ANTISENSE MOLECULES FOR TREATMENT OF *STAPHYLOCOCCUS AUREUS* INFECTION

GOVERNMENT INTEREST

[0001] This work is based in part by the Defense Advanced Research Project Agency under Phase I SBIR contract number W911QX-12-C-0072. The US government has certain rights to the invention.

FIELD OF THE INVENTION

[0002] The field of the invention relates to antisense polynucleotide reagents targeting ribosomal protein expression and useful for treatment of *Staphylococcus aureus* infection.

SUMMARY OF THE INVENTION

[0003] Provided are antisense molecules useful for treatment of *Staphylococcus aureus* infection and the inhibition of *Staphylococcus aureus* growth. The antisense molecules target S*taphylococcus aureus* ribosomal proteins and may comprise natural nucleic acid polymers and non-natural nucleic acid polymers. Non-natural nucleic acid polymers include polymers with modified backbones, such as PNA, PMO, and synthetically-modified DNA and RNA. The invention includes any type of synthetically-modified DNA or RNA that hybridizes to natural DNA and RNA. In one embodiment, the antisense molecules are in the form of a salt or a complex. In one embodiment, the antisense molecule is complexed to a cationic polymeric molecule. In another embodiment, the antisense molecule is conjugated to a cell penetrating molecule. Also provided are pharmaceutical compositions comprising the antisense molecules of the invention.

[0004] In one embodiment the invention provides an antisense molecule or salt thereof that inhibits the growth of *Staphylococcus aureus* comprising a polynucleotide sequence that is antisense to the coding region of a *Staphylococcus aureus* ribosomal protein and hybridizes to said coding region under physiological conditions. In one embodiment, the antisense molecule is 10 to 50 nucleobases in length. In another embodiment, the antisense molecule is fully complementary to a coding region of a *staphylococcus aureus*

ribosomal protein. In another embodiment, the antisense molecule is at least 80% identical to a sequence selected from the group consisting of SEQ ID NOS: 1-50. In another embodiment, the antisense molecule is an oligonucleotide. In another embodiment, the antisense molecule is substantially pure. In another embodiment, the antisense molecule comprises a modified backbone. In another embodiment, the modified backbone is a PNA backbone. In another embodiment, the antisense molecule inhibits expression of LSU ribosomal protein L15p (L27Ae) or SSU ribosomal protein S17p (S11e). In another embodiment, the antisense molecule is conjugated to a cell penetration molecule. In another embodiment, the cell penetration molecule is a peptide. In another embodiment, the peptide is a cell-penetrating peptide (CPP). In another embodiment, the antisense molecule is complexed to a delivery polymer. In another embodiment, the delivery polymer is a cationic block copolymer comprising phosphonium or ammonium ionic groups.

[0005] The invention also provides a method of inhibiting the growth of *Staphylococcus aureus,* comprising administering an effective amount of an antisense molecule or composition of the invention to a tissue containing said *Staphylococcus aureus* or suspected of containing *Staphylococcus aureus*. In one embodiment, the administering is topical administration.

[0006] The invention also provides a method of treating *Staphylococcus aureus* infection, comprising administering to an animal in need thereof an effective amount of the antisense molecule or composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1. MRSA in vitro studies. Efficacy of antibacterial nucleic acid agents is demonstrated. Peptide-PNA was tested against bacteria in culture of MRSA USA 300. Vancomycin was used to standardize these results for additional studies.

[0008] FIG. 2A – 2B. a) MRSA fluorescent overlays 2 hours post treatment with 1uM of FITC-peptide agents. b) AcB fluorescent overlays 2 hours post treatment with 1uM of FITC-peptide agents. Scale bar = 100um. This figure shows that the cell-penetrating peptides are non-toxic when used alone.

[0009] FIG. 3. Shows log-phase MRSA growth inhibition over 8 hours at 0μM, 1μM, 10μM, and 20μM concentration of PNA-peptide antisense antibiotic. Bottom left represents a positive control (FmhB) and bottom right shows a negative control.

BRIEF DESCRIPTION OF THE SEQUENCE LISTING

[0010] The polynucleotide sequences in the sequence listing include the coding sequences for *Staphylococcus aureus* ribosomal proteins. *See,* SEQ ID NOS: 65-101.

[0011] The polynucleotide sequences in the sequence listing also include antisense deoxyribonucleic acids (DNA) and/or modified nucleic acids, such as peptide nucleic acids (PNA). These sequences are capable of knockdown of expression of at least the following *Staphlyococcus aureus* ribosomal protein as set forth in Table 1:

Table 1. Antisense Polynucleotides Targeting Ribosomal Proteins

Protein Target	**Antisense Polynucleotide Sequence**
LSU ribosomal protein L10p (P0)	AGACATTCAGACACC (SEQ ID NO: 21)
LSU ribosomal protein L11p (L12e)	TAGCCACGATGTGCA (SEQ ID NO: 19)
LSU ribosomal protein L13p (L13Ae)	ACGCATAATAAT (SEQ ID NO: 8)
LSU ribosomal protein L13p (L13Ae)	TTGACGCATAATAAT (SEQ ID NO: 33)
LSU ribosomal protein L14p (L23e)	GTTGGATCATTA (SEQ ID NO: 13)
LSU ribosomal protein L14p (L23e)	TGGATCATTAGTTAA (SEQ ID NO: 42)
LSU ribosomal protein L15p (L27Ae)	TTTCATTTCGGCACC (SEQ ID NO: 1)
LSU ribosomal protein L16p (L10e)	GGTAGTAACATTATT (SEQ ID NO: 43)
LSU ribosomal protein L18p (L5e)	GATCATTTCAATACT (SEQ ID NO: 38)
LSU ribosomal protein L19p	TGATTTGTCATTATA (SEQ ID NO: 25)
LSU ribosomal protein L1p (L10Ae)	TTAGCCATTTATAGT (SEQ ID NO: 20)
LSU ribosomal protein L20p	ACTCGTGGCATA (SEQ ID NO: 6)
LSU ribosomal protein L21p	AGCAAACATACTTTG (SEQ ID NO: 31)
LSU ribosomal protein L22p (L17e)	TTCCATTAGGATGTC (SEQ ID NO: 45)
LSU ribosomal protein L23p (L23Ae)	TTCCATTATCCGAGC (SEQ ID NO: 48)
LSU ribosomal protein L27p	AACATCGGAATG (SEQ ID NO: 5)
LSU ribosomal protein L27p	TAACATCGGAATGCA (SEQ ID NO: 29)

