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PATENT APPLICATION

NOVEL LIPID FORMULATIONS FOR NUCLEIC ACID DELIVERY

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CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] The present application claims priority to U.S. Provisional Application No. 61/045,228, filed April 15, 2008, the disclosure of which is herein incorporated by reference 5 in its entirety for all purposes.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] Not applicable.

NAMES OF PARTIES TO A JOINT RESEARCH AGREEMENT

10 **[0003]** Not applicable.

REFERENCE TO A "SEQUENCE LISTING"

[0004] Not applicable.

BACKGROUND OF THE INVENTION

[0005] RNA interference (RNAi) is an evolutionarily conserved process in which 15 recognition of double-stranded RNA (dsRNA) ultimately leads to posttranscriptional suppression of gene expression. This suppression is mediated by short dsRNA, also called small interfering RNA (siRNA), which induces specific degradation of mRNA through complementary base pairing. In several model systems, this natural response has been developed into a powerful tool for the investigation of gene function (*see, e.g.,* Elbashir *et al.*, 20 *Genes Dev.*, 15:188-200 (2001); Hammond *et al.*, *Nat. Rev. Genet.*, 2:110-119 (2001)). More recently, it was discovered that introducing synthetic 21-nucleotide dsRNA duplexes into mammalian cells could efficiently silence gene expression.

[0006] Although the precise mechanism is still unclear, RNAi provides a potential new approach to downregulate or silence the transcription and translation of a gene of interest. 25 For example, it is desirable to modulate (*e.g.*, reduce) the expression of certain genes for the treatment of neoplastic disorders such as cancer. It is also desirable to silence the expression of genes associated with liver diseases and disorders such as hepatitis. It is further desirable to reduce the expression of certain genes for the treatment of atherosclerosis and its manifestations, *e.g.,* hypercholesterolemia, myocardial infarction, and thrombosis.

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[0007] A safe and effective nucleic acid delivery system is required for RNAi to be therapeutically useful. Viral vectors are relatively efficient gene delivery systems, but suffer from a variety of limitations, such as the potential for reversion to the wild-type as well as immune response concerns. As a result, nonviral gene delivery systems are receiving
5 increasing attention (Worgall *et al.*, *Human Gene Therapy*, 8:37 (1997); Peeters *et al.*, *Human Gene Therapy*, 7:1693 (1996); Yei *et al.*, *Gene Therapy*, 1:192 (1994); Hope *et al.*, *Molecular Membrane Biology*, 15:1 (1998)). Furthermore, viral systems are rapidly cleared from the circulation, limiting transfection to "first-pass" organs such as the lungs, liver, and spleen. In addition, these systems induce immune responses that compromise delivery with
10 subsequent injections.

[0008] Plasmid DNA-cationic liposome complexes are currently the most commonly employed nonviral gene delivery vehicles (Felgner, *Scientific American*, 276:102 (1997); Chonn *et al.*, *Current Opinion in Biotechnology*, 6:698 (1995)). For instance, cationic liposome complexes made of an amphipathic compound, a neutral lipid, and a detergent for
15 transfecting insect cells are disclosed in U.S. Patent No. 6,458,382. Cationic liposome complexes are also disclosed in U.S. Patent Publication No. 20030073640.

[0009] Cationic liposome complexes are large, poorly defined systems that are not suited for systemic applications and can elicit considerable toxic side effects (Harrison *et al.*, *Biotechniques*, 19:816 (1995); Li *et al.*, *The Gene*, 4:891 (1997); Tam *et al*, *Gene Ther.*,
20 7:1867 (2000)). As large, positively charged aggregates, lipoplexes are rapidly cleared when administered *in vivo*, with highest expression levels observed in first-pass organs, particularly the lungs (Huang *et al.*, *Nature Biotechnology*, 15:620 (1997); Templeton *et al.*, *Nature Biotechnology*, 15:647 (1997); Hofland *et al.*, *Pharmaceutical Research*, 14:742 (1997)).

[0010] Other liposomal delivery systems include, for example, the use of reverse micelles,
25 anionic liposomes, and polymer liposomes. Reverse micelles are disclosed in U.S. Patent No. 6,429,200. Anionic liposomes are disclosed in U.S. Patent Publication No. 20030026831. Polymer liposomes that incorporate dextrin or glycerol-phosphocholine polymers are disclosed in U.S. Patent Publication Nos. 20020081736 and 20030082103, respectively.

[0011] A gene delivery system containing an encapsulated nucleic acid for systemic
30 delivery should be small (*i.e.*, less than about 100 nm diameter) and should remain intact in the circulation for an extended period of time in order to achieve delivery to affected tissues. This requires a highly stable, serum-resistant nucleic acid-containing particle that does not interact with cells and other components of the vascular compartment. The particle should

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also readily interact with target cells at a disease site in order to facilitate intracellular delivery of a desired nucleic acid.

