyskinesia, lateral rectus palsy, stuttering, painful rigidity, tension headache, back spasm, radiculopathy, spasticity, spastic bladder, urinary detrusor-sphincter dyssynergia, achalasia, anismus, vaginismus, segmental dystonia, idiopathic dystonia, and secondary focal dystonia.
- The method of any one of the preceding embodiments, wherein the involuntary muscle contraction is associated with a focal dystonia selected from the group consisting of blepharospasm, oromandibular dystonia, facial dystonia, lingual dystonia, cervical dystonia, torticollis, spasmodic dysphonia, and task-specific dystonia.
- The method of any one of the preceding embodiments, wherein the vehicle of the invention is administered by a method selected from the group consisting of intravesical instillation, intravenous, topical, nasal spray, pulmonary inhaler, and oral administration.
- The method of any one of the preceding embodiments, wherein the carrier, excipient, or diluent comprises physiological saline.

5.5 PHARMACEUTICAL COMPOSITIONS AND ADMINISTRATION

[0146] A therapeutic agent can be incorporated into a vehicle of the invention for delivery either alone or as a part of a pharmaceutical composition. Furthermore, a vehicle of the invention can be administered to a subject either alone or as a part of a pharmaceutical composition. Therefore, the compositions of the invention include bulk drug compositions useful in the manufacture of pharmaceutical compositions (e.g., impure or non-sterile compositions) and pharmaceutical compositions (*i.e.*, compositions that are suitable for administration to a subject or patient) which can be

used in the preparation of unit dosage forms. Such compositions comprise a prophylactically or therapeutically effective amount of a vehicle of the invention and a pharmaceutically acceptable carrier and/or a prophylactically or therapeutically effective amount of one or more therapeutic agents and a pharmaceutically acceptable carrier.

[0147] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, excipient, adjuvant, or other composition with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like.

[0148] Generally, the ingredients of compositions of the invention are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0149] The compositions of the invention can be formulated as neutral or salt

forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric, mandelic acids, etc.; those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.; and those formed with organic bases such as those derived from isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[0150] Further guidance in preparing pharmaceutical formulations is found in, e.g., Gilman et al. (eds), 1990, *Goodman and Gilman's: The Pharmacological Basis of Therapeutics*, 8th ed., Pergamon Press; and *Remington's Pharmaceutical Sciences*, 17th ed., 1990, Mack Publishing Co., Easton, PA; Avis et al. (eds), 1993, *Pharmaceutical Dosage Forms: Parenteral Medications*, Dekker, New York; Lieberman et al. (eds), 1990, *Pharmaceutical Dosage Forms: Disperse Systems*, Dekker, New York.

[0151] Various methods of administering a vehicle of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intravesical, transdermal, intra-arterial, intrathecal, and enteral), epidural, and mucosal (e.g., intranasal, inhaled, sublingual, oral, and rectal routes). The vehicles of the invention may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral, nasal, rectal, intestinal, and vaginal mucosa, etc.). In preferred embodiments, the vehicles of the invention are administered through a catheter to the desired area (including, but not limited to, the bladder, genitourinary tract, gastrointestinal tract). Administration can be systemic or local and may be done together with other biologically active agents.

[0152] In a specific embodiment, vehicles of the invention are administered by intravesical instillation.

6. EXAMPLES

[0153] The examples as set forth herein are meant to exemplify the various aspects of the present invention and are not intended to limit the invention in any way.

6.1 EXAMPLE 1

[0154] Fluorescein isothiocyanate (FITC) was used as a model drug to demonstrate sustained intravesical drug delivery to the rat bladder using the a hydrogel. Bladders of normal rats were instilled with either free FITC in PBS or FITC in hydrogel. Urine was collected over time and the amount of FITC in each was measured to access the ability of the hydrogel to sustain prolonged drug exposure.

Materials and Methods

[0155] Synthesis of Polymer: Modified thermosensitive triblock co-polymer, (polyethylene glycol- poly[lactic acid-co-glycolic acid]-polyethylene glycol) PEG-PLGA-PEG was synthesized according to a protocol modified from procedures previously described (Jeong et al, 1999, Colloids and Surface B: Biointerfaces 16:185, Jeong et al., 2000, J. Biomed. Mater. Res. 50:171, and Kim et al., 2001, Pharm. Res. 50:440).

[0156] PEG-PLGA-PEG is a triblock copolymer made in two stages. First, a diblock polymer was made. Lactic acid, glycolic acid and polyethylene glycol were mixed in a particular ratio in toluene using stannous 2-ethyl hexonate as a catalyst (e.g., glycolic acid and lactic acid in the ratio of 1:3 and 3.5 gram of polyethylene glycol (mw 750)). The mixture was refluxed at 150°C for 4 hour. Toluene was removed using rotary evaporator and the diblock polymer was purified by fractional precipitation using methylene chloride and heptane and dried overnight under vacuum. The molecular weight of diblock copolymers was controlled at around 3,000~4,500. Second, a triblock polymer was made. A particular amount of previously synthesized diblock copolymer (3 g) was used for the synthesis of the triblock copolymer in a coupling reaction using hexamethylene diisocyanate (HMDI) as a catalyst in toluene at 40-50°C for 15-20 hour followed by reflux at 120°C for 5 hour. Toluene was removed from the synthesized triblock copolymer and the copolymer was purified by fractional precipitation from methylene chloride using heptane. Finally, the product was dried for about 36 h under reduced pressure up to constant weight. The triblock nature of the copolymer was confirmed by ¹H NMR and then the molecular weight of the triblock polymer was determined to be (Mw = 12052, Mn = 8532 and the polydispersity index of 1.56) by GPC. The aqueous solutions (30%, w/v) of this polymer flowed freely at room

temperature but formed a gel at 37°C (FIG. 1).

[0157] Using this modified synthesis procedure, the hydrophobicity of the triblock copolymer is increased. The reaction conditions in the first step determined the length of the PLGA block in the final product. Increased PLGA caused increased hydrophobicity.

[0158] Preparation of solutions to be instilled: A 0.02%w/v solution of free FITC in 0.1 M phosphate buffer without any polymer was instilled in control rats. The same amount of FITC in 0.1M phosphate buffer pH 7.2 was added to a 30% dispersion of PEG-PLGA-PEG polymer at room temperature by constant shaking in dark for 3h.

[0159] Instillation into the animals: Female Sprague-Dawley rats in the weight range of 200- 250g were used. Six animals were included in each treatment group. Before intravesical instillation, animals were lightly anaesthetized with halothane. Volume of instillation into the rat bladder was kept at 0.5ml of free FITC solution or FITC entrapped in hydrogel. Intravesical administration was done using a PE-50 tubing (Clay-Adams, Parsipanny, NJ), inserted into the bladder through the urethra and tied in place by a ligature around the urethral orifice. Under continuing halothane anesthesia, the urethra was left tied for 30min after instillation to prevent evacuation by the animal, as well as to allow enough time for sol-gel transformation to occur inside the bladder. All animals were given subcutaneous injection of antibiotic (Pen-strep, 30mg/kg) to prevent any infection as a result of the procedure. All protocols involving the use of animals in this study adhered to "Principles of Laboratory Animal Care" (NIH publication #85-23) and were approved by the IACUC of the University of Pittsburgh.

[0160] Urine collection. After recovery from halothane anesthesia, rats were kept in metabolic cages with food and water *ad libitum* for next 24 h to study the kinetics of FITC excretion in the urine. Urine was collected at time points chosen arbitrarily. Cumulative urine output was measured from the volume of urine collected at these time points. Animals were sacrificed by carbon dioxide euthanasia and bladders were removed and immediately frozen for cryosections.

[0161] Fluorescence measurement: Collected urine was diluted several fold to reduce the background contributed by other urine constituents. Fluorescence emitted

by FITC was measured using Perkin Elmer spectrofluorimeter at λ_{Ex} = 496nm and λ_{Em} = 512nm.

[0162] *Statistical analysis:* All values in the text and figures are mean values $\pm$ SEM. Student t test was used to test for significance between unpaired groups.