Protein Target	Antisense Polynucleotide Sequence
LSU ribosomal protein L28p	TGTTTACCCATA (SEQ ID NO: 4)
LSU ribosomal protein L2p (L8e)	TAGCCATTGTCG (SEQ ID NO: 16)
LSU ribosomal protein L2p (L8e)	AGCCATTGTCGCTTA (SEQ ID NO: 47)
LSU ribosomal protein L30p (L7e)	TTTAGCCATAACTAG (SEQ ID NO: 36)
LSU ribosomal protein L32p	TACTGCCATGATATA (SEQ ID NO: 24)
LSU ribosomal protein L34p	GTTTTACCATGCAAA (SEQ ID NO: 50)
LSU ribosomal protein L3p (L3e)	CATCGAAAGTCC (SEQ ID NO: 17)
LSU ribosomal protein L3p (L3e)	GGTCATCGAAAGTCC (SEQ ID NO: 49)
LSU ribosomal protein L5p (L11e)	CGGTTCAAAGTGGGA (SEQ ID NO: 41)
LSU ribosomal protein L6p (L9e)	TCATGTTATGGC (SEQ ID NO: 12)
LSU ribosomal protein L6p (L9e)	ACTCATGTTATGGCA (SEQ ID NO: 39)
ribosomal protein L7Ae family protein	TATACTCATTTTGGG (SEQ ID NO: 26)
SSU ribosomal protein S11p (S14e)	TTACGTGCCATT (SEQ ID NO: 9)
SSU ribosomal protein S11p (S14e)	TTTACGTGCCATTTA (SEQ ID NO: 34)
SSU ribosomal protein S12p (S23e)	GTTGGCATGTGATAT (SEQ ID NO: 22)
SSU ribosomal protein S13p (S18e)	TACGTGCCATAT (SEQ ID NO: 10)
SSU ribosomal protein S13p (S18e)	TACGTGCCATATTAA (SEQ ID NO: 35)
SSU ribosomal protein S14p (S29e) Zinc-dependent	TTTAGCCACTTAATT (SEQ ID NO: 40)
SSU ribosomal protein S15p (S13e)	AAATTGCCATAATCA (SEQ ID NO: 27)
SSU ribosomal protein S17p (S11e)	TCTTTCGCTCAC (SEQ ID NO: 14)
SSU ribosomal protein S17p (S11e)	CGCTCACTTTTGTAA (SEQ ID NO: 2)
SSU ribosomal protein S19p (S15e)	TACGAGCCATTT (SEQ ID NO: 15)
SSU ribosomal protein S19p (S15e)	GAGCCATTTGGGCGC (SEQ ID NO: 46)
SSU ribosomal protein S21p	TTTAGACATCTGTAT (SEQ ID NO: 28)
SSU ribosomal protein S3p (S3e)	TTGACCCACAGTATT (SEQ ID NO: 44)
SSU ribosomal protein S4p (S9e)	CGAGCCATAATA (SEQ ID NO: 7)
SSU ribosomal protein S4p (S9e)	GAGCCATAATAAGAC (SEQ ID NO: 32)
SSU ribosomal protein S5p (S2e)	CGAGCCATGTAT (SEQ ID NO: 11)
SSU ribosomal protein S5p (S2e)	CGAGCCATGTATTTG (SEQ ID NO: 37)

Protein Target	Antisense Polynucleotide Sequence
SSU ribosomal protein S6p	GTTCTCATTTTATAT (SEQ ID NO: 18)
SSU ribosomal protein S7p (S5e)	ACGAGGCATAAT (SEQ ID NO: 3)
SSU ribosomal protein S7p (S5e)	TTTACGAGGCATAAT (SEQ ID NO: 23)
Potential ribosomal protein	CAGTAATCATAATAA (SEQ ID NO: 30)

[0012] The sequence listing also contains control sequences of tRNA-dependent lipid II glycine ligase (FmhB): ttttccatgatttat (SEQ ID NO: 62); and Noncoding negative control (NC): aacattttggttttt (SEQ ID NO: 63).

[0013] The peptide sequences in the sequence listing include peptides that target and/or localize nucleic acids and nanoparticles to bacterial cells and promote bacterial membrane permeation. See Table 2:

Table 2. Cell Penetrating Peptides

Peptide Name	Amino Acid Sequence
KFF peptide	KFFKFFKFFK (SEQ ID NO: 51)
RFF peptide	RFFRFFRFFR (SEQ ID NO: 52)
Magainin 2	GIGKWLHSAKKFGKAFVGEIMNS (SEQ ID NO: 53)
Transportin 10	AGYLLGKINLKALAALAKKIL (SEQ ID NO: 54)
Indolicidin	ILPWKWPWWPWRR (SEQ ID NO: 61)
TAT peptide	GRKKRRQRRRPQ (SEQ ID NO: 60)
PENETRATIN 1 peptide	RQIKIWFQNRRMKWKK (SEQ ID NO: 59)
amphipathic peptide	LLIILRRRIRKQAHAHSK (SEQ ID NO: 58)
cyclic d,l-alpha-peptide	KQRWLWLW (SEQ ID NO: 57)
cyclic d,l-alpha-peptide	RRKWLWLW (SEQ ID NO: 56)
cyclic d,l-alpha-peptide	KKLWLW (SEQ ID NO: 55)

DEFINITIONS

[0014] The terms used in this disclosure have ordinary meanings as used in the art.

[0015] A polymer is a linear chain of units called monomers. In a polymer, the monomeric units may be identical or they may be different. Polymers may be natural

(made in nature) or may be synthetic. Polymers of the present invention comprise nucleic acid polymers, polypeptides, and synthetic delivery polymers.

