(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(10) International Publication Number
WO 2016/200926 A1

(43) International Publication Date
15 December 2016 (15.12.2016)

WIPO | PCT

(51) International Patent Classification:
C07D 487/00 (2006.01) *A61K 31/519* (2006.01)
A01N 43/90 (2006.01)

(21) International Application Number:
PCT/US2016/036430

(22) International Filing Date:
8 June 2016 (08.06.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/173,128 9 June 2015 (09.06.2015) US

(71) Applicant: **TECHULON INC.** [US/US]; 2200 Kraft Drive, Suite 2475, Blacksburg, Virginia 24060 (US).

(72) Inventors: **BRYSON, Joshua**; 5719 Castlecove Road, Charlotte, North Carolina 28273 (US). **MALONE, Brett**; 567 Riverbend Drive, Pearisburg, Virginia 24134 (US). **LEE, Eunkyung**; 5189 Via Capote, Thousand Oaks, California 91320 (US).

(74) Agents: **ESMOND, Robert W.** et al.; Sterne, Kessler, Goldstein & Fox P.L.L.C, 1100 New York Avenue, NW, Washington, District of Columbia 20005 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2016/200926 A1

(54) Title: PEPTIDE NUCLEIC ACID MOLECULES FOR TREATMENT OF GRAM POSITIVE BACTERIAL INFECTION

(57) Abstract: Disclosed are compositions for the treatment of Gram-positive bacteria infection and inhibition of Gram-positive bacteria growth. The compositions comprise a peptide nucleic acid linked to a cell-penetrating peptide (PNA-CPP). The PNA-CPP conjugate and compositions inhibit expression of bacterial membrane stability proteins and are optionally administered in the form of a nanoparticle compositions and antimicrobial fabrics.

PEPTIDE NUCLEIC ACID MOLECULES FOR TREATMENT OF GRAM POSITIVE BACTERIAL 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 provides to peptide nucleic acids (PNAs) conjugated to a cell-penetrating peptide. The PNA-CPP conjugates targeting bacterial membrane stability proteins are useful for treatment and inhibition of Gram positive bacterial infection.

SUMMARY OF THE INVENTION

[0003] Provided is a peptide nucleic acid (PNA) molecule conjugated to a cell-penetrating peptide (CPP). The PNA-CPP conjugate is useful for treatment of Gram positive bacterial infection and the inhibition of Gram positive bacterial growth. The PNA-CPP conjugate targets a bacterial membrane stability protein and is complementary to a coding region of *Staphylococcus aureus* multimodular transpeptidase-transglycosylase / penicillin-binding protein 1A/1B (PBP1) protein. The PNA-CPP conjugate is shown in Figure 1. In one embodiment, the PNA-CPP conjugate is substantially pure. Also provided are pharmaceutical compositions comprising the PNA-CPP conjugate of the invention.

[0004] The invention also provides a method of inhibiting the growth of Gram positive bacteria, comprising administering the PNA-CPP conjugate or composition of the invention to a tissue containing said Gram positive bacteria or suspected of containing Gram positive bacteria. In one embodiment, the administering is topical administration. In another embodiment, the composition is in the form of a hygiene wipe. In another embodiment, the composition is in the form of an antimicrobial fabric.

[0005] The invention also provides a method, comprising applying to a fabric an effective amount of the PNA-CPP conjugate or composition of the invention. In one embodiment,

the composition is a solution. In another embodiment, the composition is applied in the form of a spray to the fabric. In another embodiment, the composition is applied as a rinse to the fabric.

[0006] The invention also provides a method of treating Gram positive bacterial infection, comprising administering to an animal in need thereof an effective amount of the PNA-CPP conjugate or composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 shows the structure of the PNA-CPP conjugate.

[0008] FIG. 2 is a graph showing MRSA *in vitro* studies. Efficacy of the PNA-CPP conjugate is demonstrated. The PNA-CPP conjugate was tested against bacteria in culture of MRSA USA 300. Vancomycin was used to standardize these results for additional studies. Water and a noncoding conjugate were used as negative controls.

[0009] FIG. 3A-C are graphs showing log-phase MRSA growth inhibition over 8 hours at 0μM, 1μM, 10μM, and 20μM concentration of the PNA-CPP conjugate (FIG. 3A), non-coding PNA (FIG. 3B), and positive control Fmhb (FIG. 3C).

[0010] FIG. 4A-4E are graphs showing kill curves of the PNA-CPP conjugate against skin microbes. FIG. 4A) MRSA; FIG. 4B) MSSA; FIG. 4C) *C. diphtheria*; FIG. 4D) *S. epidermidis*; FIG. 4E) *M. luteus*.

[0011] FIG. 5 is a photograph showing efficacy of the PNA-CPP conjugate at different concentrations against an anaerobic species of bacteria (*Propionibacterium acnes*).

[0012] FIG. 6 is a table describing the dosing amounts for a single dose tolerability study of the PNA-CPP conjugate with pharmacokinetic endpoints in mice.

[0013] FIG. 7 is a table describing the dosing schedule for a multi-dose safety study of the PNA-CPP conjugate in mice.

[0014] FIG. 8A-F are bar graphs showing safety data for multiple doses of the PNA-CPP conjugate in mice. FIG. 8A-D show liver markers (FIG. 8A, ALP; FIG. 8B, ALT; FIG. 8C, AST; FIG. 8D, CPK). FIG. 8E shows kidney markers (BUN). FIG. 8F shows body weights.

[0015] FIG. 9 shows a line graph of survival data in a model of *S. aureus* blood infection in mice.

[0016] FIG. 10A-C show the *in vivo* efficacy of the PNA-CPP conjugate. FIG. 10A is a table describing the dose and treatment regimen for mice that were injected with MRSA and the PNA-CPP conjugate. FIG. 10B is a bar graph showing bacterial burden in mouse blood 1, 2, and 6 hours after treatment. FIG. 10C is a bar graph showing bacterial burden in mouse blood 24 hours after treatment.

[0017] FIG. 11 is a bar graph showing the in vitro post-antibiotic effect ("PAE") of the PNA-CPP conjugate against different species of Gram positive organisms. PAEs of the PNA-CPP conjugate and vancomycin were determined for methicillin-resistant *S. aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), *C. diptheriae*, *S. epidermidis*, and *M. luteus*. The PAE of vancomycin for *M. luteus* was not determined (ND).

[0018] FIG. 12 is a table showing the PAE Sub-MIC effect of the PNA-CPP conjugate against MRSA and MSSA strains.

[0019] FIG. 13 is a bar graph showing the susceptibility of MRSA and MSSA strains to the PNA-CPP conjugate during repeated exposure.

[0020] FIG. 14A-B are tables showing the minimal inhibitory concentration (MIC) values and minimal bactericidal concentration (MBC) values for the PNA-CPP conjugate and vancomycin against different strains of methicillin-resistant *S. aureus* (FIG. 14A) and methicillin-sensitive *S. aureus* (FIG. 14B).

[0021] FIG. 15A-B are a table and a photograph showing the MIC values and MBC values for the PNA-CPP conjugate and vancomycin against different strains of MRSA, MSSA, *C. diphtheria, S. epidermidis, M. luteus,* and *P acnes* (FIG. 15A) and comparison of CFU counts on blood agar plates for *S. epidermidis*, MSSA, and *C. diphtheria* strains (FIG. 15B).

[0022] FIG. 16A-B are photographs showing the pad drying methods employed with the model peptide (FIG. 16A) and XPS analysis of NYCO fibers coated with the model peptide (FIG. 16B).

[0023] FIG. 17A-C are photographs and Energy-dispersive X-ray spectroscopy (EDX) analysis on scanning electron microscopy (SEM) images of water treated NYCO fibers (FIG. 17A), 0.5 weight % pad dry treated NYCO fibers (FIG. 17B), and 0.5 weight % pad dry cure treated NYCO fibers (FIG. 17C).

[0024] FIG. 18A-C are photographs and tables showing the antimicrobial activity of the PNA-CPP conjugate bound to a fabric. FIG. 18A shows a disk diffusion assay of PNA-CPP coated fabrics/paper. FIG. 18B-C shows the antimicrobial activity of the PNA-CPP

conjugate and vancomycin coated cotton, nylon, and NYCON (FIG. 18B); or paper and THERAGAUZE (FIG. 18C) against skin pathogens.

[0025] FIG. 19A-D are photographs showing scanning electron microscopy (8,000x) of coated and uncoated NYCO fabric in the presence or absence of MRSA growth. FIG. 19A shows NYCO fabric only. FIG. 19B shows NYCO fabric coated with the PNA-CPP conjugate. FIG. 19C shows MRSA bacteria colony growth on uncoated NYCO fabric. FIG. 19D shows MRSA bacteria on PNA-CPP coated NYCO fabric.

[0026] FIG. 20A-B are a photograph and a bar chart showing zones of inhibition for PNA-CPP coated fabric after wash cycles of different lengths and temperatures. FIG. 20A shows an image of the zones of inhibition after incubation with the coated fabrics. FIG. 20B shows the zones of inhibition in graphical format.

DEFINITIONS

[0027] The term substantially pure means that the PNA-CPP conjugate is at least 95% homogeneous by HPLC. In another embodiment, the substantially pure PNA-CPP conjugate is 96% homogenous by HPLC. In another embodiment, the substantially pure PNA-CPP conjugate is 97% homogenous by HPLC. In another embodiment, the substantially pure PNA-CPP conjugate is 98% homogenous by HPLC. In another embodiment, the substantially pure PNA-CPP conjugate is 99% homogenous by HPLC. In another embodiment, the substantially pure PNA-CPP conjugate is 100% homogenous by HPLC.

DETAILED DESCRIPTION OF THE INVENTION

[0028] 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.