Results

[0163] Urine collected at various time points from rats in metabolic cages was used to calculate cumulative urine output over a 24h time period (FIG. 2). Mean cumulative urine output of rats instilled with free FITC was 21.35 ± 2.73 ml and that of rats instilled with FITC entrapped in hydrogel was 19.55 ± 2.72 ml (number of animal in each group was 6). No statistical significant difference was detected between the groups. The fluorescence intensity of urine measured at different time points from the collected urine was plotted (FIG. 3). Fluorescence intensity was normalized for the volume of urine collected at each time point. A significantly higher fluorescence signal was measured in the urine collected at the first time point of 2h from the rats instilled with free FITC solution ($p < 0.01$). However, at the later time point of 8, 16 and 24h, a higher signal was observed in the urine of rats instilled with FITC entrapped in hydrogel with statistical significance for later two time points ($p < 0.01$). Bladders from both groups were isolated 24h after instillation for examination under ultra violet light with the help of a dissecting microscope to detect the presence of any adhering FITC with the bladder. Bladders instilled with FITC entrapped in hydrogel exhibited bright green fluorescence of FITC whereas only background fluorescence was visible in the rats instilled with free FITC (data not shown). Within 24 h after instillation, the hydrogel did not completely fill the bladder but did attach to the inner surface of the bladder as a smooth (data not shown).

Summary

[0164] No significant difference in urine output with the treatment of hydrogel during the 24h period was observed (FIG. 2) indicating that the soft gel did not obstruct the elimination of urine from the bladder. The difference observed in the cumulative urine output between two groups did not proved to be statistically significant. The presence of the hydrogel inside the bladder after instillation of a free flowing liquid

indicates that this vehicle could produce a prolonged drug exposure to the urothelium, even after multiple voidings post-instillation of the hydrogel.

[0165] It follows naturally from the retention inside the bladder of hydrogel delivery system, that it can withstand the hostile environment of rat urinary bladder. The hydrogel could also resist being washed away by the flush of urine during the multiple voidings that occurred during 24h. The higher fluorescence signal in the control group at the initial time point of 2h demonstrated the drawback of conventional vehicles used for intravesical drug delivery (FIG. 3). Animals instilled with FITC entrapped inside a hydrogel showed a lower fluorescence at the early time point presumably because FITC did not exist in the bladder as free FITC, but rather it was sequestered in the hydrogel and was released over a period of time.

[0166] FITC contained in the hydrogel is probably released at first by diffusion, and later by the combination of both diffusion and degradation mechanisms. Moreover, the reversible nature of gelation process of a thermosensitive hydrogel can be exploited for easy removal of the gel from the bladder if desired at any time after administration by simply rinsing the bladder with sterile water at room temperature. A lower temperature of instilled water should convert from the gel state of the polymer back to its sol state allowing it to be voided in the urine.

6.2 EXAMPLE 2

[0167] The use of the hydrogel for prolonged drug release was further validated using rats with chemically induced cystitis. Misoprostol, a water soluble drug, was administered in various forms to ameliorate the symptoms of the chemically induced cystitis. Misoprostol is widely used in the clinic for the treatment of gastric and duodenal ulcer induced by chronic consumption of NSAIDs (nonsteroidal anti-inflammatory drugs) such as aspirin and ibuprofen. Misoprostol is a stable analogue of PGE₁ a cytoprotective eicosanoid (cyclooxygenase mediated arachidonic acid metabolite) having several anti-inflammatory effects, including down-regulation of the cytokine response of both macrophages and lymphocytes (Bulger and Maier, 2000, Crit. Care Med. 28:N27). The native drug is a racemate of four stereoisomers, available as a viscous liquid form, which is difficult to formulate due to its chemical instability.

Misoprostol rapidly de-esterifies to its active form, misoprostolic acid after oral administration, which is excreted in urine as its inactive metabolites with an elimination half-life of approximately 30 minutes.

Materials and Methods

[0168] Efficacy studies: Adult female Sprague Dawley rats were injected intraperitoneally with a high dose of cyclophosphamide (150mg/kg) under halothane anesthesia to induce cystitis (Cox et al, 1979, Biochem. Pharmacol. 28:2045). Rats were either instilled with saline alone, hydrogel alone, or misoprostol in saline or hydrogel. Commercially available misoprostol was used (Cytotec tablets from Searle). Volume of instillation for all animals was 0.5ml with misoprostol-treated animals receiving 50µg of misoprostol. Rats were kept in the metabolic cage after instillation for 24h followed by cystometric measurement (CMG) under urethane anesthesia (1.2g/kg, subcutaneous s/c) to measure frequency of micturition.

[0169] Cystometric Measurement. Cystometric measurements (CMG) was done as previously reported (Fraser et al., 2003, Urology 61:656). Briefly, animals were anesthetized with urethane (1.2 g/kg s.c.) before PE50 tubing (Clay-Adams, Parsippany, NJ) was inserted into the bladder through the urethra and using a three way stopcock the catheter was connected to a pressure transducer for recording intravesical pressure and to a syringe pump for infusing saline into the bladder. The catheter system was filled with 0.9% w/v saline. After the bladder was emptied, a CMG was performed to measure micturition frequency, which was the number of voiding contractions occurring during a 60 min time period of transurethral saline infusion at the rate of 0.04ml/min. After completion of CMG, bladders were isolated, fixed in Zamboni fixative and then cryosectioned for staining with haematoxylin and eosin.

Results

[0170] Cystitis induced by injection of cyclophosphamide lead to urinary incontinence in rats marked by wet and dirty perineal region around urethra. Rats instilled with saline had similar appearance during their stay in the metabolic cage. Rats kept in metabolic cages after being instilled with misoprostol entrapped in hydrogel were continent after cyclophosphamide injection.

[0171] Cystometric measurement performed under urethane anesthesia evaluated the micturition reflexes induced by saline infusion with a transurethral catheter at a rate of 0.04ml/min, which approximated the rate of physiological bladder filling with urine. CMGs performed on rats 24h after cyclophosphamide injection showed a dramatic decrease in the frequency of micturition in the rats instilled with misoprostol in hydrogel (0.04167 ± 0.01014) (FIG. 4D) compared to rats instilled with saline alone (0.1583 ± 0.04640 $n=3$ $p<0.05$) (FIG. 4A). Misoprostol alone (FIG. 4B) and hydrogel alone (FIG. 4C) offered only marginal improvement over saline instillation (FIG. 4A).

[0172] Histological assessment done by haemtoxylin and eosin staining revealed severe ulcerative cystitis in the saline instilled rats after cyclophosphamide injection (FIG. 5A). Epithelium was denuded leaving an ulcerated area with submucosal edema, inflammation and vascular ectasia and congestion. In tissue sections obtained from rats treated with misoprostol alone (FIG. 4B) and hydrogel alone (FIG. 4C), histological changes were less severe which included infiltration of inflammatory cells and edema of subepithelial tissue layer responsible for its slightly enlarged appearance. Treatment with misoprostol in hydrogel (FIG. 4D) showed drastic improvement, which was evident by lack of any ulcerations in the normal appearing epithelium with slightly enlarged appearance.

Summary

[0173] Urinary incontinence induced by cyclophosphamide was observed during the 24h metabolic cage stay of the rats. Simultaneous instillation of misoprostol in hydrogel enabled rats to maintain continence following intraperitoneal injection of cyclophosphamide. Cystometric measurements showed that misoprostol entrapped in hydrogel is available for its biological action as is evident from the decreased bladder contraction frequency in misoprostol treated rats (FIG. 4). Histological examination (FIG. 5) affirmed that the uroprotective action of misoprostol is retained when delivered intravesically using a hydrogel in rat model of cystitis.

[0174] Chemically induced cystitis is characterized by marked increase in erosions, ulcers in bladder including inflammatory cell infiltration, haemorrhages and

increased micturition frequency (Grinberg-Funes et al., 1990, J. Urol. 144:1500). A previous study done on the same model reported reduction in cyclophosphamide induced ulceration, inflammation and edema in bladder walls of male rat following long term oral administration of misoprostol (Gray et al., 1986, J. Urol. 136:497). A daily oral dose of 600 µg administered chronically for 3 months was also effective in patients with refractory interstitial cystitis (Kelly et al., 1998, Europ. Urol. 34:53). Results of the efficacy study showed that a protective effect could be seen using misoprostol in hydrogel in a single intravesical administration at a dose that was a fraction of the dose that had been administered orally.

6.3 EXAMPLE 3

[0175] The ability of the hydrogel to deliver capsaicin, a water insoluble drug, to the rat bladder was compared to liposome delivery or ethanol delivery of capsaicin.