[0016] A nucleic acid is a linear polymer of nucleotides. Nucleic acids made in nature contain deoxyribonucleotide (DNA) bases adenine, cytosine, guanine, and thymine; or ribonucleotide (RNA) bases adenine, cytosine, guanine, and uracil. As used herein, polynucleotide and oligonucleotide refer to a nucleic acid molecule and include genomic DNA, cDNA, RNA, or mRNA of any length. Nucleic acid, polynucleotide, oligonucleotide are terms that may be used interchangeably.

[0017] Modified nucleic acids are non-natural polymers that hybridize to natural DNA and RNA with sequence specificity according to Watson-Crick base paring rules. Examples of modified nucleic acids are phosphorothioate-oligodeoxynucleotides (PS-ODNs), locked nucleic acids (LNAs), 2'-O-methyloligoribonucleotides (2'O-Mes), phosphorodiamidate morpholino oligonucleotides (PMOs), and peptide nucleic acids (PNAs). Modified nucleic acids have modified backbones and are generally more resistant to degradation than natural nucleic acids. The invention includes any type of synthetically-modified DNA or RNA that hybridizes to natural DNA and RNA. *See, e.g.*, U.S. Pat. Nos. 5,116,195, 5,539,082, 5,527,675, 5,623,049, 5,714,331, 5,736,336, 5,773,571, 5,786,461, 5,811,232, 5,837,459, 5,874,564, 5,891,625, 5,972,610, 5,986,053, 6,107,470, 6,174,870, 7,098,192, 7,696,345, 8,124,745, 8,354,093, 8,357,664, Wagner *et al., Nucl. Acid Res. 19:*5965-71 (1991); and Koshkin *et al., Tetrahedron 54*:3607-30 (1998).

[0018] Antisense molecules of the invention may also be composed of non-natural polymers that hybridize to natural nucleic acids. Atypical nucleoside bases may also be employed, such as methylated bases, phosphorylated bases, inosine, thiouridine, pseudouridine, dihydrouridine, queuosine, and wyosine, among others. Examples of such antisense polymers comprising atypical bases are disclosed in U.S. Pat. Nos. 7,875,733, 7,919,612, 7,939,677, 8,314,229, 8,372,969, and 8,377,898.

[0019] The term antisense polynucleotide refers to a nucleic acid molecule that is complementary to at least a portion of a target nucleotide sequence of interest and hybridizes to the target nucleotide sequence under physiological conditions. Antisense molecules specifically hybridize with one or more nucleic acids encoding a preselected target nucleic acid. The terms target nucleic acid and nucleic acid encoding the target encompass DNA encoding the target, RNA (including pre-mRNA and mRNA)

transcribed from such DNA, and also cDNA derived from such RNA. The hybridization of an antisense compound with its target nucleic acid interferes with the normal function of the nucleic acid. This modulation of function of a target nucleic acid by compounds which specifically hybridize to it is generally referred to as antisense. The functions of DNA to be interfered with include replication and transcription. The functions of RNA to be interfered with include all vital functions such as, for example, translocation of the RNA to the site of protein translation, translation of protein from the RNA, splicing of the RNA to yield one or more mRNA species, and catalytic activity which may be engaged in or facilitated by the RNA. The overall effect of such interference with target nucleic acid function is modulation of the expression of the target. In the context of the present invention, modulation means either an increase (stimulation) or a decrease (inhibition) in the expression of a gene. In the context of the present invention, inhibition is the form of modulation of gene expression.

[0020] Polynucleotides are described as complementary to one another when hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides.

[0021] The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity and similarity between two sequences can be accomplished using a mathematical algorithm (see e.g., Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). In a preferred embodiment, the percent identity between two sequences is determined based on alignments generated with the Clustal W algorithm (Thompson, J. D. *et al.*, 1994, *Nucleic acids Res. 22*:4673-4680). This algorithm is incorporated into many commercial software packages, in this case the alignX software program in the Vector NTI suite (version 8.0). Default Clustal W parameters were used to generate pairwise alignments from which percent identity values were calculated (gap opening penalty of 10; gap

extension penalty of 0.1). The percent identity is defined as the number of identical bases divided by the total number of bases and multiplied by 100. If sequences in the alignment are of different lengths (due to gaps or extensions), the length of the longest sequence will be used in the calculation, representing the value for total length.

[0022] Proteins are polymers containing one or more chains of amino acids bonded together by peptide bonds. Proteins typically fold into a three dimensional form, facilitating a biological function.

[0023] A polypeptide is a polymer of amino acids bonded together by peptide bonds. The terms protein and polypeptide and peptide are generally used interchangeably, although polypeptides and peptides are generally shorter in length than proteins.

[0024] The terms charged, uncharged, cationic and anionic refer to the predominant state of a chemical moiety at near-neutral pH, e.g. about 6 to 8. In one embodiment, the term refers to the predominant state of the chemical moiety at physiological pH, that is, about 7.4. Thus, a cationic backbone linkage is predominantly positively charged at pH 7.4.

[0025] The term substantially pure means that the antisense molecule is substantially free from other materials such as other nucleic acids, proteins, lipids, carbohydrates, and other materials with which it may be naturally associated. In one embodiment, substantially pure antisense molecules are 95-95% homogeneous by HPLC. In another embodiment, substantially pure antisense molecules are 99-100% homogenous by HPLC.

DETAILED DESCRIPTION OF THE INVENTION

[0026] The present invention may be understood by reference to the following detailed description of the embodiments of the invention and examples included herein. The terminology used herein is for the purpose of describing embodiments of the invention and is not intended to be limiting.

[0027] Specific aspects of the invention include antisense molecules that are useful for the treatment of *Staphylococcus aureus* infection and/or inhibit the growth of *Staphylococcus aureus* comprising an antisense molecule that is antisense to a S*taphylococcus* aureus ribosomal protein coding region under physiological conditions. In one embodiment, the antisense molecule hybridizes to a S*taphylococcal aureus* ribosomal coding region selected from the group consisting of SEQ ID NOS: 65-101. In one embodiment, the antisense molecule contains 10-50 nucleobases, *i.e.,* is a 10-50-mer.