[0029] Specific aspects of the invention include a PNA-CPP conjugate that is useful for the treatment of Gram positive bacterial infection and/or inhibiting the growth of Gram positive bacteria. The PNA-CPP conjugate hybridizes to a coding region of

S*taphylococcal aureus* multimodular transpeptidase-transglycosylase / penicillin-binding protein 1A/1B (PBP1) protein.

[0030] The PNA-CPP conjugate of the invention comprises a cell penetration peptide (CPP). The cell penetration peptide may have one or more functions to facilitate cell targeting and/or membrane permeation of Gram positive bacteria in a host. The cell penetration peptide provides for membrane disruption of bacteria provides specificity and reduces toxicity. The PNA-CPP conjugate shown in Figure 1.

[0031] Bulk 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. In one embodiment, the PNA molecule is conjugated to the 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.

[0032] In one embodiment of the invention, the PNA-CPP conjugate is part of a composition comprising a buffer. We found that the PNA-CPP conjugate exhibited greater antimicrobial activity in a composition comprising a basic pH. Thus, suitable buffers in the composition of the invention provide a basic pH when dissolved or dispersed in water. In some embodiments, the buffer has a pKa of greater than about 6. See, for example, "Handbook of Pharmaceutical Excipients," 5th ed., Rowe *et al.* (eds.) (2006); and SIGMA Life Sciences, "Products for Life Science Research," Product Catalog (2008-2009). The composition may comprise one or more buffers. Such buffers include—but are not limited to—phosphate buffers, carbonate buffers, ethanolamine buffers, borate buffers, imidazole buffers, tris buffers, and zwitterionic buffers (e.g., HEPES, BES, PIPES, Tricine, and other so-called "Good's Buffers"). See, for example, Good *et al.*, "Hydrogen Ion Buffers for Biological Research," *Biochemistry*, 5(2):467–477

(1966). In one particular embodiment, the buffer is a carbonate, such as sodium bicarbonate or carbonate. In another particular embodiment, the buffer is imidazole. In another embodiment, the buffer is Tris(hydroxymethyl)aminomethane ("Tris").

[0033] In one embodiment of the invention, the buffer has a pKa between about 6 and about 14, between about 7 and about 13, between about 8 and about 12, between about 9 and about 11, and between about 10 and about 11. In another embodiment, the buffer has a pKa between about 6 and about 9, between about 7 and about 9, and between about 8 and about 9. In another embodiment, the buffer has a pKa between about 6 and about 13, between about 6 and about 12, between about 6 and about 11, between about 6 and about 10, between about 6 and about 9, between about 6 and about 8, and between about 6 and about 7. In one embodiment the buffer has a pKa of 6.37. In another embodiment, the buffer has a pKa of 6.95In another embodiment, the buffer has a pKa of 8.1. In another embodiment, the buffer has a pKa of 10.25.

[0034] In another embodiment of the invention, the PNA-CPP conjugate 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 PNA-CPP conjugate 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.

[0035] 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.

[0036] In one embodiment, the PNA-CPP conjugate is part of a composition comprising delivery or carrier polymers. In another embodiment, the PNA-CPP conjugate is part of nanoparticle polyplexes capable of transporting 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.

[0037] 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.

[0038] 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.

[0039] *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.

[0040] 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.

[0041] In one embodiment, the PNA-CPP conjugate 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 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.

[0042] 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.

[0043] In one embodiment, the PNA-CPP conjugate is 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. Gernnaro 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.

[0044] Sterile injectable solutions are prepared by incorporating the PNA-CPP conjugate 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.

[0045] In one embodiment, the composition comprising the PNA-CPP conjugate is in contact with a fabric. The fabric may comprise natural fibers, synthetic fibers, or both. Examples of textile fabrics include, but are not limited to, nylon, cotton, nylon-cotton blends, wool, silk, linen, polyester, rayon, and worsted. In one particular embodiment of the invention, the fabric is cotton. In another embodiment, the fabric is nylon. In another embodiment, the fabric is a nylon-cotton blend. The ratio of nylon to cotton in the nylon-cotton blend fabric can be between about 1:99 and about 99:1, between about 10:90 and about 90:10, between about 20:80 and about 80:20, between about 30:70 and about 70:30, between about 40:60 and about 60:40, and between about 45:55 and about 55:45. In a preferred embodiment, the fabric is a 50:50 nylon-cotton blend.

[0046] In another embodiment of the invention, the fabric has a high tensile strength-to-weight ratio. In one embodiment, the fabric with a high tensile-to-weight ratio is a fabric comprising aramid fibers. In a particular embodiment, the aramid fiber is a para-aramid fiber (e.g., the para-aramid fiber commercially known as KEVLAR). In another particular embodiment, the aramid fiber is a meta-aramid fiber (e.g., the meta-aramid fiber commercially known as NOMEX).

[0047] In certain embodiments, the antimicrobial fabric is capable of treating a Gram-positive bacteria infection or inhibiting growth of a Gram-positive bacteria after the fabric has been washed. In some embodiments, the antimicrobial fabric is capable of treating a Gram-positive bacteria infection or inhibiting growth of a Gram-positive bacteria after between about 10 and about 60 wash cycles, between about 20 and about 50 wash cycles,

between about 20 and about 40 wash cycles, between about 20 and about 30 wash cycles, and between about 20 and about 25 wash cycles. In another embodiment, the duration of a wash cycle is between about 10 minutes and about 90 minutes, between about 10 minutes and about75 minutes, between about 10 minutes and about 60 minutes, between about 10 minutes and about 45 minutes, between about 10 minutes and about 30 minutes, and between about 10 minutes and about 15 minutes. In another embodiment, the water temperature in the wash cycles is between about 16°C and about 60°C, between about 27°C and about 49°C, or between about 37° and about 44°C. In one particular embodiment, the antimicrobial fabric is capable of treating a Gram-positive bacteria infection or inhibiting growth of a Gram-positive bacteria following Laundry Test Method AATCC 147 from American Association of Textile Chemists and Colorists (AATCC).

[0048] In another embodiment, provided is a composition comprising the PNA-CPP conjugate. The composition may be in the form of solution that can be applied to a fabric, e.g., by rinsing, dipping, or spraying. The fabric can be an antimicrobial fabric or a non-antimicrobial fabric. In one embodiment, application of the solution to the fabric provides a fabric that is capable of treating a Gram-positive bacteria infection or inhibiting growth of a Gram-positive bacteria. In other embodiments, application of the solution to the fabric increases the fabric's capability of treating a Gram-positive bacteria infection or inhibiting growth of a Gram-positive bacteria. In a particular embodiment, application of the solution to an antimicrobial fabric with low antimicrobial activity increases the antimicrobial activity of the fabric.

[0049] In other embodiments of the invention, provide is a wound healing dressing comprising the PNA-CPP conjugate. In one embodiment, the wound healing dressing is an adhesive dressing. In another embodiment, the wound healing dressing is a non-adhesive dressing. In one embodiment, the dressing comprises a foam, gel, or cream. In another embodiment, the dressing comprises a fiber based material (*e.g.,* gauzes or waddings). In one embodiment, the fiber-based material is cotton. In another embodiment, the fiber-based material is rayon. In another embodiment, the fiber-based material is a gel-forming fiber, such as a carboxymethylated cellulosic material. In another embodiment, the fiber-based material is a synthetic polymer. In another

embodiment, the wound healing dressing is THERAGAUZE (Soluble Systems, LLC, Newport News, VA).

[0050] The invention also provides a method of treating Gram positive bacterial infection and a method of inhibiting the growth of Gram positive bacteria. The Gram positive bacteria may include, but are not limited to, methicillin-resistant strains of *Staphylococcus aureus* (MRSA) and methicillin-susceptible strains of *Staphylococcus aureus* (MSSA). The Gram positive bacteria may also include, but are not limited to, other *Staphylococcus spp.* (*e.g.,* vancomycin-resistant *Staphylococcus aureus* ("VRSA") and *S. epidermidis*); *Bacillus spp.* (*e.g., B. anthracis*); *Clostridium spp.* (*e.g., C. botulinum, C. dificile, C. perfringens,* and *C. tetani*); *Corynebacterium spp.* (*e.g., C. diptheriae*); *Enterococcus spp.* (*e.g.,* vancomycin-resistant *Enterococcus spp.* ("VRE"), *E. faecalis,* and *E. faecium*); *Lysteria spp.* (*e.g., L. monocytogenes*); *Micrococcus spp.* (*e.g., M. luteus*); *Mycobacterium spp.* (*e.g., M. leprae and M. tuberculosis*); *Propionibacterium spp. (e.g., Propionibacterium acnes*) and *Streptococcus spp.* (*e.g., S. pneumoniae, S. pyogenes,* and *S. agalactiae*). In one embodiment, the animal undergoing treatment for Gram positive bacterial infection exhibits one or more symptoms of Gram positive bacterial infection including puss production in the infected area, acne, boils, abscesses, carbuncles, stys, cellulitis, diarrhea, botulism, and gas gangrene. The animal may also exhibit signs of sepsis or pneumonia.

[0051] In one embodiment, the PNA-CPP conjugate is administered by intravenous injection. In another embodiment, the PNA-CPP conjugate is administered by intramuscular injection. In another embodiment, the PNA-CPP conjugate is administered by peritoneal injection. In another embodiment, the PNA-CPP conjugate is administered topically, *e.g.* to a tissue suspected to be infected by Gram positive bacteria. In another embodiment, the PNA-CPP conjugate is administered orally. When administered orally, the PNA-CPP conjugate may be formulated as part of a pharmaceutical composition coated with an enteric coating that will protect the PNA-CPP conjugate from the acid environment of the stomach and release the PNA-CPP conjugate in the upper gastrointestinal tract. In another embodiment, the PNA-CPP conjugate may be formulated as part of a sustained release formulation that will release the PNA-CPP conjugate on a substantially continuous basis over a period of time.