Materials and methods

[0176] Preparation of liposome: Liposomes were prepared as previously reported (Fraser et al., 2003, Urology 61:656). Briefly, phosphatidylcholine, cholesterol and capsaicin in 2:1:1 mole ratio were coated inside a glass tube and dried in vacuum overnight to form a thin film inside the tube. The lipids were hydrated with normal saline the next day to form multilamellar vesicles.

[0177] Preparation of Hydrogel: The triblock hydrogel was prepared as described previously in Example 1. Required amount of PEG-PLGA-PEG copolymer was dispersed in 0.1 M phosphate buffer pH 7.4 to form a 30% w/v aqueous dispersion at room temperature. Aqueous dispersion of the copolymer was prepared by constant vortex at room temperature. For copolymers with capsaicin, the prepared copolymer dispersion was then added to a glass tube containing 1 mM capsaicin that had been purified from its stock solution solvent of ethanol by air drying.

[0178] Preparation of Ethanolic Solution: Capsaicin was added from its stock solution to normal saline containing 30% ethanol to produce a 1mM of capsaicin solution.

[0179] Instillation of Drugs: 1mM capsaicin entrapped in lipid bilayer of liposome,

dispersed in the polymer, or dissolved in ethanolic saline was instilled intravesically into the female Sprague-Dawley rats (200-300 g) (n= 8 for each group) under halothane anesthesia. The volume for intravesical instillation was 0.5 ml for each formulation of capsaicin. Subsequent to instillation, the urethra was ligated for 30 min to prevent evacuation and to allow enough time for gel formation in the bladder. Subsequently, bladders were emptied by pressing the lower abdomen and washed with 0.5 ml of saline. All the animals were also treated with antibiotic (Pen-strep, 30mg/kg, s.c.) to prevent infection.

[0180] Cystometric measurements (CMG): CMG was essentially performed as previously described in Example 2 48 h after intravesical instillation. After the bladder was emptied, a CMG was performed by filling with a constant infusion (0.04 ml/min) of saline. to measure the amplitude, pressure threshold, and frequency of reflex bladder contractions per minute. Measurements in each animal represented the average of 3 to 5 bladder contractions.

[0181] Histopathological analysis: After CMG, whole bladders were harvested, fixed in 10% buffered formalin, and cryopreserved. Tissue blocks were blind coded and sectioned (20 μ m thickness) for haematoxylin and eosin staining and immunohistochemistry of CGRP staining.

[0182] Statistical analysis: Quantitative data are expressed as means plus or minus standard error. Multiple comparisons among the different groups were analyzed by a single-factor ANOVA, followed by post hoc comparisons with Newman Keuls test, according to the Graph Pad prism v. 3.0 (GraphPad Software, San Diego, CA). Differences among groups were considered significant at $p < 0.05$.

Results

[0183] Capsaicin was administered to normal rat bladders while the animals were rats under urethane anesthesia and CMG measurements are shown in FIG. 6. Administration of capsaicin either in liposomes or 30% ethanol was able to produce blockade of micturition reflex (FIG. 6F and 6G, respectively). Absence of periodic bladder contractions in the CMG represents blockade of micturition reflex following capsaicin treatment, and raised plateau in intravesical pressure reflect urinary retention.

The mean bladder contraction frequency was considered zero for such animals, which was observed in 4 and 6 rats of the capsaicin treated groups using liposomes or 30% ethanol, respectively (n=8). The remaining rats in capsaicin treated groups using liposomes and ethanol showed decreased mean bladder contraction frequency with no significant difference between the two groups (0.01 ± 0.006 vs 0.01 ± 0.007 , $p > 0.05$) (FIG. 7).

[0184] Administration of capsaicin in hydrogel significantly reduced mean bladder contraction frequency compared to hydrogel alone (0.10 ± 0.021 vs 0.25 ± 0.033) (FIG. 6E and 6D, and FIG. 7). A similar significant difference of mean bladder contraction frequency was observed in ethanol treated groups in presence and absence of capsaicin (0.01 ± 0.00654 vs 0.12 ± 0.021) (FIG. 6B, 6G, and FIG. 7). However, liposome treated groups failed to show significant difference in the mean bladder contraction frequency in presence and absence of capsaicin (0.01 ± 0.007 vs 0.08 ± 0.025) (FIG. 6C, 6F, and FIG. 7). Mean bladder contraction frequency (0.25 ± 0.033) of hydrogel treated rats in absence of capsaicin was lower but not significantly different from saline treated rats (0.28 ± 0.02491) ($p > 0.05$) (FIG. 6A, 6D, and FIG. 7). Other CMG parameters such as pressure threshold and amplitude of bladder contractions were not affected by capsaicin treatment in various vehicles (data not shown).

[0185] None of the animals in the study showed any signs of urinary tract infection after instillation of the intravesical solutions. However, some of the treatments did irritate the rat bladders. The bladders of rats treated with 30% ethanol showed sign of severe redness throughout the bladder tissue, which turned to bleeding within the walls and ulceration at the bladder lumen both in the presence and absence of capsaicin (FIG. 8D and 8C, respectively) in contrast to the bladders of untreated rats or rats treated with saline (FIG. 8A and 8B, respectively). Only dilatation of blood vessels at the bladder dome was visible in the bladders of rats treated with liposomes alone or hydrogel alone in absence of capsaicin (FIG. 8E and 8G), which become more prominent in the presence of (FIG. 8F and 8H, respectively).

[0186] CGRP staining was performed to assess the amount of depletion of CGRP from nerve fibers by capsaicin. Capsaicin caused significant depletion of CGRP

when administered in either 30% ethanol or liposomes (FIG. 9B and 9D, respectively). However, capsaicin administered in hydrogel failed to produce the depletion observed in the ethanol and liposome treatment groups (FIG. 9F).

[0187] The mucosal lining of bladders of rats treated with various vehicles either with or without capsaicin was assessed. Bladders of rats treated with hydrogel alone or liposome alone demonstrated similar bladder mucosal histology (FIG. 10C and 10E, respectively). Intravesical instillation of 30% ethanol alone revealed distinct histological changes including, thinning and denuding of epithelium, submucosal edema, and vascular congestion (FIG. 10A). With the addition of capsaicin to the 30% ethanol, these changes were further aggravated and acute mucosal injury was visible (FIG. 10B). By comparison, histological changes produced by capsaicin delivered using either liposomes or hydrogel appeared mild with urothelium remaining intact (FIG. 10D and 10F).

Summary

[0188] Traditional anticholinergic therapies of hyperactive bladder target the efferent branch of micturition reflex. Treatment on the afferent branch of micturition reflex by using C-fiber neurotoxin capsaicin is an attractive alternative that would avoid systemic anticholinergic side effects. The role of capsaicin-sensitive bladder afferents in micturition control and bladder irritation is collectively supported by both close apposition of VR-1-expressing fibers with bladder smooth muscle cells as well as urothelial expression of VR-1 (Birder et al., 2001, PNAS 98:13396 and Chuang et al., Am J Physiol Regul Integr Comp Physiol 281:R1302). Intravesical instillation of capsaicin and resiniferatoxin for treatment of detrusor overactivity not only reduces the problem of systemic neurological toxicity, but also eliminates their significant first pass effect (Donnerer et al., 1990, Naunyn Schmiedebergs Arch Pharmacol 342:357). The presently accepted vehicle for intravesical capsaicin is 30% ethanol in saline. Unfortunately, the ethanol vehicle alone was observed to be substantially as irritating to the bladder mucosa as capsaicin itself in a study done on spinal cord injured patients (deSeze et al., 1998, Neurourol Urodyn 17:513). The pain and autonomic dysreflexia reported by some patients during and sometimes after the instillations has hindered

wider application of vanilloids in clinical use.

[0189] In this study, normal adult female rats were used to investigate the efficacy of capsaicin entrapped in the lipid bilayer of liposomes and in the hydrophobic matrix of thermosensitive hydrogel. Micturition in such rats with an intact neuraxis is dependent upon a spinobulbospinal reflex activated by A δ bladder afferents and their employment as animal model for comparison of efficacy for capsaicin formulations under urethane anesthesia is based on extensive information published by various laboratories Maggi et al., 1990, J Auton Nerv Syst 30:247, Mallory et al., 1989, Am J Physiol 257:R410, and Chuang et al., 2001, Am J Physiol Regul Integr Comp Physiol 281:R1302). Given this knowledge of micturition control of normal rats under urethane anesthesia, a blockade of the micturition reflex was determined to be assessed as a successful endpoint of capsaicin delivery from various vehicles. Moreover, by using normal rats, the variable influence of disease on the uptake of capsaicin from the different delivery systems can be avoided. Damaged urothelium is more permeable than normal urothelium and it is possible that pathological conditions will induce a variation in study designed to examine capsaicin uptake from various vehicles.