In another embodiment, the antisense molecule is a 10-25-mer, a 12-20-mer, a 12-15-mer, a 11-mer, a 12-mer, a 13-mer, a 14-mer, a 15-mer, a 16-mer, a 17-mer, an 18-mer, a 19-mer, a 20-mer, a 21-mer, a 22-mer, a 23-mer, a 24-mer, a 25-mer, a 26-mer, a 27-mer, a 28-mer, a 29-mer, or a 30-mer. The nucleotide sequence for the antisense molecule is chosen at a binding location that preferably spans the start codon. Proprietary software scans window sizes 10 bases, 11 bases, 12 bases, 13 bases, 14 bases, 15 bases, 16 bases, 17 bases, 18 bases, 19 bases, and/or 20-40 bases (as a non-limiting example) including the start codon and ranks self-folding potential by base content. The software algorithm may be programmed to span the start codon. Alternatively, the algorithm may be programmed to optionally span the start codon region. Selection of antisense sequence can be finalized manually from these data or through an automated process derived from empirical data and parameter weighting. These antisense molecules against ribosomally-expressed genes are substantially orthogonal to the human transcriptome. In one embodiment, the antisense molecules have base lengths exhibiting features such as Tm greater than 37°C, low self-folding, and significant start codon overlap.

[0028] In another embodiment, the invention provides a polynucleotide sequence at least 80% identical to a sequence selected from SEQ ID NO: 1-50. Specifically, the sequences may contain one or more substitutions, additions, deletions, and/or insertions with natural or non-natural nucleotides, such that the target gene modulation activity is not substantially diminished. Variants exhibit at least about 80%, 81%, 82%, 83%, 84% 85%, 86%, 87%, 88%, or 89% sequence identity; and another embodiment at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to a sequence selected from the group consisting of SEQ ID NOS: 1-50. The percent identity may be readily determined by comparing sequences of the polynucleotides to the corresponding portion of the target polynucleotide, using any method including using computer algorithms well known to those of ordinary skill in the art. Algorithms include the Align or the BLAST algorithm (Altschul, 1991 J. Mol. Biol. 219:555-565; Henikoff and Henikoff, 1992, *Proc. Natl. Acad. Sci. USA 89*:10915-10919).

[0029] In one embodiment of the invention, the active ingredient is coupled to a targeting/cell penetration molecule. In one aspect of the invention, the targeting molecule comprises a peptide. The peptide may comprise a cell penetration peptide (CPP). Peptides utilized may have one or more functions to facilitate cell targeting and/or membrane permeation. In particular, the therapeutic polynucleotides of the invention can be

delivered to *Staphylococcus aureus* in a host by conjugating peptides to the antisense molecule. The ability to conjugate antisense molecules to peptides for membrane disruption of bacteria provides specificity and reduces toxicity. Examples of cell penetration peptides include those having SEQ ID NOS: 51-61. Additional examples cell penetration peptides and methods to link them to antisense molecules are described in U.S. Pat. Nos. 8,354,387, 8,354,093, 8,313,778, 8,299,236, 8,242,081, 8,211,468, 8,207,293, 8,138,383, 8,044,019, 8,039,587, 7,943,581, and 7,879,813. In another embodiment, the cell penetrating peptides is derived from HIV tat, herpes virus VP22, the Drosphila Antennapedia homeobox gene product, signal sequences, fusion sequences or protegrin I as disclosed in U.S. Pat. 8,338,366. The antisense molecule-peptide conjugate may be prepared by methods of solid-phase synthesis, where cysteine serves as the linker between peptide and DNA. Other methodologies known in the art may be used (See for example, Dirksen, A., *et al.*, *J. Am. Chem. Soc.* 2006. *128*, 15602-3).

[0030] CPPs useful in the invention are peptides of diverse origins. Cationic nucleic acid-carrier peptides form productive nanoparticles when mixed with the synthetic polymers of the invention. One example is the peptide KFFKFFKFFK (SEQ ID NO: 51) described in Xie *et al.*, *Molecular Therapy* 2004, *10*, 652–659. Additional peptides may include TAT peptide and PENETRATIN. The TAT peptide, GRKKRRQRRRPQ (SEQ ID NO: 60), is derived from the transactivator of transcription (TAT) of human immunodeficiency virus and is a CPP. CPPs overcome the lipophilic barrier of cell membranes and deliver large molecules and particles inside the cell for their biological actions. PENETRATIN peptide is a 16-amino acid peptide of sequence RQIKIWFQNRRMKWKK (SEQ ID NO: 59) corresponding to the third helix of the homeodomain of Antennapedia protein.

[0031] Useful CPPs also encompass cyclic d,l-αpeptides, such as, KQRWLWLW (SEQ ID NO: 57), RRKWLWLW (SEQ ID NO: 56), and KKLWLW, (SEQ ID NO: 55) as described in Fernandez-Lopez *et al.*, *Nature* 2001, 412, 452-455. These peptides have antibiotic properties of their own, and also function as carriers of cargo for internal cellular delivery. Additionally, amphipathic peptides LLIILRRRIRKQAHAHSK (SEQ ID NO: 58) and transportin 10 (TP10), AGYLLGKINLKALAALAKKIL (SEQ ID NO: 54), described in Nekhotiaeva *et al. FASEB J.* 2010, 394-396, form productive nanoparticles. Tryptophan rich peptides, such as Magainin 2 peptide, GIGKWLHSAKKFGKAFVGEIMNS (SEQ ID NO: 53), which was isolated from the African clawed frog (Karas *et al, Biochemistry* 2002, *41,*10723-31), are additional CPPs

useful in the present invention. Furthermore, Indolicidin, ILPWKWPWWPWRR (SEQ ID NO: 61), which was isolated from bovine neutrophils, is another CPP useful in the present invention. These and other peptides of similar sequence and properties are recognized by one of skill in the art as functional alternatives and are encompassed by the present invention. Furthermore, these peptides may be modified to improve function as desired or needed.

