

ionic agents for delivery of therapeutic polynucleotides molecules include, but are not limited to, surfactants, liposomes, peptides, polymers, micelles, nanoparticles or combinations thereof. Polynucleotides can include plasmid DNA, synthetic natural or chemically modified oligonucleotides used for gene silencing via antisense or RNA interference mechanisms, ribozymes, aptamers, microRNAs, decoys, etc. The delivery vector including an antisense molecule has an electrostatic charge ratio of about -5 to +5 equivalents and a particle size of about 50 nm to about 300 nm.

[0014] A third aspect of the invention provides a pharmaceutical composition comprising the delivery vector as described above and a pharmaceutically acceptable carrier. The composition can further comprise a therapeutic polynucleotide such as an antisense DNA or RNA molecule a plasmid DNA or antiRNA.

[0015] A fourth aspect of the invention provides a method of treatment for human or animal diseases including but not limited to cancer and heterotopic ossification. Such method includes silencing the expression of genes, such as oncogenes in cancer cells, by administering to a patient a therapeutically effective amount of the delivery vector as described above further comprising an siRNA targeted to an appropriate gene. In cancer, the gene targeted for silencing might be an anti-apoptotic gene, such as BCL-2. In heterotopic ossification, the target gene might be one such as Runx2 that is in an osteogenic pathway. Without limiting the range that may be required for a particular therapeutic application, the dose of siRNA can be between about 0.1 to about 25 mg/kg, preferably about 0.1 to about 10 mg/kg, more preferably between about 0.1 to about 1 mg/kg.

[0016] A further aspect of the invention is directed to a graft copolymer polyelectrolyte complex comprising:

[0017] (1) an anionic graft copolymer comprising:

[0018] (i) a backbone comprising a poly(alkyl acrylic acid); and

[0019] (ii) one or more polyetheramine pendent chains covalently attached to said copolymer backbone as amides of the acrylic acid groups, wherein said pendent chains predominantly comprise ethylene oxide repeating units;

[0020] wherein said copolymer has a graft density between about 0.5 and about 25 mole percent;

[0021] (2) one or more anionic, cationic or polyelectrolyte therapeutic agents; and

[0022] (3) optionally, a liposome which optionally comprises an additional therapeutic agent;

wherein when said therapeutic agent is an antisense molecule, a liposome comprising said additional therapeutic agent is also present.

[0023] The pendent chains of the graft copolymer polyelectrolyte complex can further comprise one or more ligands that target a specific cell, tissue or surface. In one embodiment, one or more of said ligands targets a microbial biofilm or a planktonic microbe. In another embodiment, one or more of said ligands comprises a phosphonate molecule that targets bone tissue.

[0024] Another aspect of the present invention is directed to a functional nanoparticle comprising the above graft copolymer polyelectrolyte complex, wherein the nanoparticle provides in vitro, ex vivo or in vivo delivery via oral, enteral, parenteral or topical routes of administration of the therapeutic agent.

[0025] Yet another aspect of the invention is directed to a method of preparing a graft copolymer-polyelectrolyte complex comprising the steps of:

[0026] (1) providing an aqueous mixture of an anionic graft copolymer comprising:

[0027] (i) a backbone comprising a poly(alkyl acrylic acid); and

[0028] (ii) one or more polyetheramine pendent chains covalently attached to said copolymer backbone as amides of the acrylic acid groups, wherein said pendent chains predominantly comprise ethylene oxide repeating units;

[0029] wherein said copolymer has a graft density between about 0.1 and about 25 mole percent;

[0030] (2) adding one or more polyelectrolytes to form a polyelectrolyte-copolymer mixture;

[0031] (3) optionally adding an aqueous mixture containing a liposome which optionally comprises an additional therapeutic agent, to form a liposome-containing polyelectrolyte-copolymer mixture; and

[0032] (4) allowing said polyelectrolyte-copolymer mixture or said liposome-containing polyelectrolyte-copolymer mixture to self-assemble in the aqueous medium to form said complex, which further forms nanoparticles.

[0033] Still another aspect of the invention is directed to a method of treating a patient in need thereof with a polyelectrolyte therapeutic agent comprising the steps of:

[0034] (1) formulating the complex or the nanoparticle of any of the above with one or more pharmaceutically acceptable carriers to provide a pharmaceutical composition; and

[0035] (2) administering said pharmaceutical composition to said patient via oral, parenteral, enteral or topical routes in an amount effective to treat said patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] FIG. 1 presents a diagram of a delivery complex of the invention.

[0037] FIG. 2 presents a diagram of the morphological structure of a typical liposome-polymer carrier system of the invention (antisense oligonucleotide; AON).

[0038] FIG. 3 presents a diagram of the various components of a typical graft copolymer polyelectrolyte complex (cationic antimicrobial peptide).

[0039] FIGS. 4a and 4b present results of studies on cellular uptake and antisense activity, respectively, of DOTAP/ODN complexes in the presence of PPAA, PPAA-g-JEFFAMINE® or PPAA-g-PEO in CHO-d1EGFP cells 24 hours post-treatment.

[0040] FIGS. 5a and 5b present results of studies on cellular uptake and antisense activity, respectively, of DOTAP/ODN complexes in the presence of PPAA, PPAA-g-JEFFAMINE® or PPAA-g-PEO in U87-d1EGFP cells 24 hours post-treatment.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0041] One aspect of the invention provides innovative graft polymers designed for the efficient delivery of antisense molecules into biological cells and for maintaining the biological activity of the antisense molecules while in serum and other aqueous environments. Such graft polymers can com-