[0190] Delivery of capsaicin by hydrogels and were compared to delivery of capsaicin by a positive control (30% ethanol in saline). The three vehicles (e.g., liposomes, hydrogels and 30% ethanol) were also examined for any clinical or irritative effect) in absence of any capsaicin. CMGs done after 48 h following intravesical instillation demonstrated that liposomal capsaicin was as successful as ethanolic capsaicin in blocking the micturition reflex in urethane anaesthetized normal rats.

[0191] In a previous study, a large dose of capsaicin (50 mg/kg s.c.) elicited an acute block of bladder activity that persisted for 8-15 h (Cheng et al., 1993, Am J Physiol 265:R132). The longer duration of capsaicin (48 h) effect observed in the present study might be explained by high local concentration of capsaicin following intravesical administration. The prolonged desensitizing effect of capsaicin in the present work corroborates the earlier report documenting the initial excitation produced by capsaicin following its intravesical delivery using liposomes of similar composition (Tyagi et al., 2002, XIVth World Congress of Pharmacology: Meeting Abstracts 44(No.

2):26.6(A30)).

[0192] Hydrogel with capsaicin was effective in reducing the bladder contraction frequency compared to vehicle controls of hydrogel alone or liposomes alone, but failed to produce overflow continence (unlike administration of capsaicin in liposomes or 30% ethanol, Santicioli et al., 1985, J Urol 133:700). The difference seen with of capsaicin delivery in hydrogel treated animals may be related to capsaicin's water insoluble nature.

[0193] At the cellular level, capsaicin acts by releasing CGRP stored in afferent fibers (Tucci et al., 2002, J Pharm Pharmacol 54:1111) and it was reported to produce reduction in suburothelial nerve densities in the bladder of patients with detrusor hyper-reflexia. This may explain its prolonged beneficial effect in these patients (Dasgupta et al., 2000, BJU Int 85:238). Capsaicin delivered in liposome and ethanol vehicles was able to reduce the CGRP staining in bladders removed following the completion of CMG studies. haematoxylin and eosin staining on formalin fixed bladder sections revealed disruptive effect of ethanolic vehicle on urothelium even in absence of capsaicin. These results agree with those reported previously (Byrne et al, 1998, J Urol 159:1074) as well as explain the discomfort felt by the patients following intravesical administration in an ethanol vehicle.

[0194] The contents of all patents, patent applications, published articles, books, reference manuals, texts and abstracts cited herein are hereby incorporated by reference in their entirety to more fully describe the state of the art to which the present invention pertains.

[0195] The present invention has been described in detail including the preferred embodiments thereof. However, it will be appreciated by those skilled in the art, upon consideration of this disclosure, that modifications and improvements may be made thereon without departing from the spirit and scope of the invention as set forth in the description and claims.

WHAT IS CLAIMED IS:

1. A method of treating hyperactive bladder in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat the hyperactive bladder.
2. The method of claim 1, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol- poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).
3. The method of claim 2, wherein the amount of PLGA is at least 3000 block length.
4. The method of claim 1, wherein at least one of the one or more therapeutic agents is soluble in water.
5. The method of claim 1, wherein at least one of the one or more therapeutic agents is not soluble in water.
6. The method of claim 5, wherein at least one of the one or more therapeutic agents is a vanilloid.
7. The method of claim 6, wherein the vanilloid is selected from the group consisting of capsaicin, resiniferatoxin, and tinyatoxin.
8. The method of claim 1, wherein the hydrogel further comprises an adhesive.
9. The method of claim 8, wherein the adhesive is a mucoadhesive.
10. The method of claim 1, wherein at least one of the one or more the therapeutic agents is an antisense nucleic acid.
11. The method of claim 10, wherein the antisense nucleic acid hybridizes to a nucleotide sequence encoding nerve growth factor receptor.

12. The method of claim 1, wherein at least one of the one or more therapeutic agents is a botulinum toxin.

13. The method of claim 12, wherein the botulinum toxin is selected from the group consisting of botulinum toxins A through G.

14. The method of claim 1, wherein in the hyperactive bladder is associated with a medical condition selected from the group consisting of anxiety, aging, infection, diabetes mellitus, brain tumor, spinal cord tumor, spinal cord injury, stroke, ruptured intervertebral disk, demyelinating disease, degenerative disease of the nervous system, irritation, inflammation, micturition pattern alteration, and incontinence.

15. A method of treating bladder pain in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat the bladder pain.

16. The method of claim 15, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol- poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

17. The method of claim 16, wherein the amount of PLGA is at least 3000 block length.

18. The method of claim 15, wherein at least one of the one or more therapeutic agents is soluble in water.

19. The method of claim 15, wherein at least one of the one or more therapeutic agents is not soluble in water.

20. The method of claim 19, wherein at least one of the one or more therapeutic agents is a vanilloid.

21. The method of claim 20, wherein the vanilloid is selected from the group consisting of capsaicin, resiniferatoxin, and tinyatoxin.

22. The method of claim 15, wherein the hydrogel further comprises an adhesive.

23. The method of claim 22, wherein the adhesive is a mucoadhesive.

24. The method of claim 15, wherein at least one of the one or more therapeutic agents is an antisense nucleic acid.

25. The method of claim 24, wherein the antisense nucleic acid hybridizes to a nucleotide sequence encoding nerve growth factor receptor

26. The method of claim 15, wherein the bladder pain is associated with a medical condition selected from the group consisting of interstitial cystitis, bladder hyperactivity, irritation, inflammation, micturition pattern alteration, and incontinence.

27. A method of treating pain associated with bladder cancer in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat the pain.

28. The method of claim 27, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol-poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

29. The method of claim 28, wherein the amount of PLGA is at least 3000 block length.

30. The method of claim 27, wherein at least one of the one or more therapeutic agents is soluble in water.

31. The method of claim 27, wherein at least one of the one or more therapeutic agents is not soluble in water.

32. The method of claim 31, wherein at least one of the one or more therapeutic agents is a vanilloid.

33. The method of claim 32, wherein the vanilloid is selected from the group consisting of capsaicin, resiniferatoxin, and tinyatoxin.

34. The method of claim 27, wherein the hydrogel further comprises an adhesive.

35. The method of claim 34, wherein the adhesive is a mucoadhesive.

36. The method of claim 27, wherein the hydrogel is coadministered with an anticancer agent.

37. The method of claim 27, wherein at least one of the one or more therapeutic agents is an anticancer agent.

38. The method of either claim 36 or 37, wherein the anticancer agent is selected from the group consisting of vincristine, doxorubicin, mitoxantrone, camptothecin, cisplatin, bleomycin, cyclophosphamide, methotrexate, streptozotocin, actinomycin D, vincristine, vinblastine, cystine arabinoside, anthracyclines, alkylating agents, platinum compounds, antimetabolites, nucleoside analogs, methotrexate, purine and pyrimidine analogs, adriamycin, daunomycin, mitomycin, epirubicin, 5-FU, and aclacinomycin.

39. The method of claim 27, wherein the cancer is selected from the group consisting of transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma.

40. The method of claim 27, wherein the cancer is associated with a condition selected from the group consisting of bladder hyperactivity, irritation, inflammation, micturition pattern alteration, and incontinence.

41. A method of treating pain associated with a urinary system infection in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat the pain.

42. The method of claim 41, wherein the infection is in a urinary system component selected from the group consisting of kidney, ureter, urethra, bladder, urethral sphincter, and bladder neck sphincter.

43. The method of claim 41, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol-poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

44. The method of claim 43, wherein the amount of PLGA is at least 3000 block length.

45. The method of claim 41, wherein at least one of the one or more therapeutic agents is soluble in water.

46. The method of claim 41, wherein at least one of the one or more therapeutic agents is not soluble in water.

47. The method of claim 46, wherein at least one of the one or more therapeutic agents is a vanilloid.

48. The method of claim 47, wherein the vanilloid is selected from the group consisting of capsaicin, resiniferatoxin, and tinyatoxin.