[0012] Recent work has shown that nucleic acids can be encapsulated in small (*e.g.,* about 70 nm diameter) "stabilized plasmid-lipid particles" (SPLP) that consist of a single plasmid encapsulated within a bilayer lipid vesicle (Wheeler *et al.*, *Gene Therapy*, 6:271 (1999)). These SPLPs typically contain the "fusogenic" lipid dioleoylphosphatidylethanolamine (DOPE), low levels of cationic lipid, and are stabilized in aqueous media by the presence of a poly(ethylene glycol) (PEG) coating. SPLPs have systemic application as they exhibit extended circulation lifetimes following intravenous (i.v.) injection, accumulate preferentially at distal tumor sites due to the enhanced vascular permeability in such regions, and can mediate transgene expression at these tumor sites. The levels of transgene expression observed at the tumor site following i.v. injection of SPLPs containing the luciferase marker gene are superior to the levels that can be achieved employing plasmid DNA-cationic liposome complexes (lipoplexes) or naked DNA.

[0013] Thus, there remains a strong need in the art for novel and more efficient methods and compositions for introducing nucleic acids such as siRNA into cells. In addition, there is a need in the art for methods of downregulating the expression of genes of interest to treat or prevent diseases and disorders such as cancer and atherosclerosis. The present invention addresses these and other needs.

BRIEF SUMMARY OF THE INVENTION

[0014] The present invention provides novel, serum-stable lipid particles comprising one or more active agents or therapeutic agents, methods of making the lipid particles, and methods of delivering and/or administering the lipid particles (*e.g.,* for the treatment of a disease or disorder).

[0015] In preferred embodiments, the active agent or therapeutic agent is fully encapsulated within the lipid portion of the lipid particle such that the active agent or therapeutic agent in the lipid particle is resistant in aqueous solution to enzymatic degradation, *e.g.,* by a nuclease or protease. In other preferred embodiments, the lipid particles are substantially non-toxic to mammals such as humans.

[0016] In one aspect, the present invention provides lipid particles comprising: (a) one or more active agents or therapeutic agents; (b) one or more cationic lipids comprising from about 50 mol % to about 85 mol % of the total lipid present in the particle; (c) one or more non-cationic lipids comprising from about 13 mol % to about 49.5 mol % of the total lipid

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present in the particle; and (d) one or more conjugated lipids that inhibit aggregation of particles comprising from about 0.5 mol % to about 2 mol % of the total lipid present in the particle.

[0017] More particularly, the present invention provides serum-stable nucleic acid-lipid particles (SNALP) comprising a nucleic acid (*e.g.,* one or more interfering RNA molecules such as siRNA, aiRNA, and/or miRNA), methods of making the SNALP, and methods of delivering and/or administering the SNALP (*e.g.,* for the treatment of a disease or disorder).

[0018] In certain embodiments, the nucleic acid-lipid particle (*e.g.,* SNALP) comprises: (a) a nucleic acid (*e.g.,* an interfering RNA); (b) a cationic lipid comprising from about 50 mol % to about 85 mol % of the total lipid present in the particle; (c) a non-cationic lipid comprising from about 13 mol % to about 49.5 mol % of the total lipid present in the particle; and (d) a conjugated lipid that inhibits aggregation of particles comprising from about 0.5 mol % to about 2 mol % of the total lipid present in the particle.

[0019] In one preferred embodiment, the nucleic acid-lipid particle (*e.g.,* SNALP) comprises: (a) an siRNA; (b) a cationic lipid comprising from about 56.5 mol % to about 66.5 mol % of the total lipid present in the particle; (c) cholesterol or a derivative thereof comprising from about 31.5 mol % to about 42.5 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 1 mol % to about 2 mol % of the total lipid present in the particle. This preferred embodiment of nucleic acid-lipid particle is generally referred to herein as the "1:62" formulation.

[0020] In another preferred embodiment, the nucleic acid-lipid particle (*e.g.,* SNALP) comprises: (a) an siRNA; (b) a cationic lipid comprising from about 52 mol % to about 62 mol % of the total lipid present in the particle; (c) a mixture of a phospholipid and cholesterol or a derivative thereof comprising from about 36 mol % to about 47 mol % of the total lipid present in the particle; and (d) a PEG-lipid conjugate comprising from about 1 mol % to about 2 mol % of the total lipid present in the particle. This preferred embodiment of nucleic acid-lipid particle is generally referred to herein as the "1:57" formulation.

[0021] The present invention also provides pharmaceutical compositions comprising a lipid particle described herein (*e.g.,* SNALP) and a pharmaceutically acceptable carrier.

[0022] In another aspect, the present invention provides methods for introducing an active agent or therapeutic agent (*e.g.,* nucleic acid) into a cell, the method comprising contacting the cell with a lipid particle described herein such as a nucleic acid-lipid particle (*e.g.,* SNALP).

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