[0052] Animals that may be treated with the PNA-CPP conjugate according to the invention include any animal that may benefit from treatment with the PNA-CPP

conjugate. Such animals include mammals such as humans, dogs, cats, cattle, horses, pigs, sheep, goats and the like.

[0053] The PNA-CPP conjugate is administered in an amount that is effective for the treatment of Gram positive bacterial infection or inhibition of the growth of Gram positive bacteria. The amount may vary widely depending on the mode of administration, the species of Gram positive bacteria, the age of the animal, the weight of the animal, and the surface area of the mammal. The amount of PNA-CPP 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 PNA-CPP conjugate, salt and/or complex thereof may range anywhere from 1 to 99 weight percent. In another embodiment, the amount of PNA-CPP conjugate, salt and/or complex thereof may range anywhere from 1 to 10 weight percent.

EXAMPLE I

[0054] **Synthesis of PNA-CPP Conjugate:** The PNA agent was prepared using heterogenous solid-phase peptide synthesis techniques and purified with HPLC.

[0055] 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, a peptide derived from bacterial-infecting organisms that can penetrate these bacterial cell walls is attached to the PNA molecule to enhance nucleic acid entry into the bacterium.

[0056] The PNA sequence was synthesized using high-fidelity synthesizers made by NEO-Bio Group, Cambridge, MA. The polynucleotide was then coupled to the cell-penetrating peptide (CPP) which permits permeation of bacterial membranes and polynucleotide entry. In the present invention, solid-phase synthetic methodology for peptide-PNA coupling was employed where cysteine served as the linker between peptide and PNA.

[0057] In addition to the PNA-CPP conjugate, a positive control from literature (FmhB); and a noncoding sequence for use as a negative control (NC) was also synthesized. Each PNA was coupled to the cell penetrating peptide (CPP) motif KFFKFFKFFK. The structure of the PNA-CPP conjugate of the invention is shown in FIG. 1.

[0058] Mass spectrometric analysis of the PNA-CPP conjugate was performed to confirm successful synthesis. The purity of the PNA-CPP conjugate was established using HPLC. Purity of about 99.9% was achieved for the PNA-CPP conjugate. Increased purity and simplicity of manufacture of the PNA-CPP conjugate provides advantages over other nucleotide-peptide conjugates with respect to cGMP-compliant manufacture in battlefield arenas.

EXAMPLE II

[0059] **MRSA *in vitro* studies:** Demonstration of anti-MRSA effects of the PNA-CPP conjugate 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 are 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 give slightly higher sensitivity. There is a correlation seen with the lower dilution titrations and a faster time to higher absorbance value. A_{550} is established as the optimal measurement to assess propagation in vancomycin titration and Minimum Inhibitory Concentration (MIC) assays.

[0060] Vancomycin titrations were established to determine a suitable test range. An 800 μg/ml stock solution was diluted tenfold in TSB to 80 μg/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.

[0061] 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}.

[0062] 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 are subsequently analyzed for bacterial growth via A_{600}.

[0063] A wide range of concentrations were tested for the PNA-CPP conjugate. 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 PNA-CPP conjugate and non-coding sequence control were diluted in a range from 20 μM, 10 μM, 1 μM, and 0 μM with sterile RNase-free, DNase-free water. Inhibition of MRSA growth was observed over a wide range of PNA-CPP concentrations. The results are shown in FIG. 3A. FIG. 3B and 3C are graphs showing negative and positive controls, respectively.

[0064] The time course was carried out using MRSA strain USA 300. Freshly-thawed MRSA at a 1:100 dilution in TSB is added to wells containing the PNA-CPP conjugate. An additional positive control, vancomycin at 12.5 μg/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. Inhibition of MRSA growth was observed over time at a 5 μM concentration. The results are shown in FIG. 2.

[0065] In log-phase growth, inhibition was observed at concentrations as low as ~1 μM for the PNA-CPP conjugate.

[0066] When cell-penetrating peptides were conjugated to FITC and added to cells in culture, the cells remained alive over time periods of the cell culture experiments.

EXAMPLE III

[0067] The *in vitro* antimicrobial efficacy of the PNA-CPP conjugate was tested against skin microbes in culture. As a model for *in vitro* studies, testing was carried out using a BIOSCREEN-C instrument and the Clinical and Laboratory Standards Institute (CLSI) protocol. Vancomycin was used as a positive control.

[0068] Kill curves for the PNA-CPP conjugate were generated using different bacteria strains. Kill curve analysis is a dose-response experiment whereby bacteria are subjected to increasing amounts of antibiotic to determine the minimum concentration needed to inhibit bacterial growth. Kill curves are performed when a new antimicrobial is being assayed to establish the compound's potency and to understand the pharmacodynamics of the drug's impact on bacterial growth.

[0069] The following reference strains were purchased from American Type Culture Collection (ATCC), Manassas, VA: Methicillin-resistant *Staphylococcus aureus* (MRSA) *Staphylococcus aureus* subspecies aureus Rosenbach (ATCC® BAA-1556™); Methicillin-sensitive *Staphylococcus aureus* (MSSA) *Staphylococcus aureus* subspecies aureus Rosenbach (ATCC® BAA-1721™); *Corynebacterium diphtheriae* (Kruse) Lehmann and Neumann (ATCC® 14779™); *Staphylococcus epidermidis* (Winslow and Winslow) Evans (ATCC® 12228™); *Micrococcus luteus* (ATCC® 49732); and *Propionibacterium acnes* (Gilchrist) Douglas and Gunter (ATCC® 11827™).

[0070] Lyophilized cultures from ATCC were revived and grown in tryptic soy broth (TSB). Glycerol stocks were prepared and stored in liquid nitrogen for future studies. The following protocol was used for the kill curve analysis of the bacterial strains against RANT1:

[0071] A loop full of bacteria from glycerol stock was inoculated in TSB and allowed to grow for overnight at 37°C. The culture's optical density (OD_{600}) was adjusted to 1.0 in a spectrophotometer and diluted in Mueller-Hinton broth (MHB) or Tryptic soy broth (TSB) to obtain 10^5 bacteria/100 microliters. Serial dilutions of the PNA-CPP conjugate were prepared using sterile water and different concentrations of the PNA-CPP conjugate (ranging from 0 μg to 54 μg per 100 μl) were incubated with the bacterial cultures. The growth of bacteria was monitored using a BIOSCREEN-C spectrophotometer at 37°C with intermittent agitation. The absorbance values were measured in every five minutes interval for 20-28 hours, dependent on bacterial growth. The optical densities of bacterial

culture in presence/absence of the PNA-CPP conjugate were plotted as a function of time to generate kill curves as shown in FIG. 4A-E.

[0072] Growth of each bacteria was inhibited by the PNA-CPP conjugate at low concentrations: 3.4 μg/100 μl (*S. epidermidis*, FIG. 4D), 1.7 μg/100 μl (MRSA. FIG. 4A), 1.56 μg/100 μl (MSSA, FIG. 4B), 0.21 μg/100 μl (*C. diphtheria,*FIG. 4C), and 0.02 μg/100 μl (*M. luteus*, FIG. 4E).

[0073] The *in vitro* antimicrobial activity of the PNA-CPP conjugate was also tested against clinical isolates of MRSA (n=10) and MSSA (n=10). These results showed that the PNA-CPP conjugate had a similar killing effect against all 20 clinical isolates. Cultures treated with the PNA-CPP conjugate showed a significant growth inhibition at a concentration range of 1.70–3.40 μg/100 μl.

EXAMPLE IV

[0074] The antimicrobial effect of the PNA-CPP conjugate against *Propionibacterium acnes* was investigated *P. ac*nes grows under anaerobic conditions. Therefore, the antimicrobial effect of the PNA-CPP conjugate against *P. acnes* was tested in microfuge tubes in an anaerobic chamber. *P. acnes* cultures were treated with serial dilutions of the PNA-CPP conjugate and incubated anaerobically. After incubation, tubes were examined for formation of a pellet of cells. The results are shown in FIG. 5.

[0075] A lack of pellet formation after overnight incubation was observed at a PNA-CPP conjugate concentration of 0.85 μg/100 μl.

EXAMPLE V

[0076] To dissolve the PNA-CPP conjugate, it was 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 preparation was heated to 80°C under gentle agitation for an additional 6h, after which clear solutions were obtained. The initial solution is tested via DLS to look at for potential self-assembly between the PNA-CPP 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.

[0077] 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 PNA-CPP conjugate of the present invention was combined with serum-stable phosphonium-block copolymers to form polyplexes. This diblock copolymer forms a supramolecular assembly with negatively-charged PNA. The particle forms a core-shell type morphology with a neutral polyethylene glycol (PEG) brush on the surface. Polyplex hydrodynamic diameter is measured on a Zetasizer (Nano ZS) dynamic light scattering (DLS) instrument (Malvern Instruments, Worcestershire, UK). As a size comparison, a PNA-peptide conjugate without carrier polymer, was measured at 1 mg/ml in tris buffer solution at pH = 9. This PNA-peptide conjugate with diblock-*Poly*[(ethylene glycol)$_9$ methyl ethyl methacralate][stirylphosphonium] at three concentrations exhibited size ranges from 40 nm – 300 nm.

[0078] Formation of nanoparticles with the PNA-peptide conjugates was dependent on physical factors. Because the PNA region is negatively charged and the KFFKFFKFFK 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 + /PNA phosphate -) and [PNA-peptide conjugate] ≤ 0.5 mg/ml and lower. When concentrations exceeded 0.5 mg/ml, dynamic light scattering (DLS) analysis indicated 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.