49. The method of claim 41, wherein the hydrogel further comprises an adhesive.

50. The method of claim 49, wherein the adhesive is a mucoadhesive.

51. The method of claim 41, wherein the infection is bacterial.

52. The method of claim 51, wherein the hydrogel is coadministered with an antibacterial agent.

53. The method of claim 51, wherein at least one of the one or more therapeutic agents is an antibacterial agent.

54. The method of either claim 52 or 53, wherein the antibacterial agent is selected from the group consisting of lincomycin, erythromycin, dirithromycin, clindamycin, clarithromycin, azithromycin, ticarcillin, piperacillin, mezlocillin, carbenicillin indanyl, bacampicillin, ampicillin, amoxicillin, amoxicillin-clavulanic acid, ampicillin-sulbactam, benzylpenicillin, cloxacillin, dicloxacillin, methicillin, oxacillin, penicillin G, penicillin V, piperacillin plus tazobactam, ticarcillin plus clavulanic acid, amikacin, gentamicin, kanamycin, neomycin, netilmicin, streptomycin, tobramycin, tetracycline, oxytetracycline, minocycline, methacycline, doxycycline, and demedocycline.

55. The method of claim 41, wherein the infection is associated with a condition selected from the group consisting of bladder hyperactivity, pain, irritation, inflammation, micturition pattern alteration, and incontinence.

56. A method of treating a urinary system obstruction in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat the obstruction.

57. The method of claim 56, wherein the obstruction is in a urinary system component selected from the group consisting of kidney, ureter, urethra, bladder, urethral sphincter, and bladder neck sphincter.

58. The method of claim 56, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol-poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

59. The method of claim 58, wherein the amount of PLGA is at least 3000 block length.

60. The method of claim 56, wherein at least one of the one or more therapeutic agents is botulinum toxin.

61. The method of claim 60, wherein the botulinum toxin is selected from the group consisting of botulinum toxins A through G.

62. The method of claim 56, wherein the urinary obstruction is associated with a condition selected from the group consisting of urinary detrusor-sphincter dyssynergia, pain, irritation, and inflammation.

63. A method of treating involuntary muscle contraction in a urinary system component in a mammalian subject comprising administering to the subject in need thereof a pharmaceutical composition comprising i) a thermosensitive hydrogel and ii) one or more therapeutic agents in an amount effective to treat to treat the involuntary muscle contraction.

64. The method of claim 63, wherein the involuntary muscle contraction affects a muscle selected from the group consisting of bladder muscle, urethral sphincter muscle, and bladder neck sphincter muscle.

65. The method of claim 63, wherein the involuntary muscle contraction is associated with a condition selected from the group consisting of spastic bladder and urinary detrusor-sphincter dyssynergia.

66. The method of claim 63, wherein the thermosensitive hydrogel is a triblock co-polymer comprising polyethylene glycol-poly[lactic acid-co-glycolic acid]-polyethylene glycol (PEG-PLGA-PEG).

67. The method of claim 66, wherein the amount of PLGA is at least 3000 block length.

68. The method of claim 63, wherein at least one of the one or more therapeutic agents is botulinum toxin.

69. The method of claim 68, wherein the botulinum toxin is selected from the group consisting of botulinum toxins A through G.