[0032] Bulk peptide and polynucleotide synthesis can be carried out by contract manufacturers, such as Neo Group, Inc. (Cambridge, MA) using standard methodologies including solid-scaffold protection/deprotection synthesis via high fidelity synthesizers. The peptide-PNA or peptide-DNA component is the therapeutic molecule which enters the pathogen and disrupts its genetic regulation.

[0033] In one embodiment, an antisense molecule is conjugated to a CPP using well known conjugation methods that employ succinimidyl-6-hydrazinonicotinateacetonehydrazone to succinimidyl-4-formylbenzoate coupling chemistry. This is a specific, well-behaved, and highly efficient conjugation method for peptide-DNA coupling. In order to covalently couple peptides to nucleic acids, the peptides are prepared for reaction by modifying the N-terminal with a reactive group. In one embodiment, the N-terminal of the peptide is modified with S6H (succinimidyl-6-hydrazinonicotinateacetonehydrazone). N-protected peptides are desalted and dissolved in dry DMF. Next, S6H is added in 2x molar excesses to a stirring solution and allowed to react at room temperature for 2 hours. Workup follows procedures known in the art, such as that described by Dirksen *et al. J. Am. Chem. Soc.* 2006 128, 15602-3. Other methods of coupling peptides to nucleic acids known in the art may be used.

[0034] An FITC assay may be utilized to monitor cellular uptake of peptides. Peptides were conjugated to fluorescein isothiocyanate (FITC) to monitor uptake using florescence microscopy. FIG. 2A-2B show assay results for several peptides as tested in MRSA (FIG. 2A) and AcB (FIG. 2B) (fluorescent overlays 2 hours post-treatment with 1 μM of FITC-peptide agents, scale bar = 100 μm). For MRSA, the helical cationic peptides with KFF and RFF motifs are effective for cellular entry. Also, Magainin-FITC is effective for entry into MRSA. There do not appear to be any bactericidal effects from the peptides at the tested concentration (1 μM) in any of the micrographs presented in FIG 2A - 2B.

[0035] In another embodiment of the invention, the antisense molecule is combined with a delivery polymer. The polymer-based nanoparticle drug delivery platform is adaptable to a diverse set of polynucleotide therapeutic modalities. In one aspect of the invention, the delivery polymer is cationic. In another aspect of the invention, the delivery polymer comprises phosphonium ions and/or ammonium ions. In another example of the invention, the antisense molecule is combined with a delivery polymer, and the composition forms nanoparticles in solution. In a further embodiment, nanoparticle polyplexes are stable in serum and have a size in the range of about 30 nm – 5000 nm in diameter. In one embodiment, the particles are less than about 300 nm in diameter. For example, the nanoparticles are less than about 150 nm in diameter.

[0036] In one embodiment, the delivery vehicle comprises a cationic block copolymer comprising phosphonium or ammonium ionic groups as described in PCT/US12/42974. In one embodiment, the polymer is diblock-*Poly*[(ethylene glycol)$_9$ methyl ethyl methacralate][stirylphosphonium]. In another embodiment of the invention, the delivery polymer comprises glycoamidoamines as described in Tranter et al. *Amer Soc Gene Cell Ther*, Dec 2011; polyhydroxylamidoamines, dendritic macromolecules, carbohydrate-containing polyesters, as described in US20090105115; and US20090124534. In other embodiments of the invention, the nucleic acid delivery vehicle comprises a cationic polypeptide or cationic lipid. An example of a cationic polypeptide is polylysine. See U.S. Pat. 5,521,291.

[0037] In one embodiment, the antisense molecules are part of a composition comprising delivery or carrier polymers. In another embodiment, the antisense molecules are part of nanoparticle polyplexes capable of transporting antisense molecules with stability in serum. The polyplex compositions comprise a synthetic delivery polymer (carrier polymer) and biologically active compound associated with one another in the form of particles having an average diameter of less than about 500 nm, such as about 300 nm, or about 200 nm, preferably less than about 150 nm, such as less than about 100 nm. The invention encompasses particles in the range of about 40 nm – 500 nm in diameter.

[0038] In one embodiment, the delivery or carrier polymer comprises a cationic block copolymer containing phosphonium or ammonium ionic groups as described in PCT/US12/42974. In another embodiment of the invention, the delivery or carrier polymer comprises glycoamidoamines as described in Tranter *et al. Amer Soc Gene Cell Ther*, Dec 2011; polyhydroxylamidoamines, dendritic macromolecules, carbohydrate-

containing polyesters, as described in US20090105115; and US20090124534. The polyglycoamidoamine (PGAA) polymer system, which is a proprietary, localized and biodegradable nanoparticle system, represents another delivery or carrier polymer. Poly(galactaramidoamine) is an efficient cationic polymeric vehicle with low cytotoxicity (Wongrakpanich *et al. Pharmaceutical Development and Technology*, January 12, 2012). The nanoparticle delivery system disclosed in Hemp *et al. Biomacromolecules*, 2012 13:2439-45 represents another delivery or carrier polymer useful in the present invention.

[0039] In other embodiments of the invention, the delivery or carrier polymer comprises a cationic polypeptide or cationic lipid. Polymers, such as *poly*-L-lysine (PLL), *poly*ethyleneimine (PEI), chitosan, and their derivatives are also encompassed by the invention. Nucleic acid delivery using these compounds relies on complexation driven by electrostatic interactions between the gene and the polycationic delivery agent. Polymer-DNA complexes condense into particles on the order of 60 nm – 120 nm in diameter. Polymers such as linear PEI and PLL have high transfection rates in a variety of cells.

[0040] *In vivo* nucleic acid delivery has size constraints requiring a sufficiently small polyplex to enable long circulation times and cellular uptake. In addition, polyplexes must resist salt- and serum-induced aggregation. Serum stability is generally associated with a particle size of about sub-150 nm hydrodynamic radius or below maintainable for 24 h. The nanoparticles of the invention, which comprise nucleic acid therapeutic and delivery polymer, have the hydrodynamic radius and material properties for serum stability. In particular, the delivery polymer, when combined with the nucleic acid, protects the therapeutic cargo under physiological conditions. The delivery polymers are designed to have characteristics of spontaneous self-assembly into nanoparticles when combined with polynucleotides in solution.