EXAMPLE VI

[0079] To investigate safety of the PNA-CPP conjugate, *in vitro* toxicity studies were carried out in human keratinocyte cultures. Human keratinocytes were procured from ATCC (ATCC- PCS-200-011) and propagated according the manufacturer's recommended protocol. Keratinocytes were seeded on 96-well plates at a density of 10,000 cells per well, providing approximately 70% confluence. Cells were incubated in supplier recommended media (Dermal cell basal media with keratinocytes growth kit), for

24 h at 37°C in a 5% CO_2 environment. The PNA-CPP conjugate was added at a concentration of 10 μM. Plates were briefly swirled and incubated for additional an additional 20 h. The LDH Cytotoxicity Assay kit (Pierce-Thermo Scientific) was used to determine the cytotoxicity of the PNA-CPP conjugate in treated keratinocytes.

[0080] Cytotoxicity was determined by the manufacturer's recommendation. All the samples were tested in triplicate. Untreated cells were used as a control for maximum LDH activity, and cells treated with sterile water were used as a control for spontaneous LDH activity. Media alone was used as negative control. Treated cells (mixed with 10 μl lysis buffer) and untreated cells (mixed with 10 microliter of water) were incubated for 45 minutes at 37°C with 5% CO_2. Fifty microliters of samples were transferred to fresh wells and mixed with 50 microliters of Reaction Mixture and incubated an additional 30 minutes at room temperature protected from light. Fifty microliters of stop solution were added to the wells and absorbance was recorded at 490 and 680 nm. To determine the LDH activity, the OD_{680} absorbance value was subtracted from the OD_{490} value before calculation of percentage cytotoxicity. The percentage cytotoxicity was calculated using the following formula:

$$\%\ \text{Cytotoxicity} = \frac{(\text{Treated cells LDH activity}) - (\text{Spontaneous LDH activity})}{(\text{Maximum LDH activity}) - (\text{Spontaneous LDH activity})} \times 100$$

[0081] *In vitro* cytoxicity of the PNA-CPP conjugate against human keratinocytes was 4.01%, indicating no significant toxicity for 10 μM concentration of PNA-CPP.

EXAMPLE VII

[0082] To assess the safety of the PNA-CPP conjugate, a single dose tolerability study in mice was performed. As shown in FIG. 6, mice were divided into four groups of 15 animals per group. Each group was given a single intravenous injection of either vehicle control (PBS) or PNA-CPP conjugate at a dose of 1 mg/kg, 3.3 mg/kg, or 10 mg/kg.

[0083] After the injections, sera and tissues were collected as indicated in FIG. 6. Samples were collected 15 min., 30 min., 1 hour, 4 hours, and 12 hours after administration of the PNA-CPP conjugate. These samples can be assessed for biological safety markers and PNA-CPP conjugate biodistribution analyses.

[0084] Animals were observed for outward signs of toxicity after the injections. All animals survived the treatments and showed no outward signs of toxicity. These results indicate that the PNA-CPP conjugate is safe *in vivo* when administered in a single dose as high as 10 mg/kg.

EXAMPLE VIII

[0085] To further assess the safety of the PNA-CPP conjugate, multi-dose safety studies were performed in mice. Mice were divided into four groups of 10 animals per group. Each group was given multiple intravenous injections of either vehicle control (PBS) or the PNA-CPP conjugate according to the dosing schedule shown in FIG. 7.

[0086] After the injections, animals were monitored for outward signs of toxicity, such as body weight, appetite, and grooming. Depending on the treatment group, animals were sacrificed after three, four, or seven days and blood samples were collected to measure liver and kidney biological markers (FIG. 7).

[0087] No mortality was observed in any treatment group. All animals showed normal appetite and grooming behavior. Furthermore, no significant changes in body weights were observed for any treatment group (FIG. 8A-E). Liver (ALT, ALP, AST, CPK) and kidney (BUN) markers appeared normal in all treatment groups. These results indicate that the PNA-CPP conjugate is safe *in vivo* when administered in multiple doses as high as 10 mg/kg.

EXAMPLE IX

[0088] To assess the *in vivo* efficacy of the PNA-CPP conjugate, a *Staphylococcus aureus* blood infection mouse model was developed. FIG. 9 shows the survival data for mice administered increasing doses of methicillin resistant *S. aureus* (MRSA). Mice were administered either a vehicle control (4% hog gastric mucin) or 4 x 10^6, 2 x 10^7, or 1 x 10^8 colony forming units (CFU) of MRSA. As shown in FIG. 9, the LD_{50} in this mouse model was approximately 2 x 10^7 CFU.

[0089] After establishing the blood infection mouse model, the efficacy of the PNA-CPP conjugate was assessed. Mice were divided into three groups of 10 animals per group. Each group was intravenously injected with MRSA in a volume of 0.2 ml at a

concentration of 2 x 10^7 CFU/ml. Each group was then treated with (i) vehicle control (PBS); (ii) PNA-CPP conjugate at a dose of 10 mg/kg; or (iii) vancomycin (2 mg/kg), which served as a positive control. PNA-CPP conjugate treatments were administered twice daily for four days.

[0090] FIGS. 10B and 10C shows the bacterial burden in mouse blood 1, 2, 6, and 24 hours after treatment. As shown in FIG. 10B-C, treatment with the PNA-CPP conjugate reduced the levels of MRSA in the blood compared to the control group. These results indicate that the PNA-CPP conjugate is efficacious *in vivo*.

EXAMPLE X

[0091] The post antibiotic effect ("PAE") of the PNA-CPP conjugate was assessed for various bacteria strains and compared to the PAE of vancomycin. PAE is an antimicrobial pharmacodynamic parameter that is a measure of the suppression of bacterial growth after a brief exposure (1-2 hrs) to the antibiotic. The sub-MIC effect ("SME") of an antibiotic is the effect of sub-inhibitory levels of the antibiotic on bacteria that have not been previously exposed to the antibiotic. Similarly, the PAE-sub-MIC effect (PAE-SME) of an antibiotic is the effect of sub-inhibitory levels of the antibiotic on bacteria that were previously exposed to inhibitory levels of the antibiotic. The PAE and PAE-SME of an antibiotic could be used to determine the frequency of drug administration during *in vivo* studies.

[0092] The PAE was determined by the viable plate count method. Bacteria strains shown in FIG. 11 were grown in 3 ml Meuller Hinton Broth (MHB)/Tryptic Soy Broth (TSB) overnight. Absorbance of each culture density was measured by OD_{600} and adjusted to 1.0 (approximately 1 x 10^9 colony forming units (CFUs) with MHB. The culture was then diluted 1:100 in MHB to obtain a culture of 1 x 10^7 CFUs.

[0093] 200 µl of the diluted bacterial culture were transferred to 1.5 ml microcentrifuge tubes. Each culture was exposed to 54 µg/100 µl of PNA-CPP conjugate or 40 µg/100 µl vancomycin for one hour. Control samples included the inoculum with no antibiotic. During exposure, all samples were incubated at 35°C for one hour.

[0094] After exposure to the antibiotic, cultures were diluted 1:1,000 in pre-warmed broth to remove the antibiotic. Antibiotic removal was confirmed by comparison of growth

curves of a control culture containing no antibiotic. Viable counts were determined before exposure and immediately after dilution (0 h) and then every 0.5 hrs until 8 hrs.

[0095] PAE was defined as follows: PAE = T − C, where T is the time required for the viable counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ unit above the counts observed immediately after dilution and C is the corresponding time for the control sample growth.

[0096] As shown in FIG. 11, Gram positive bacteria treated with the PNA-CPP conjugate required more time to increase CFU counts compared to the same strains treated with vancomycin. The PAE of the PNA-CPP conjugate against *M. luteus* and *S. epidermidis* is 3.5 hrs and 5 hrs, respectively. However the PAE of the PNA-CPP conjugate against MRSA, MSSA and *C. diphtheria* is in 1.5-2 hrs. The PAE value of the PNA-CPP conjugate against the skin pathogens is comparable and corroborate with MIC and MBC values.

EXAMPLE XI

[0097] To determine the PAE-Sub-MIC effect (PAE-SME) of the PNA-CPP conjugate against MRSA and MSSA, bacterial cultures were exposed for 1 hour to the 10 μM concentration of the conjugate and the drug was removed by further dilution (1:1000) in pre-warmed culture media. The diluted cultures were further exposed to sub-MIC of the PNA-CPP conjugate—i.e., 0.2X, 0.4X, 0.6X and 0.8X. Viable counts of MRSA and MSSA were determined by plating the cultures on agar plates. This test was performed to determine the emergence of drug resistance as well as the lower concentration of drug that can be used as a correlate for *in vivo* drug testing. The PA-SME was defined as PA-SME =T_{pa} − C, where T_{pa} is the time for cultures previously exposed to the PNA-CPP conjugate and then reexposed to different sub-MICs to increase by 1$\log_{10}$ above the counts determined immediately after dilution and C is the corresponding time for the unexposed control. The PAE-SME of the PNA-CPP conjugate against MRSA and MSSA was over 24 hrs (FIG. 12). This indicates that the PNA-CPP conjugate can be used at 24 hrs of interval subjected to systemic *in vivo* testing.

EXAMPLE XII

[0098] The emergence of drug resistance is an important parameter to consider during *in vitro* testing of a new drug. ATCC reference strains of MRSA and MSSA were subjected to a continuous multiday treatment of the PNA-CPP conjugate to assess development of drug-resistance. Cultures were treated in the presence of the PNA-CPP conjugate (54 μg/100 μl) or water only and cultured for five days. Aliquot of cultures were diluted in TSB broth and plated on MH plates to determine the CFUs/ml. The remaining cultures were centrifuged to pellet the bacteria and re-suspended in fresh culture media with drug or water as control and grown overnight at 37°C. This process was continued for 5 days. On day four the entire culture of MSSA was plated to determine the CFUs/ml and MRSA culture was continued for additional one day to observe the emergence of resistance colonies. The water treated cultures were diluted 1:10000 every day and continued the experiment until day 5. The results are shown in FIG. 13.