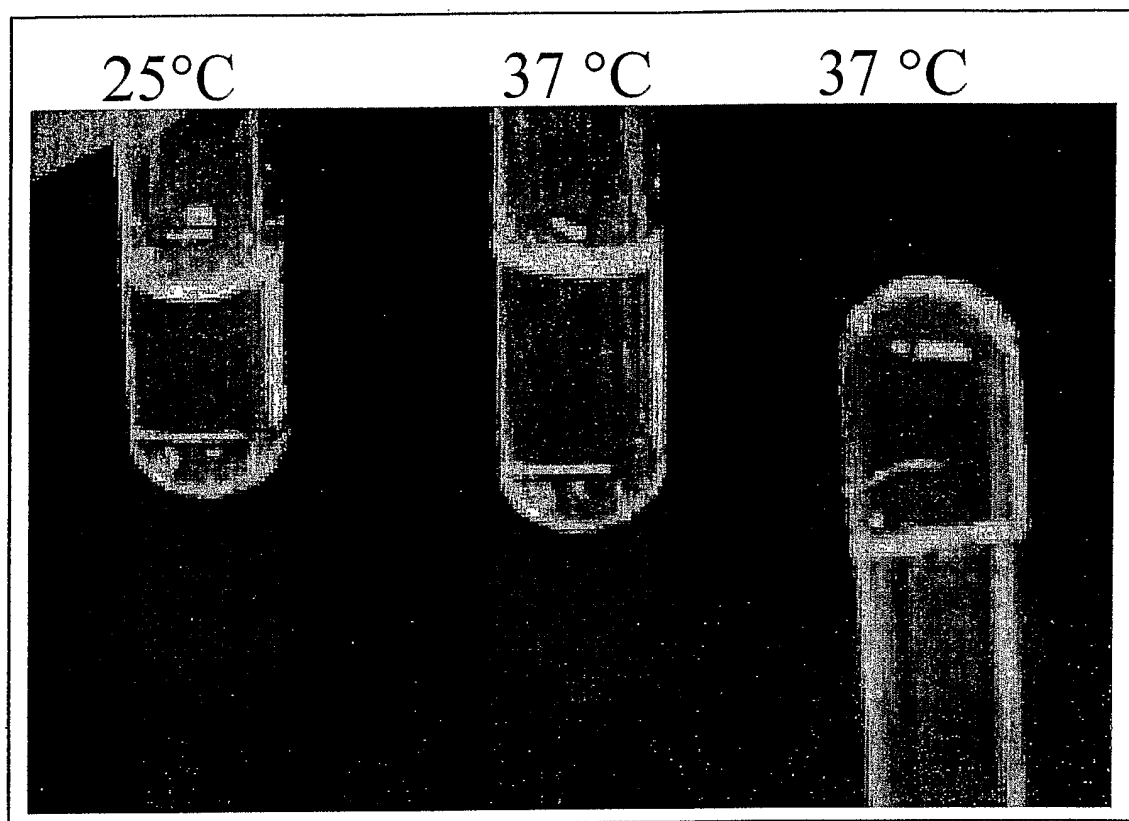


FIG. 1

2/10

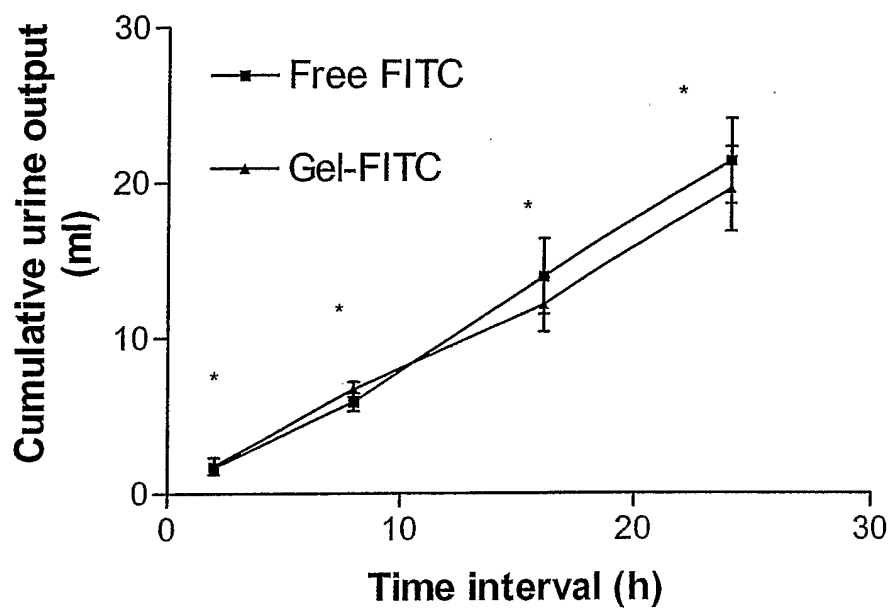


FIG. 2

3/10

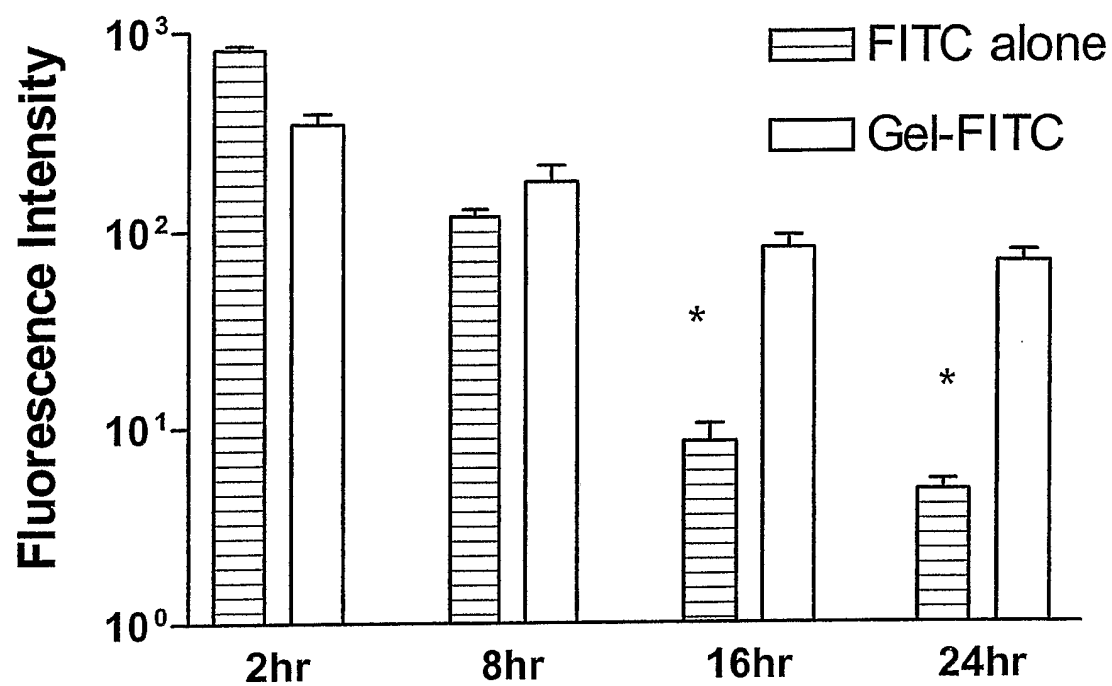
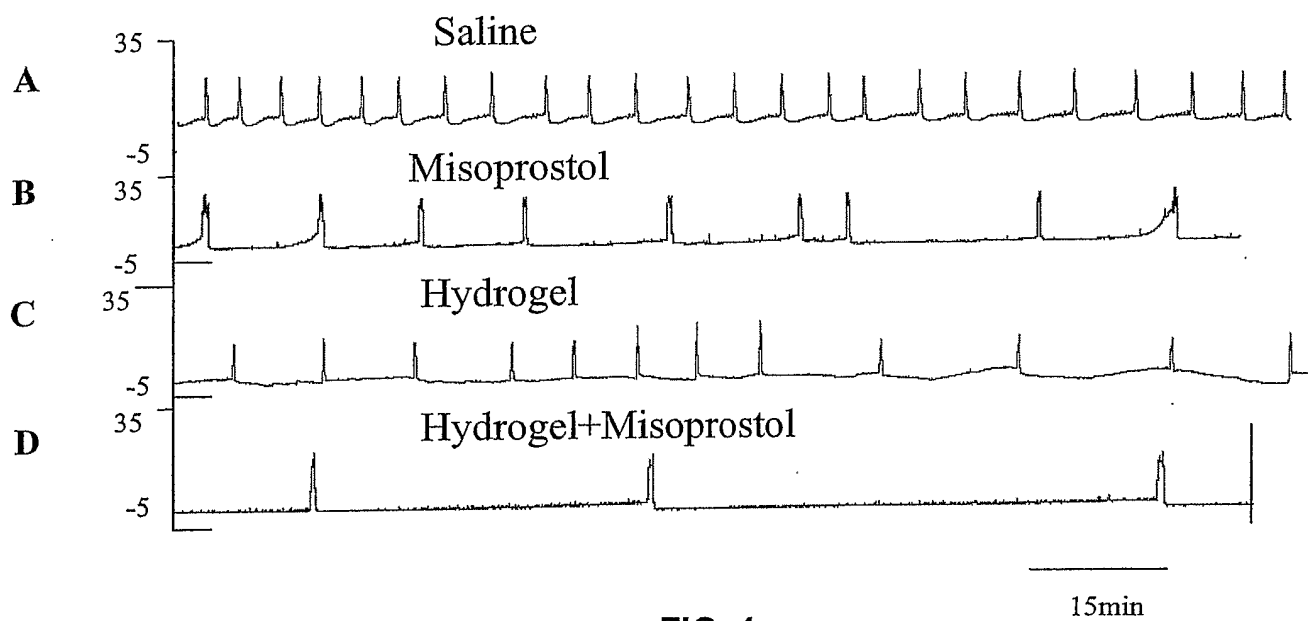