[0041] The invention also contemplates other delivery polymers that form serum-stable nanoparticles. The invention is not limited to the type of delivery polymer and may be adaptable to nucleic acid characteristics, such as length, composition, charge, and presence of coupled peptide. The delivery polymer may also be adaptable for material properties of the resultant nanoparticle, such as hydrodynamic radius, stability in the host bloodstream, toxicity to the host, and ability to release cargo inside a host cell.

[0042] In one embodiment, the antisense molecule or penetrating peptide conjugate thereof is administered in the form of a salt. The salt may be any pharmaceutically acceptable salt comprising an acid or base addition salt. Examples of pharmaceutically

acceptable salts with acids include those formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, hydroiodic acid, hydrofluoric acid, phosphorous acid, and the like. Also included are salts that are formed with organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, alkanedioic acids, aromatic acids, aliphatic and. aromatic sulfonic acids, etc. and include, for example, acetic acid, trifluoroacetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, maleic acid, malonic acid, succinic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid, and the like. Exemplary salts thus include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, nitrates, phosphates, monohydrogenphosphates, dihydrogenphosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, trifluoroacetates, propionates, caprylates, isobutyrates, oxalates, malonates, succinate suberates, sebacates, fumarates, maleates, mandelates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, phthalates, benzenesulfonates, toluenesulfonates, phenylacetates, citrates, lactates, malates, tartrates, methanesulfonates, and the like. Also contemplated are salts of amino acids, such as arginates, gluconates, and galacturonates (see, for example, Berge S. M. et al., "Pharmaceutical Salts," *Journal of Pharmaceutical Science, 66*:1-19 (1997). Acid addition salts of basic antisense molecules may be prepared by contacting the free base forms with a sufficient amount of the desired acid to produce the salt according to methods and techniques with which a skilled artisan is familiar.

[0043] Pharmaceutically acceptable base addition salts are formed by addition of an inorganic base or an organic base to the free acid. Pharmaceutically acceptable base addition salts may be formed with metals or amines, such as alkali and alkaline earth metals or organic amines. Salts derived from inorganic bases include, but are not limited to, sodium, potassium, lithium, ammonium, calcium, magnesium, iron, zinc, copper, manganese, aluminum salts and the like. Salts derived from organic bases include, but are not limited to, salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines and basic ion exchange resins, for example, isopropylamine, trimethylamine, diethylamine, triethylamine, tripropylamine, ethanolamine, diethanolamine, 2-dimethylaminoethanol, 2-diethylaminoethanol, dicyclohexylamine, lysine, arginine, histidine, caffeine, procaine,

N,N-dibenzylethylenediamine, chloroprocaine, hydrabamine, choline, betaine, ethylenediamine, ethylenedianiline, N-methylglucamine, glucosamine, methylglucamine, theobromine, purines, piperazine, piperidine, N-ethylpiperidine, polyamine resins and the like.

[0044] The antisense molecules are administered as part of a pharmaceutical composition comprising a pharmaceutically acceptable diluent, excipient or carrier. Suitable diluents, excipients and carriers are well known in the art and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro Ed., 1985). The pharmaceutical dosage forms suitable for injection or infusion can include sterile aqueous solutions or dispersions or sterile powders comprising the active ingredient which are adapted for the extemporaneous preparation of sterile injectable or infusible solutions or dispersions, optionally encapsulated in liposomes. In all cases, the ultimate dosage form must be sterile, fluid and stable under the conditions of manufacture and storage. The liquid carrier or vehicle can be a solvent or liquid dispersion medium comprising, for example, water, saline, ethanol, a polyol (for example, glycerol, propylene glycol, liquid polyethylene glycols, and the like), vegetable oils, nontoxic glyceryl esters, and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the formation of liposomes, by the maintenance of the required particle size in the case of dispersions or by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, buffers or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0045] Sterile injectable solutions are prepared by incorporating the antisense molecule in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filter sterilization. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying techniques, which yield a powder of the active ingredient plus any additional desired ingredient present in the previously sterile-filtered solutions.

[0046] The invention also provides a method of treating *Staphylococcus aureus* infection and a method of inhibiting the growth of *Staphylococcus aureus.* In one embodiment, the *Staphylococcus aureus* is a methicillin-resistant (MRSA) strain. In one embodiment, the animal undergoing treatment for *Staphylococcus aureus* infection exhibits one or more symptoms of *Staphylococcus aureus* infection including puss production in the infected area, boils, abscesses, carbuncles, stys, and/or cellulitis. The animal may also exhibit signs of sepsis or pneumonia.

[0047] In one embodiment, the antisense molecules are administered by intravenous, intramuscular, or peritoneal injection. In another embodiment, the antisense molecules are administered topically, *e.g.* to a tissue suspected to be infected by *Staphylococcus aureus*. In another embodiment, the antisense molecules are administered orally. When administered orally, the antisense molecules may be formulated as part of a pharmaceutical composition coated with an enteric coating that will protect the antisense molecules from the acid environment of the stomach and release the antisense molecules in the upper gastrointestinal tract. In another embodiment, the antisense molecules may be formulated as part of a sustained release formulation that will release the antisense molecules on a substantially continuous basis over a period of time.

[0048] Animals that may be treated with the antisense molecules according to the invention include any animal that may benefit from treatment with the antisense molecules. Such animals include mammals such as humans, dogs, cats, cattle, horses, pigs, sheep, goats and the like.

[0049] The antisense molecules are administered in an amount that is effective for the treatment of *Staphylococcus aureus* infection or inhibition of the growth of *Staphylococcus aureus*. The amount may vary widely depending on the mode of administration, the age of the animal, the weight of the animal, and the surface area of the mammal. The amount of antisense molecule, conjugate, salt and/or complex thereof may range anywhere from 1 pmol/kg to 1 mmol/kg. In another embodiment, the amount may range from 1 nmol/kg to 10 mmol/kg. When administered topically, the amount of antisense molecule, conjugate, salt and/or complex thereof may range anywhere from 1 to 99 weight percent. In another embodiment, the amount of antisense molecule, conjugate, salt and/or complex thereof may range anywhere from 1 to 10 weight percent.