[0099] Cultures treated with the PNA-CPP conjugate showed a significant reduction in CFUs (6-8 $\log_{10}$) reduction compared to untreated controls. MSSA colonies disappeared after three days of treatment with the PNA-CPP conjugate, which indicated that the PNA-CPP conjugate is 100% effective against MSSA. MRSA cultures were taken out to day 5 before no colony growth was observed.

EXAMPLE XIII

[0100] The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of the PNA-CPP conjugate were assessed for different strains of MRSA and MSSA using Clinical and Laboratory Standards Institute (CLSI) protocols. Strains of *C. diphtheria*, *S. epidermidis*, *M. luteus*, and *P. acnes* were also tested.

[0101] The MIC and MBC of the PNA-CPP conjugate and/or vancomycin were determined by 96-well plate microdilution method. Eleven two-fold serial dilutions of each compound were made in a final volume of 10 μl sterile water. Each well was inoculated with ~10^5 bacteria at the initial time of incubation, prepared from an overnight culture (OD_{600}=1.0). The MIC was defined as the lowest concentration of compound that prevented bacterial growth after incubating 20 to 22 hours at 35°C. Twenty microliters of the culture were further diluted from each well (control and PNA-CPP treated) and spread

on Mueller-Hinton (MH) plates to determine the MBC of the PNA-CPP conjugate. Similarly, MIC and MBC of PNA-CPP against clinical isolates were also determined by CLSI protocol.

[0102] The results are shown in FIG. 14A-B and FIG 15A-B. The MICs of the PNA-CPP conjugate against clinical isolates of MRSA and MSSA are much lower in comparison to ATCC reference strains. Similarly, the MBCs of the PNA-CPP conjugate against MRSA and MSSA clinical isolates are significantly lower than the ATCC reference strains. However, the MIC and MBC of vancomycin against MRSA and MSSA clinical isolates and ATCC reference strains remained same.

[0103] These studies also demonstrated that antimicrobial activity of the PNA-CPP conjugate is highly specific and selective against skin pathogens in comparison to normal skin flora. As shown in FIG. 15B, *C. diphtheria*, MSSA and *S. epidermidis* were exposed overnight to 1.70 μg/100 μl of the PNA-CPP conjugate and colony forming units were determined by plating on blood agar plates. MSSA and *C. diphtheria* are highly susceptible to the PNA-CPP conjugate in comparison to the normal skin flora *S. epidermidis* strain (FIG. 15B).

EXAMPLE XIV

[0104] The binding properties of the PNA-CPP conjugate to fabric were investigated. To examine the long-lasting binding of peptides or the PNA-CPP conjugate on different fabrics, a model peptide was designed to serve as a proxy to develop methodologies to analyze the deposition of peptide on fabrics. The peptide sequence of the model peptide was a Lysine-Phenylalanine-Phenylalanine-Cysteine-Cysteine-Glutamine sequence. Lysine provides a positive charge, the cysteine groups provide an element for analysis with X-ray photoelectron spectroscopy (XPS), Energy-dispersive X-ray spectroscopy (EDX) in conjunction with scanning electron microscopy (SEM). The sulfur atoms present in the model peptide are useful for the analysis of coated fibers and the phenylalanine groups are useful for the analysis of released peptide.

[0105] Standard methods for coating fabrics include the pad-dry and pad-dry-cure processes, both of which were performed for the model peptide. The first step in these processes was to soak pieces of fabric in solutions of the model peptide for 30 minutes with agitation every 10 minutes. The fabric was soaked in 0.1 and 0.5 wt % solutions of

the model peptide in water. For the pad-dry process, the fabric samples were transferred to a 95 °C oven for 1 h. The pad-dry-cure process involved placing the fabric samples in a 95 °C oven for 90 seconds, followed by a 150 °C oven for 60 seconds. The protocol is shown in FIG. 16A.

[0106] X-ray photoelectron spectroscopy (XPS) is a surface-sensitive quantitative spectroscopic technique that measures the elemental composition of the model peptide coated on nylon-cotton blend (NYCO) fabric. It was determined that model peptide is not stable after autoclaving coated fabrics through the loss of sulfur found on the cysteine residues (FIG. 16B). This is an important finding when considering the type of sterilization options for the antimicrobial fabric of the invention.

[0107] EDX elemental analysis was performed to evaluate the presence of sulfur groups on the fibers. This method is similar to that done with XPS analysis, but increases the depth of the sample probed. The increased depth allowed by EDX is useful since the fabric fibers are woven and are overlapping to form many z-layers. EDX analysis allows evaluation of the peptide's coating manner—i.e., a thin coat over the entire surface or a deeper coating that penetrates between the woven fibers. EDX analysis was performed concurrently with SEM in order to visualize the physical presence of the model peptide on the fabric. The results are shown in FIG. 17A-C. No sulfur peak was present when testing the control 50/50 NYCO fibers (FIG. 17A). When analyzing the 50/50 NYCO fibers treated with 0.5 weight % model peptide and pad dried or pad dry cured, a sulfur peak appears where the new rough, bumpy coating is present on the fibers (FIG. 17B-C). This sulfur peak correlates to the model peptide.

EXAMPLE XV

[0108] Adherence of the PNA-CPP conjugate on fabric was assessed. The commercially available fabrics; 100% cotton, 100% cotton, and NYCO (a 50:50 blend of nylon and cotton) were obtained from marketplace. An additional fabric, THERAGAUZE (Soluble Systems, LLC, Newport News, VA), was also assayed. THERAGAUZE is an FDA-approved sterile polymer wound care dressing that differentially regulates moisture across the wound site. Filter papers and different fabrics (nylon, cotton and NYCO) were cut into 7mm disks and autoclaved for sterilization. The PNA-CPP conjugate was diluted in sterile water to obtain a series of concentrations (in μg/100 μl) of 54, 27, 13.5, 6.79, 5.43,

2.72 and 0 (water only as an untreated control). Different concentrations of the PNA-CPP conjugate were applied onto sterilized paper and fabrics aseptically and allowed to air-dry overnight. A vancomycin disk of 64μg was used as a positive control. Disks coated in water only were used as negative controls. Fabrics coated with the PNA-CPP conjugate were placed on a lawn of bacteria at 10^6 CFUs/ml. Plates were incubated overnight at 37°C. The bacterial growth inhibition zones were observed and recorded as shown in FIG. 18A-B. FIG. 18A shows the results for MSSA bacteria. FIG. 18B-C shows the results for additional strains of skin pathogens.

EXAMPLE XVI

[0109] The *in vitro* antimicrobial activity of a PNA-CPP antimicrobial fabric was also assessed using scanning electron microscopy (SEM). The PNA-CPP conjugate at a concentration of 271.75 μg/100 μl was coated aseptically on NYCO fabric and allowed to air-dry overnight. Uncoated and coated fabrics were cultured with 1.0 x 10^6 CFU/ml of MRSA overnight at 37°C. The fabrics were fixed with fixative solution (5% glutaraldehyde, 4.4% formaldehyde, 2.75% picric acid and 0.05% sodium cocodylate). Prior to fixation, aliquots of bacterial cultures from the coated fiber and uncoated fiber samples were plated on agar to determine the CFU counts. The fixed fabrics were prepped for scanning electron microscopy (SEM). The results are shown in FIG. 19A-D.

[0110] FIG. 19A-D show the SEM images of coated and uncoated fabrics with and without bacteria at 8,000x magnification. SEM revealed that the PNA-CPP conjugate remained bound to NYCO fabric. The uncoated NYCO fabric co-cultured with MRSA revealed the presence of bacterial colonies attached to the fibers (FIG. 19C). Fabric coated with the PNA-CPP conjugate showed significant reduction in the number MRSA colonies attached to the fibers (FIG. 19D). The enumerated CFU/ml of the uncoated fabric was determined to be 2.1x10^8 CFU/ml. The CFU counts from the PNA-CPP coated fabric resulted in zero viable colonies present.

EXAMPLE XVII

[0111] The stability PNA-CPP coated fabric was assessed using Association of Textile Chemists and Colorist (AATCC) protocol 147 for laundering and washing of fabric. This

study determined the ability of the PNA-CPP conjugate to withstand washing under different temperatures. NYCO disks (10 mm) were coated with the PNA-CPP conjugate or water only and dried overnight. Coated fabric disks were soaked in water for one hour with or without agitation using a magnetic stir bar (1,000 rpm). The disks were incubated at three different temperatures: room temperature, 37°C & 44°C. Fabric samples were collected at 15, 30, 45 and 60 minutes. Fabric disks were allowed to aseptically air dry prior to being placed on agar plates with a lawn of MRSA bacteria (10^4 CFUs). Plates were incubated overnight at 37°C and zones of clearance were observed (FIG. 20A) and analyzed (FIG. 20B).