FIG. 3

4/10



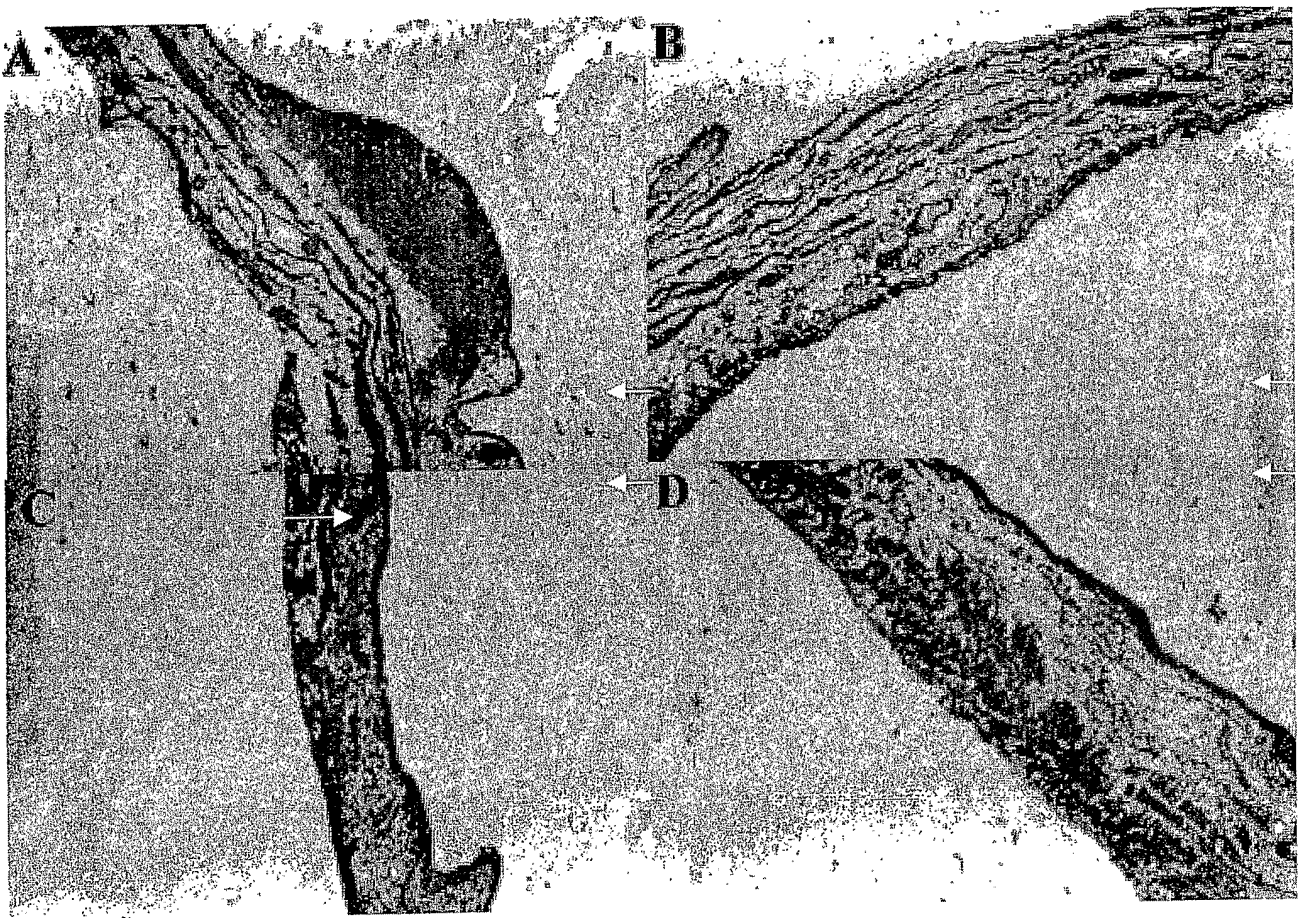


FIG. 5

6/10

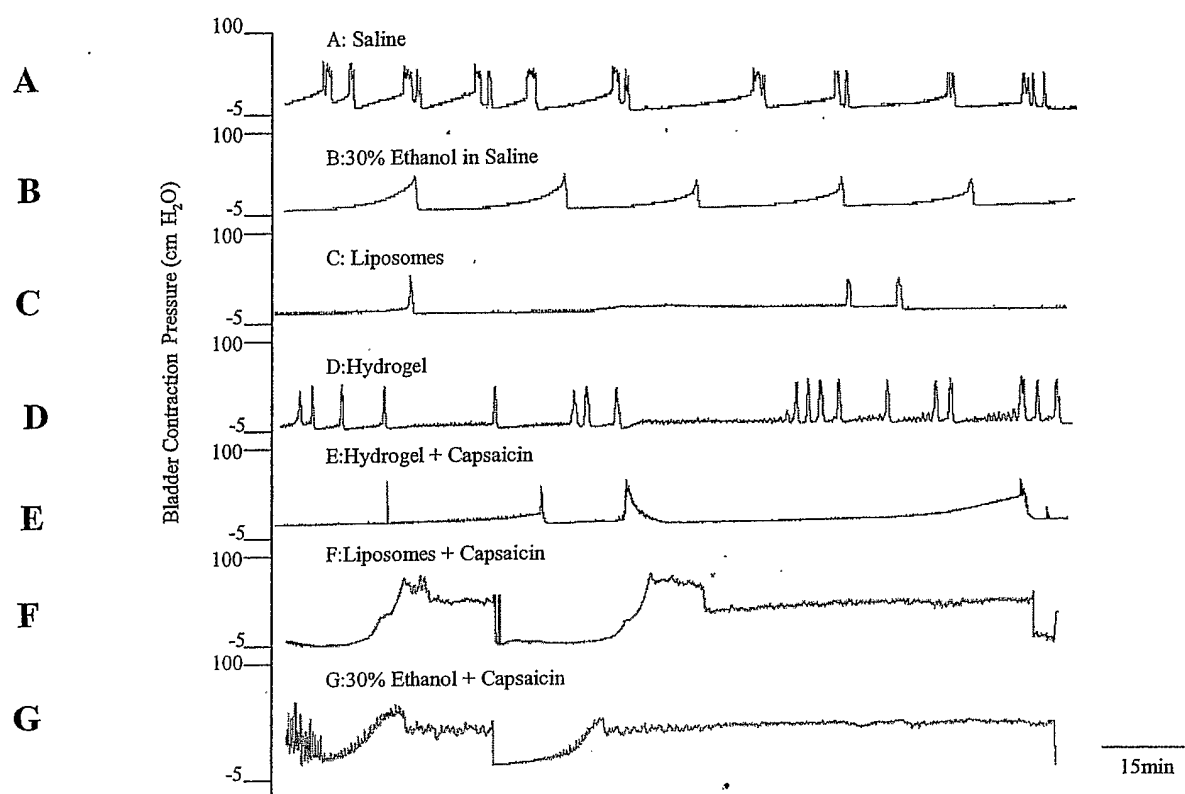


FIG. 6

7/10

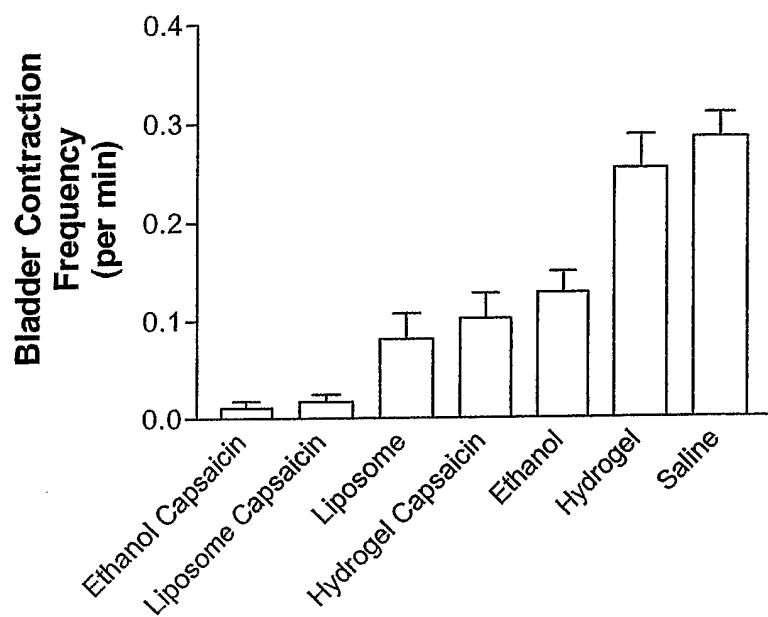
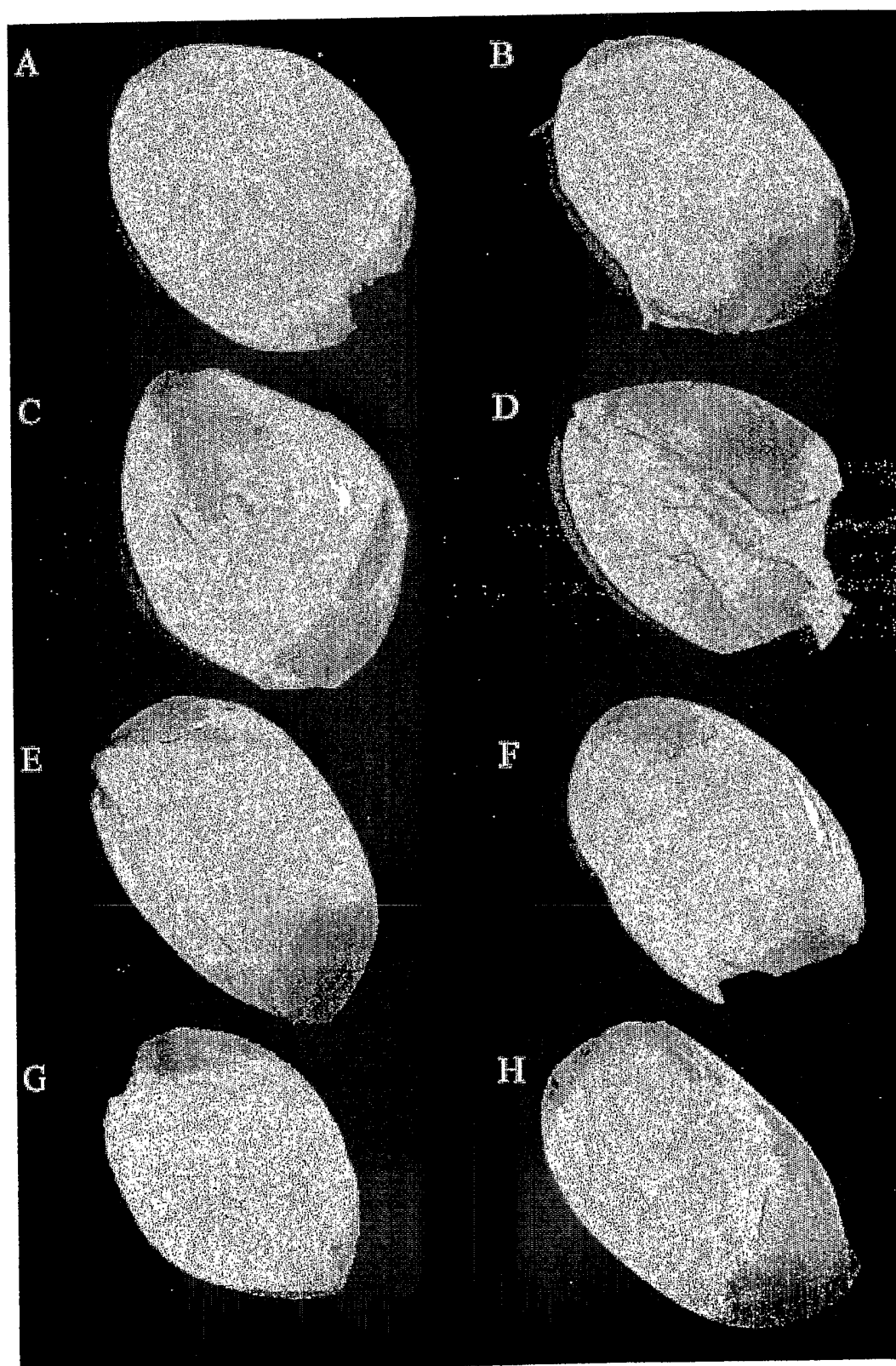