EXAMPLE I

[0050] **Synthesis of Peptide-PNA Conjugate:** All PNA agents were prepared using heterogenous solid-phase peptide synthesis techniques and purified with HPLC.

[0051] Although direct dosing with naked polynucleotides has been used to inhibit pathogenesis of MRSA in culture, a significant barrier for nucleic acid therapy in humans is the bacterial cell wall. To overcome the cell wall barrier, peptides derived from bacterial-infecting organisms that can penetrate these bacterial cell walls can be attached to nucleic acids or modified nucleic acids to enhance nucleic acid entry into the bacterium.

[0052] DNA sequences were synthesized using high-fidelity synthesizers made by NEO-Bio Group, Cambridge, MA. The polynucleotide was then coupled to peptides which permit permeation of bacterial membranes and polynucleotide entry. In the present invention, solid-phase synthetic methodology for peptide-DNA coupling was employed where cysteine served as the linker between peptide and DNA.

[0053] In a specific embodiment, antisense 15-mer DNA and PNA analogs were synthesized for testing in cell culture. A positive control from literature (FmhB); and a noncoding sequence for use as a negative control (NC) were also synthesized. Each polynucleotide was coupled to the cell penetrating peptide (CPP) motif KFFKFFKFFK (SEQ ID NO: 51).

[0054] Both PNA-CPP and DNA-CPP candidates were synthesized and tested. Mass spectrometric analysis of each conjugate was performed to confirm successful synthesis. The purity of the PNA-peptide and DNA-peptide candidates was established using HPLC. Purity of about 99.9% was achieved for PNA-peptide; while >87% was achieved for DNA-peptide. DNA-peptides yielded a higher degree of impurity likely due to the steps required to make the DNA and CPP peptide separately and then conjugate them before a final purification step. Conversely, synthesis of the PNA agents yielded purity levels of about 99%. Increased purity and simplicity of manufacture of PNA-peptide therapeutics provides advantages over DNA-peptide candidates with respect to cGMP-compliant manufacture in battlefield arenas.

EXAMPLE II

[0055] An FITC assay was utilized to monitor cellular uptake of peptides. Peptides were conjugated to fluorescein isothiocyanate (FITC) to monitor uptake using florescence microscopy. FIG. 2A-2B shows assay results for several peptides as tested in MRSA (FIG. 2A) and AcB (FIG. 2B) (fluorescent overlays 2 hours post-treatment with 1 μM of FITC-peptide agents, scale bar = 100 μm). For MRSA, the helical cationic peptides with KFF and RFF motifs are effective for cellular entry. Also, Magainin-FITC is effective for entry into MRSA. There do not appear to be any bactericidal effects from the peptides at the tested concentration (1 μM) in any of the micrographs presented in FIG 2A - 2B.

EXAMPLE III

[0056] **MRSA *in vitro* studies:** Demonstration of sequence-specific effects of PNA-peptide molecules on MRSA was carried out in MRSA USA 300. MRSA USA 300 is a major source of community-acquired infections in the US, Canada and Europe. Clone FPR3757 is a multidrug-resistant USA 300 strain that is available from ATCC as both the culture (ATCC® BAA- 1556TM) and the genomic DNA (ATCC® BAA- 1556D-5). MRSA USA 300 strain is well characterized which allows for reliable benchmarking. MRSA growth curves were generated by inoculating freshly thawed frozen bacterial stocks at different dilutions ranging from 1:3000, 1:1500, 1:600 and 1:300 in Tryptic Soy Broth (TSB, Becton-Dickinson). Absorbance readings were taken hourly at 600 nm (A_{600}) and 550 nm (A_{550}) using a Biomate 3S spectrophotometer (Thermo Scientific) to establish optimal measurement settings and characterize bacterial growth kinetics. Readings at 550 nm gave slightly higher sensitivity. There was a correlation seen with the lower dilution titrations and a faster time to higher absorbance value. A550 was established as the optimal measurement to assess propagation in vancomycin titration and Minimum Inhibitory Concentration (MIC) assays.

[0057] Vancomycin titrations were established to determine a suitable test range. An 800 ug/ml stock solution was diluted tenfold in TSB to 80 ug/ml and further serial diluted to 40, 20, 10, 5, and 2.5 μg/ml in TSB, respectively. MRSA USA 300 strain was cultured to an early log phase OD 550 value of 0.111 and treated with the 80-2.5μg/ml range of

vancomycin. Absorbance measurements at 550 nm were taken hourly over a 4-hour time period.

[0058] Minimum inhibitory concentration (MIC) analyses were performed as described in Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 7th ed.; Approved Standard M7-A7; CLSI: Wayne, PA, USA, 2006; volume 26, No. 2. Vancomycin and methicillin were used as controls. MIC was determined as the lowest concentration of agent that inhibits bacterial growth detected at A_{600}.

[0059] Time-kill analyses were performed as described in Haste et al. *J. Antibiot.* 2010, *63*, 219–224. Agents at various concentrations were aliquoted into the Falcon tubes. Four ml of bacteria at 5E5 cfu/ml were added to the tubes. Tubes were incubated in a shaker at 37^0C, and at 0, 2, 4, and 8 h were subsequently analysed for bacterial growth via A_{600}.

[0060] Sequence-specific effects of polynucleotide-peptide agents against MRSA: A wide range of concentrations were tested for the PNA-peptide antisense sequences determined from bioinformatics. FmhB was used as a positive control from the literature (Xie et al., *Molecular Therapy,* 2004, 10, 652–659) and a non-encoding sequence with a terminal $(KFF)_3K$ motif was used as a negative control (NC) to indicate bactericidal effects imparted by peptide membrane disruption. Sequence-specific inhibition was demonstrated by treating bacteria during lag phase to determine growth inhibition and potential recovery at later time points. The candidate agents and non-coding sequence control were diluted in a range from 20 μM, 5 μM, 1 μM, 250 μM, and 25 μM with sterile RNase-free, DNase-free water. Inhibition of MRSA growth was observed over a wide range of PNA-peptide concentrations.