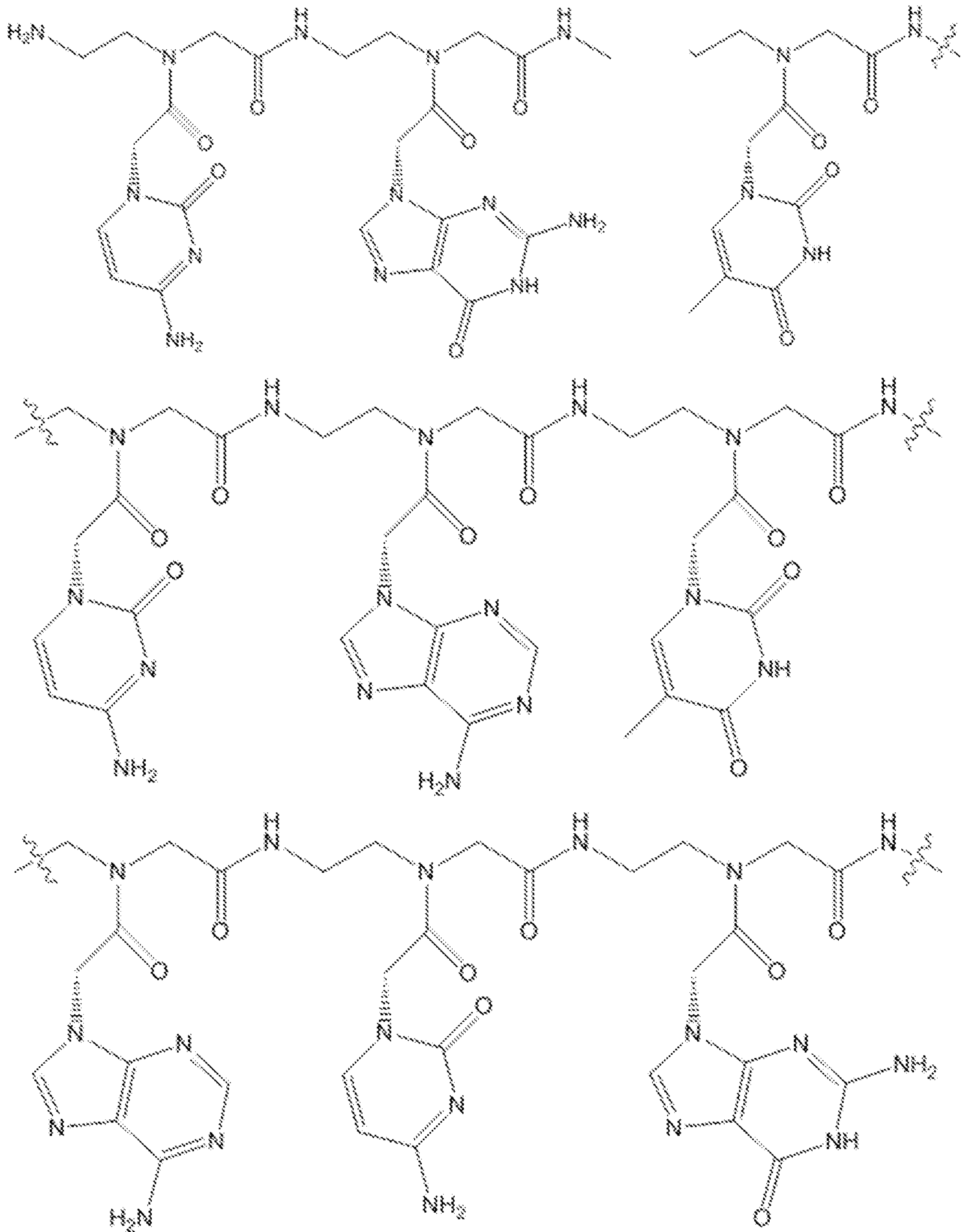
[0112] These results show that the PNA-CPP antimicrobial fabric retains antimicrobial activity after wash cycles of varying lengths and temperatures.

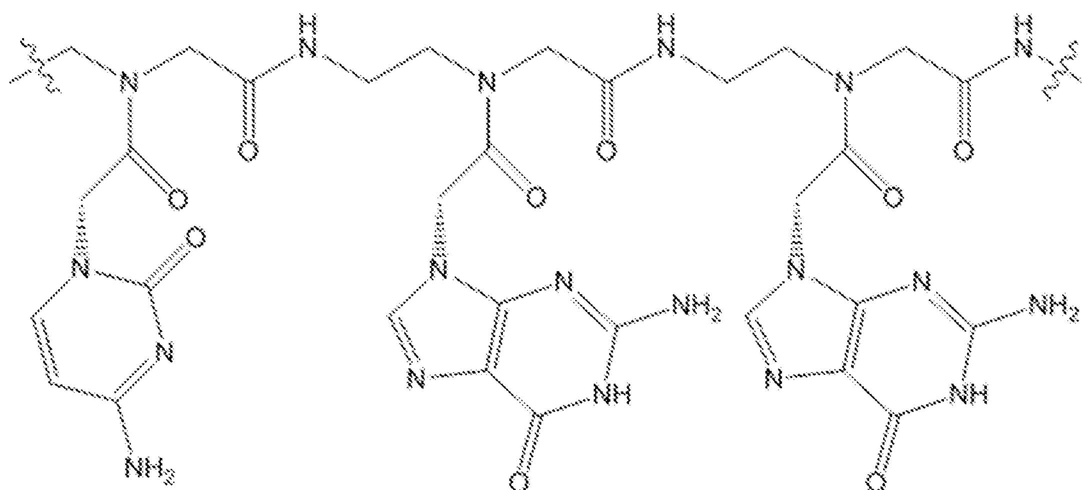
[0113] All patents, patent applications and publications cited herein are fully incorporated by reference.

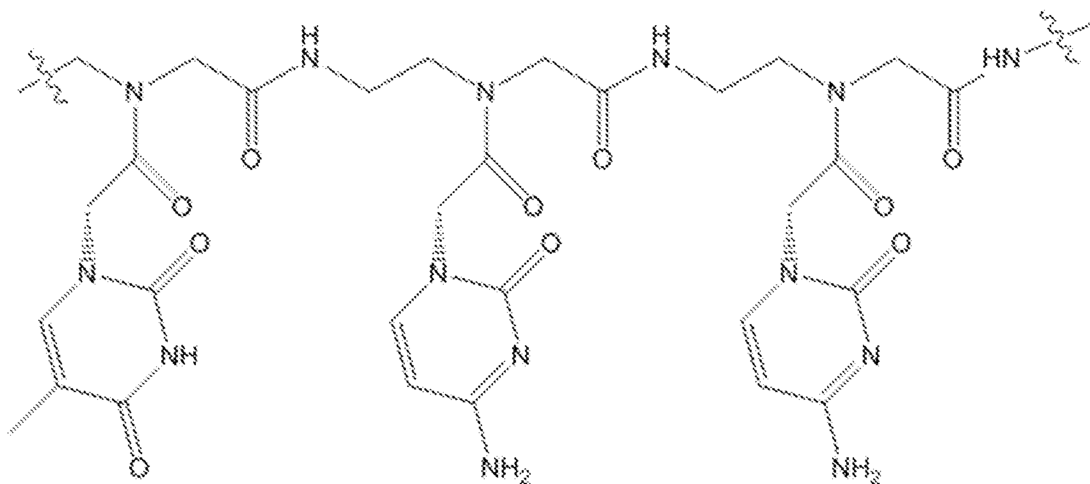
WHAT IS CLAIMED IS:

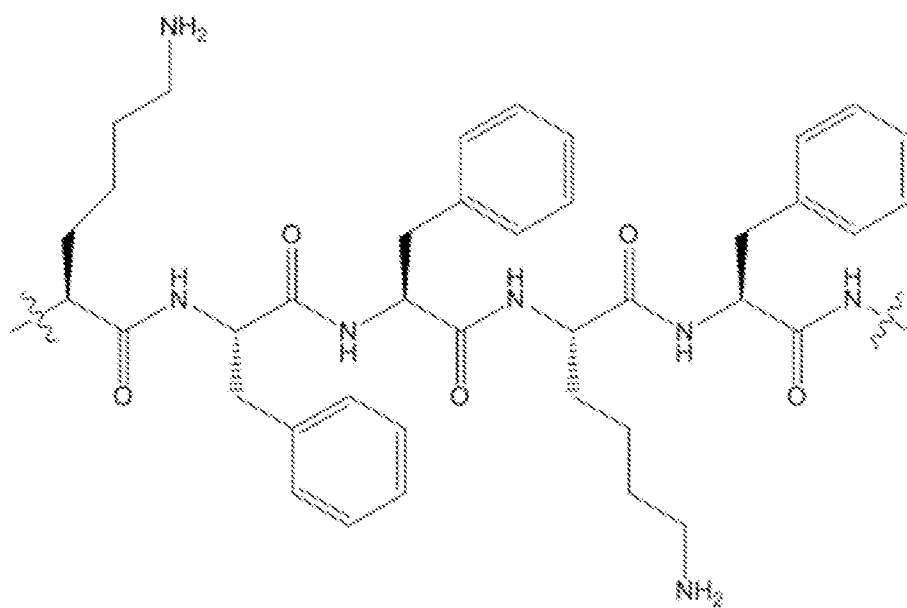
1. A compound of Formula I:

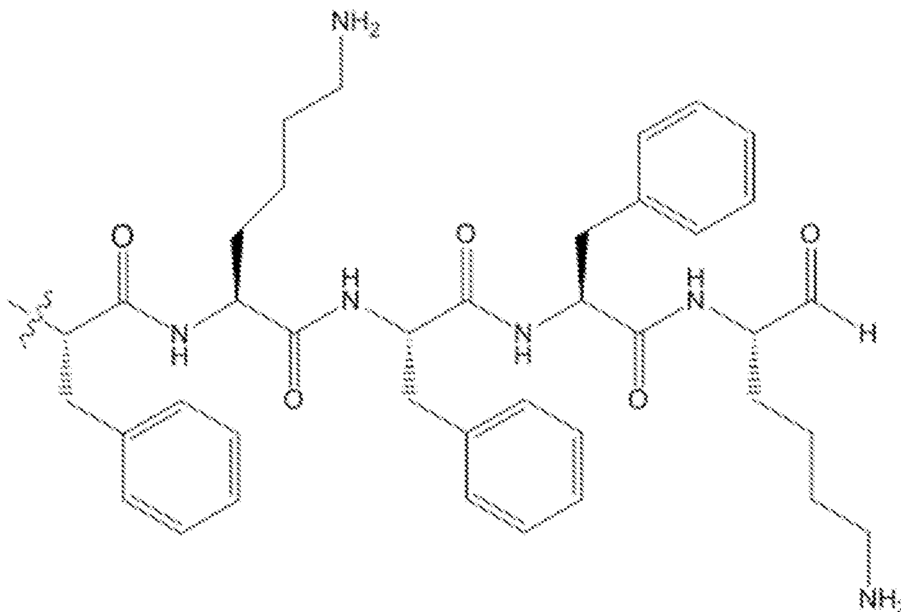
Formula I




H
N
NH2
NH


H
N
HN
NH
NH2


NH2
H
N
O



or a pharmaceutically acceptable salt thereof.