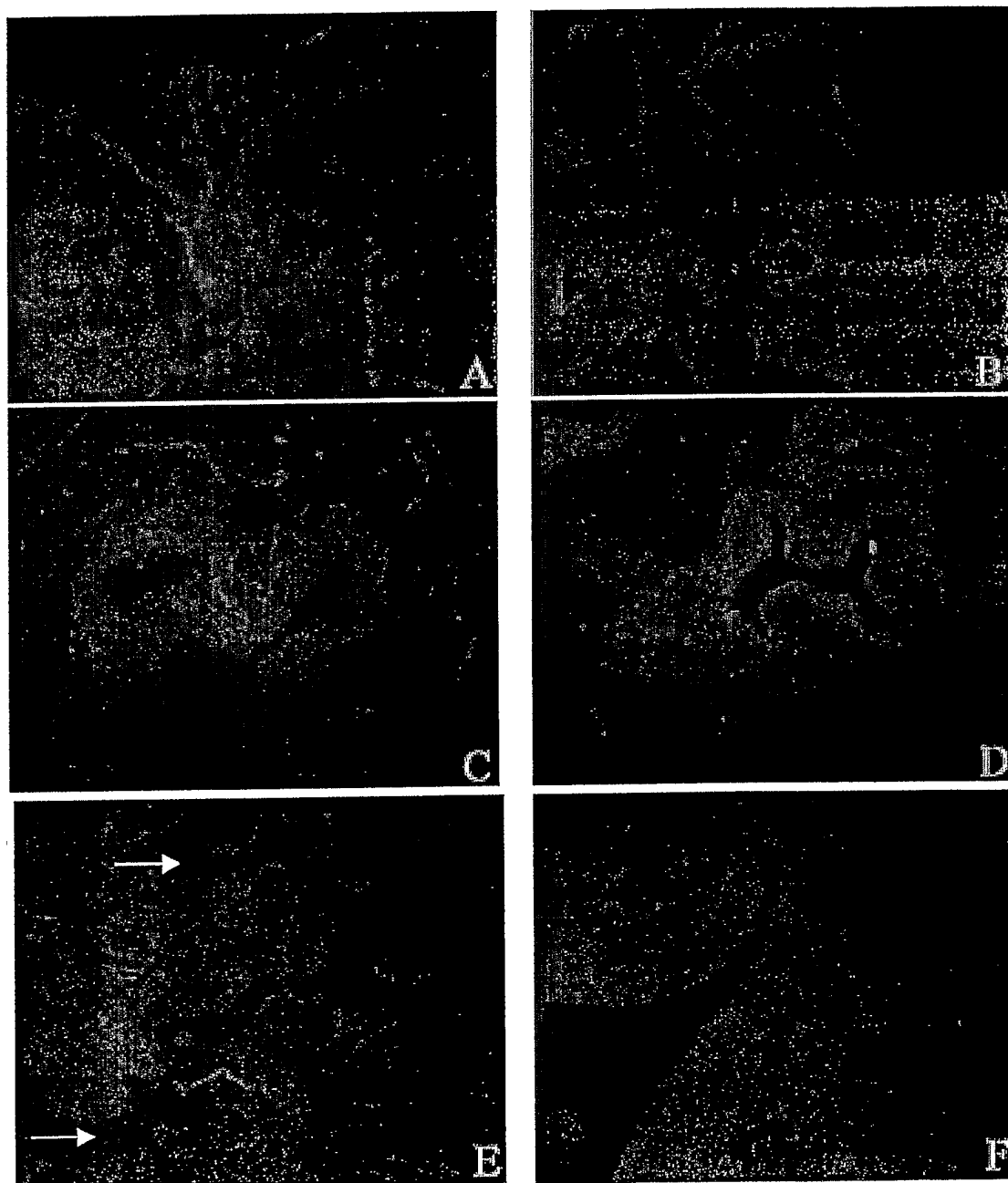
FIG. 7

FIG. 8



9/10

FIG. 9



10/10.

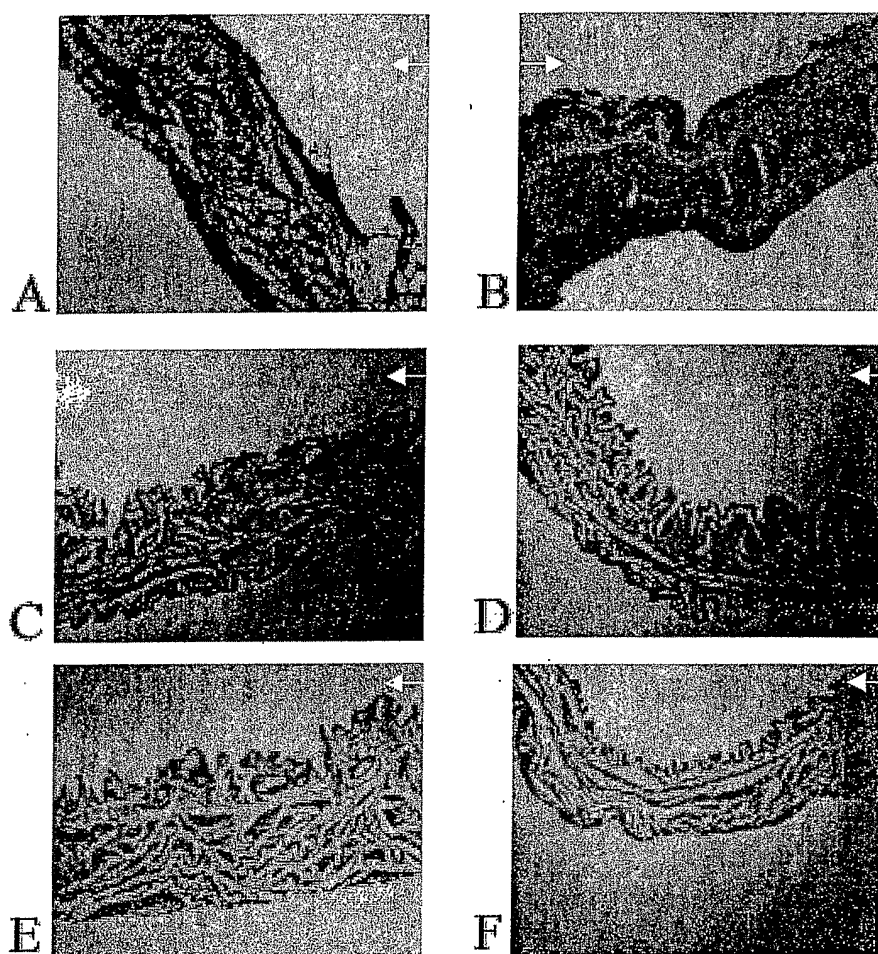


FIG. 10

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/41473

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61F 13/00

US CL : 424/449, 443

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)

U.S. : 424/449, 443

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)

Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2002/0115992 A1 (UTLEY et al.) 22 August 2002; abstract; paragraphs 0010, 0042, 0045, 0055, 0057, 0064, 0068.	1, 3-15, 17-27, 29-42, 44-57, 59-65, 67-69 ----- 2, 16, 28, 43, 58, 66
X --- Y	US 2003/0203030 A1 (ACHTON et al.) 30 October 2003; abstract; paragraphs 0020, 0021, 0059, 0064, 0124, 0217.	1, 3-15, 17-27, 29-42, 44-57, 59-65, 67-69 ----- 2, 16, 28, 43, 58, 66
Y	US 2004/0241244 A1 (LIN et al.) 02 December 2004; paragraphs 0024, 0025, 0113, 0127.	2, 16, 28, 43, 58, 66



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

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

"E" earlier application or patent 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

16 June 2005 (16.06.2005)

Date of mailing of the international search report

06 JUL 2005

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/41473

Continuation of Item 4 of the first sheet:

Examiner suggests

"HYDROGEL VEHICLE FOR DRUG DELIVERY"

Continuation of B. FIELDS SEARCHED Item 3:

WEST, ALL DATA BASES

SEARCH TERMS: hydrogel, bladder, active agents