[0061] The time course shown in FIG. 3 was carried out using MRSA strain USA 300. Freshly-thawed MRSA at a 1:100 dilution in TSB was added to wells containing the individual PNA-peptide molecules. An additional positive control, vancomycin at 12.5 ug/ml, and a negative control, water only, were also assayed. The samples were allowed to incubate at 37 °C with 225 RPM orbital shaking and measured at two-hour time intervals, over an 8-hour time course. As FIG. 1 illustrates, inhibition of MRSA growth was observed over time at a 5 μM concentration.

[0062] In log-phase growth, inhibition is observed at concentrations as low as ~1 μM for PNA-peptide conjugates (FIG. 3) and as low as ~10 μM for DNA-peptide conjugates.

[0063] FIGS 2A – 2B demonstrate the non-toxicity of the cell-penetrating peptides. When conjugated to FITC and added to cells in culture, the cells remain alive over time periods of the cell culture experiments.

EXAMPLE IV

[0064] To dissolve the DNA-peptide conjugates, they were dispersed in tris buffer at an elevated pH = 9. The conjugates were then gently agitated for 24 h at 40°C. After this time period cloudiness was still observed, so the conjugates were heated to 80°C under gentle agitation for an additional 6h, after which clear solutions were obtained. The initial solution was tested via DLS to look at for potential self-assembly between the DNA-peptide conjugates. As exhibited with many charged polymers there was self-aggregation observed in solution, showing broad polydisperse aggregates in the 300nm to 1-micron range.

[0065] Particle size plays an important role in determining blood circulation time and clearance. It is also a predictor of tissue permeation, clearance potential, and selectivity. Polymer-containing particles have been validated with siRNA and DNA, are capable of protecting nucleic acids from nuclease degradation, and can be engineered for colloidal stability in the bloodstream. The antisense molecule-peptide conjugates of the present invention were combined with serum-stable phosphonium-block copolymers to form polyplexes. This diblock copolymer forms a supramolecular assembly with negatively-charged DNA. The particle forms a core-shell type morphology with a neutral polyethylene glycol (PEG) brush on the surface. Polyplex hydrodynamic diameter was measured on a Zetasizer (Nano ZS) dynamic light scattering (DLS) instrument (Malvern Instruments, Worcestershire, UK). As a size comparison, a DNA-peptide conjugate (S11e-KFFKFFKFFK (SEQ ID NO: 51) without carrier polymer, was measured at 1 mg/ml in tris buffer solution at pH = 9. This DNA-peptide conjugate with diblock-*Poly*[(ethylene glycol)$_9$ methyl ethyl methacralate][stirylphosphonium] at three concentrations exhibited size ranges from 40 nm – 300 nm.

[0066] Formation of nanoparticles with the DNA-peptide conjugates is dependent on physical factors. Because the DNA region is negatively charged and the KFFKFFKFFK (SEQ ID NO: 51) region is positively charged, the conjugates exhibit strong intramolecular associations in solution. A wide range of formulation conditions were

evaluated. Optimal particles form at charge-to-charge ratios of 2-4 (phosphonium + /DNA phosphate -) and [DNA-peptide conjugate] $\leq$ 0.5 mg/ml and lower. When concentrations exceed 0.5 mg/ml, dynamic light scattering (DLS) analysis indicates that large aggregates form. The DLS data indicates that pre-formulation concentration influences the final nanoparticle size range, with 0.5 mg/ml forming the largest nanoparticles clustering around 90 nm – 100 nm; and 0.1 mg/ml forming particles as small as 40 nm diameter.

[0067] To dissolve the DNA-peptide conjugates, they were dispersed in tris buffer at an elevated pH = 9. The conjugates were then gently agitated for 24 h at 40°C. After this time period cloudiness was still observed, so the conjugates were heated to 80°C under gentle agitation for an additional 6 h, after which clear solutions were obtained. The initial solution was tested via DLS to look at for potential self-assembly between the DNA-peptide conjugates. As exhibited with many charged polymers there was self-aggregation observed in solution, showing broad polydisperse aggregates in the 300 nm to 1 μm range.

[0068] All patents, patent applications and publications cited herein are fully incorporated by reference.

CLAIMS

1. An antisense molecule or salt thereof that inhibits the growth of *Staphylococcus aureus* comprising a polynucleotide sequence that is antisense to the coding region of a *Staphylococcus aureus* ribosomal protein and hybridizes to said coding region under physiological conditions, wherein said antisense molecule is 10 to 50 nucleobases in length and has a sequence that is at least 95% identical to a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 18-27, 29-46 and 48-50, and wherein said antisense molecule is conjugated to a cell penetration molecule.

2. The antisense molecule of claim 1, that has a sequence selected from the group consisting of SEQ ID NOs: 1, 2, 18-27, 29-46 and 48-50.

3. A pharmaceutical composition comprising the antisense molecule of claim 1 and a pharmaceutically acceptable diluent, excipient, or carrier, wherein the antisense molecule is 99-100% homogeneous by high-performance liquid chromatography (HPLC).

4. The antisense molecule of claim 1, wherein the antisense molecule comprises a modified backbone.

5. The antisense molecule of claim 4, wherein the modified backbone is a PNA backbone.

6. The antisense molecule of claim 1, which inhibits expression of LSU ribosomal protein L15p (L27Ae) or SSU ribosomal protein S17p (S11e), wherein the antisense molecule has a sequence selected from the group consisting of SEQ ID NOs: 1 and 2.

7. The antisense molecule of claim 1, wherein said cell penetration molecule is a peptide.

8. A composition comprising an antisense molecule of claim 1, complexed to a delivery polymer.

9. The composition of claim 8, wherein said delivery polymer is a cationic block copolymer comprising phosphonium or ammonium ionic groups.