2. The compound of claim 1, which is substantially pure.

3. A composition comprising the compound of claim 1 or 2, and a pharmaceutically acceptable carrier.

4. The composition of claim 3, wherein said carrier comprises a polymer complexed to said compound.

5. The composition of claim 4, wherein said delivery polymer is a cationic block copolymer comprising phosphonium or ammonium ionic groups.

6. A composition comprising the compound of claim 1 or 2 or composition of claim 3 or 4 and a buffer.

7. The composition of claim 6, wherein the buffer is selected from the group consisting of phosphate buffers, carbonate buffers, imidazole buffers, Tris buffers, and zwitterionic buffers.

8. The composition of claim 6 or 7, wherein the buffer is a carbonate buffer.

9. The composition of claim 6 or 7, wherein the buffer is sodium bicarbonate.

10. The composition of claim 6 or 7, wherein the buffer is imidazole.

11. The composition of claim 6, wherein the buffer has a pKa between about 6 and about 14.

12. The composition of claim 11, wherein the buffer has a pKa of 6.37.

13. The composition of claim 11, wherein the buffer has a pKa of 6.95.

14. A method of inhibiting the growth of Gram positive bacteria, comprising administering the compound of claim 1 or 2 or the composition of any one of claims 3-13 to a tissue containing said Gram positive bacteria or suspected of containing Gram positive bacteria.

15. The method of claim 14, comprising topical administration of the compound or composition.

16. The method of claim 14, wherein the composition is in the form of a hygiene wipe.

17. The method of claim 14, wherein the composition is in the form of a topical dressing.

18. The method of claim 14, wherein the composition is in the form a fabric.

19. The method of claim 18, wherein the fabric is nylon, cotton, a nylon-cotton blend, wool, silk, linen, polyester, rayon, or worsted.

20. The method of claim 19, wherein the fabric is a nylon-cotton blend with a ratio of nylon to cotton between about 1:99 and about 99:1, between about 10:90 and about 90:10, between about 20:80 and about 80:20, between about 30:70 and about 70:30, between about 40:60 and about 60:40, and between about 45:55 and about 55:45.

21. A method, comprising applying to a fabric an effective amount of the compound of claim 1 or 2 or composition of any one of claims 3-13.

22. The method of claim 21, wherein the composition is in the form of solution.

23. The method of claim 22, wherein the solution is applied as a rinse, dip, or spray.

24. A method of treating a Gram positive bacterial infection, comprising administering to an animal in need thereof an effective amount of the compound of claim 1 or 2 or composition of any one of claims 3-13.

25. The method of any one of claims 14-24, wherein the Gram positive bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-susceptible *Staphylococcus aureus* (MSSA), vancomycin-resistant *Staphylococcus aureus* ("VRSA"), *Staphylococcus epidermidis, Bacillus anthracis, Clostridium botulinum, Clostridium dificile, Clostridium perfringens, Clostridium tetani, Corynebacterium diptheriae,* vancomycin-resistant *Enterococcus spp.* ("VRE"), *Enterococcus faecalis, Enterococcus faecium, Lysteria monocytogenes, Micrococcus luteus, Mycobacterium leprae, Mycobacterium tuberculosis, Propionibacterium acnes, Streptococcus pneumoniae, Streptococcus pyogenes,* or *Streptococcus agalactiae*.

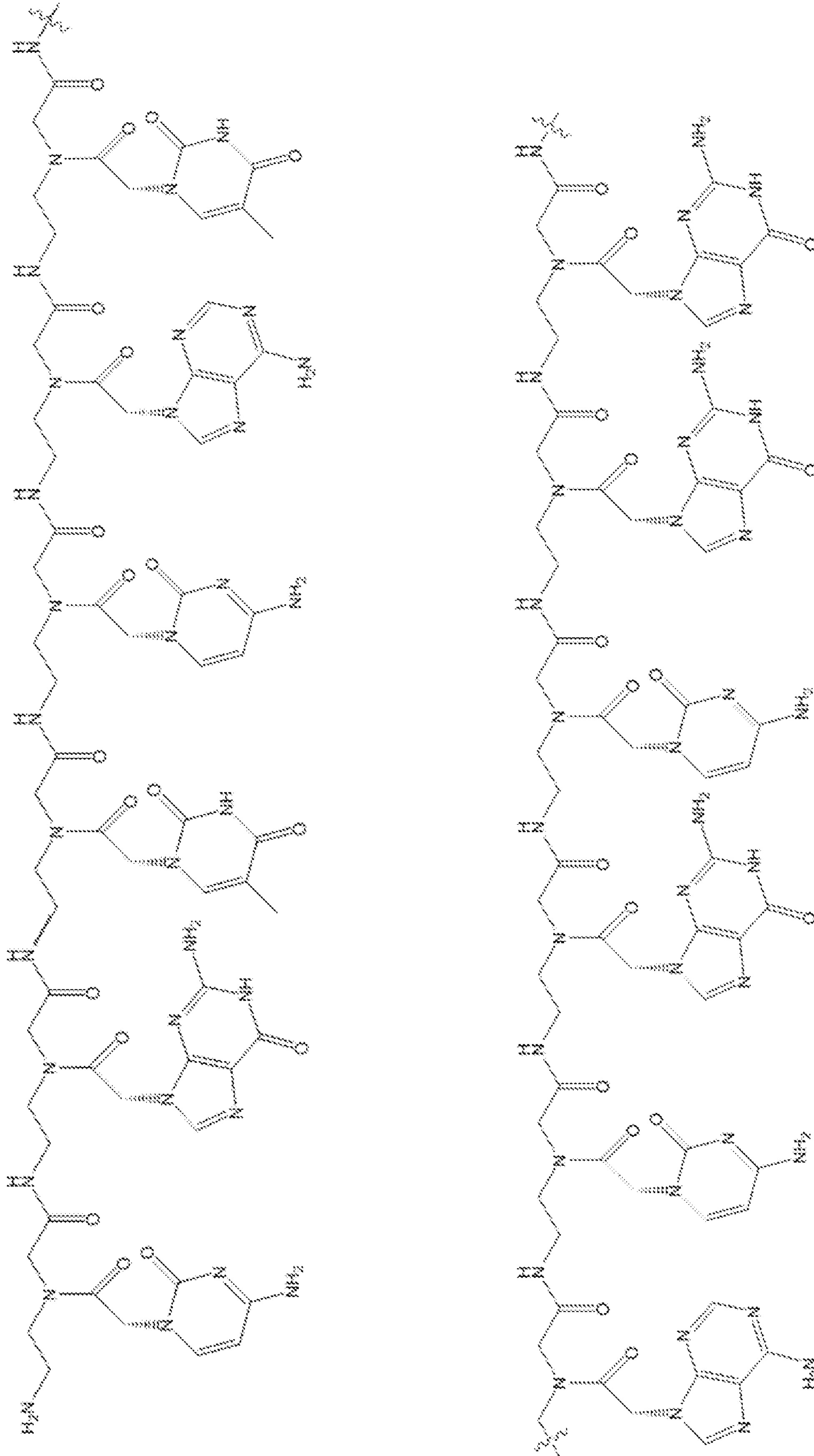


FIGURE 1

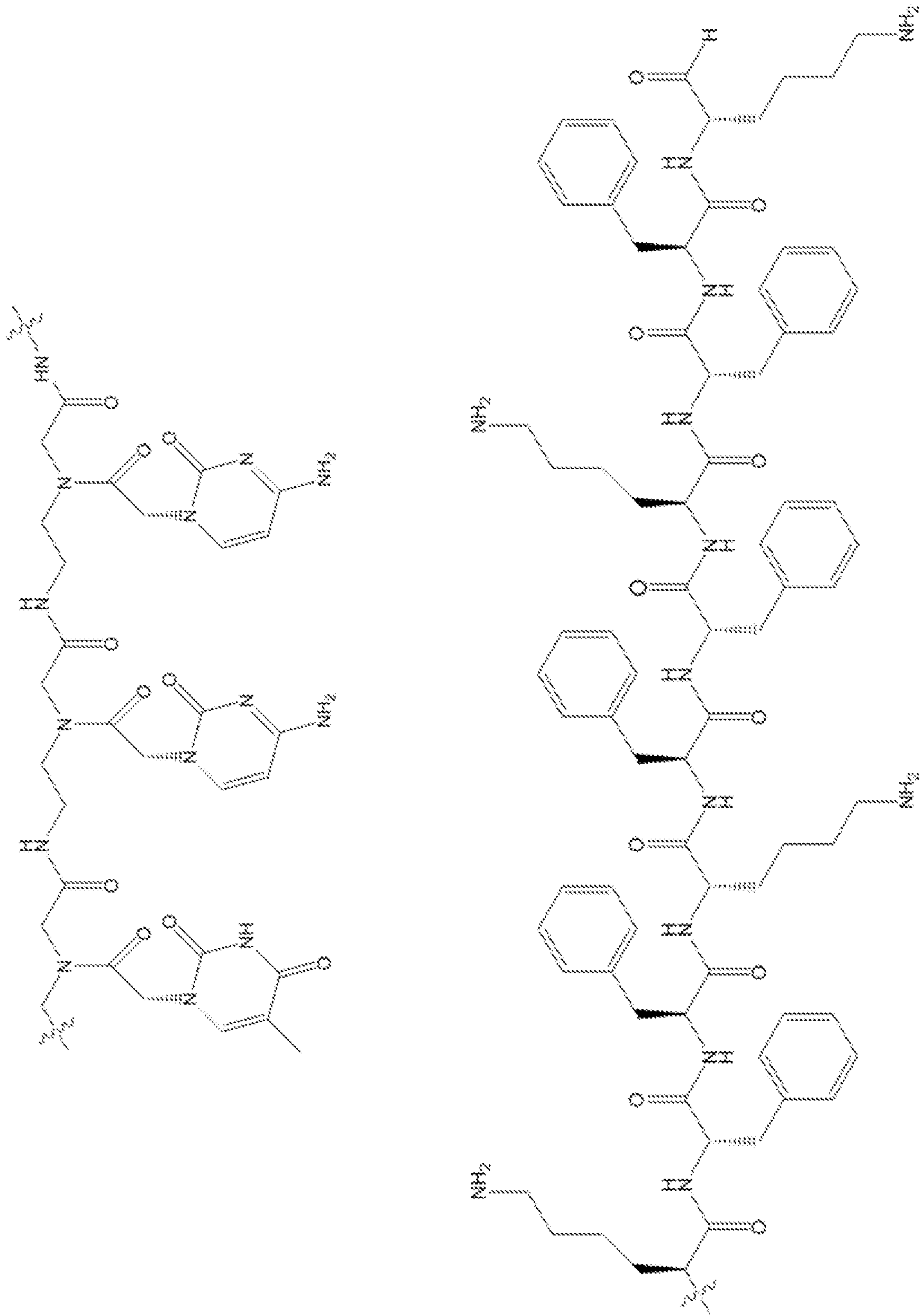


FIGURE 1 (continued)

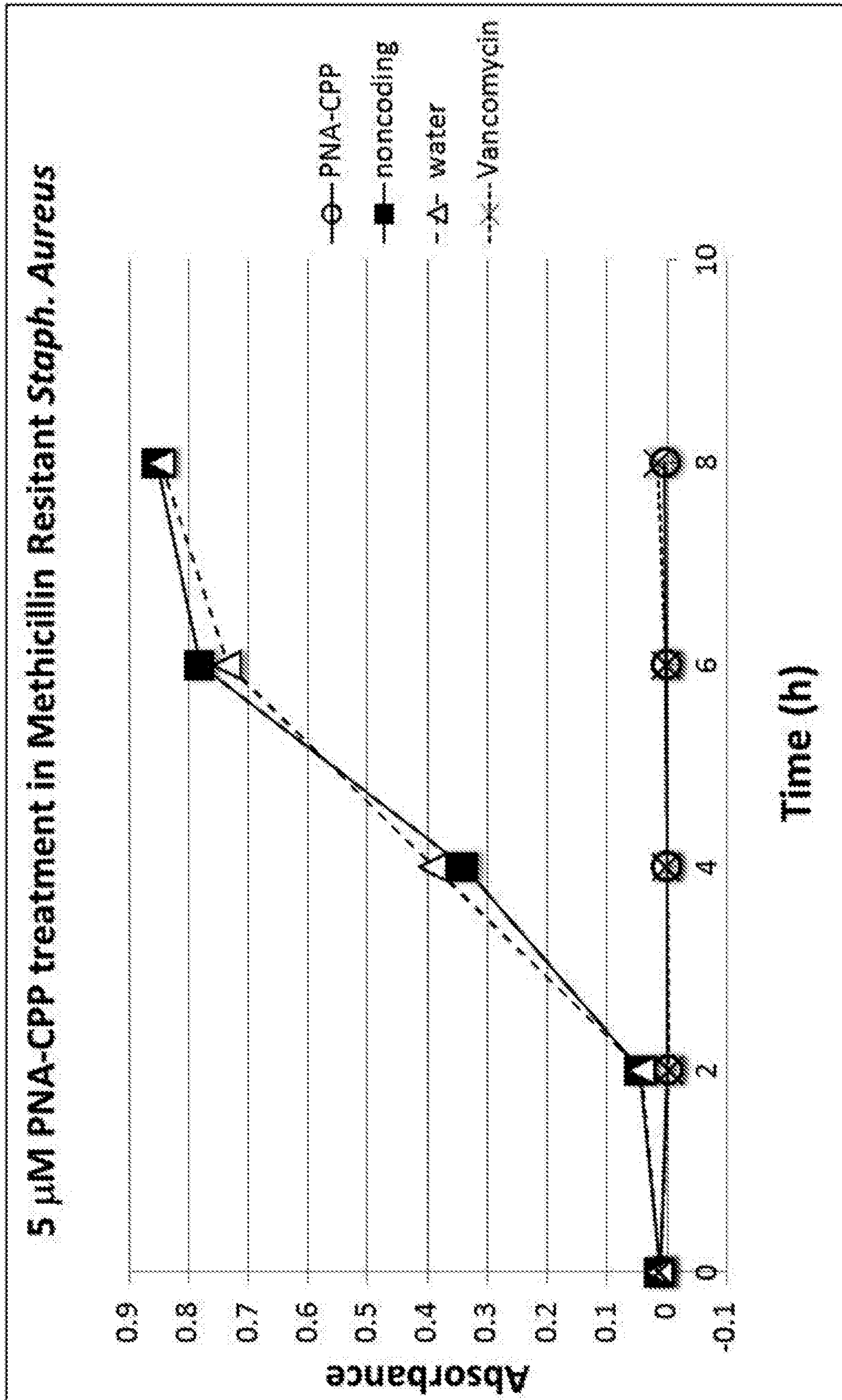


FIGURE 2

FIGURE 3A

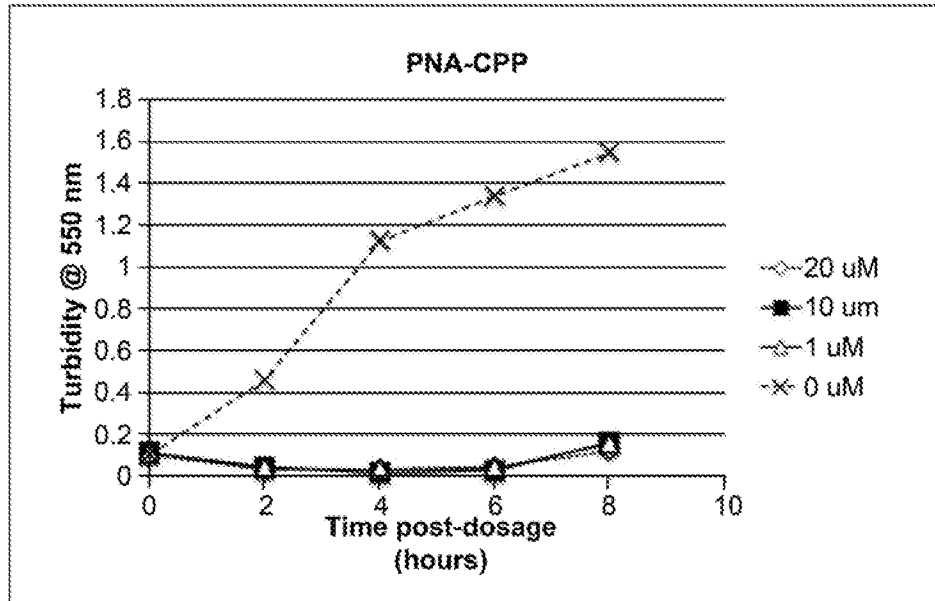


FIGURE 3B

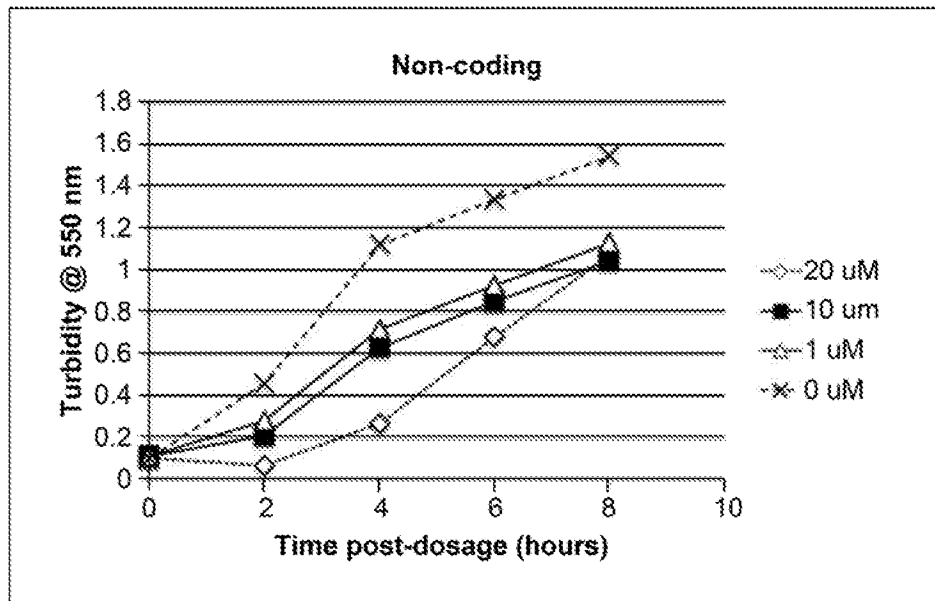


FIGURE 3C

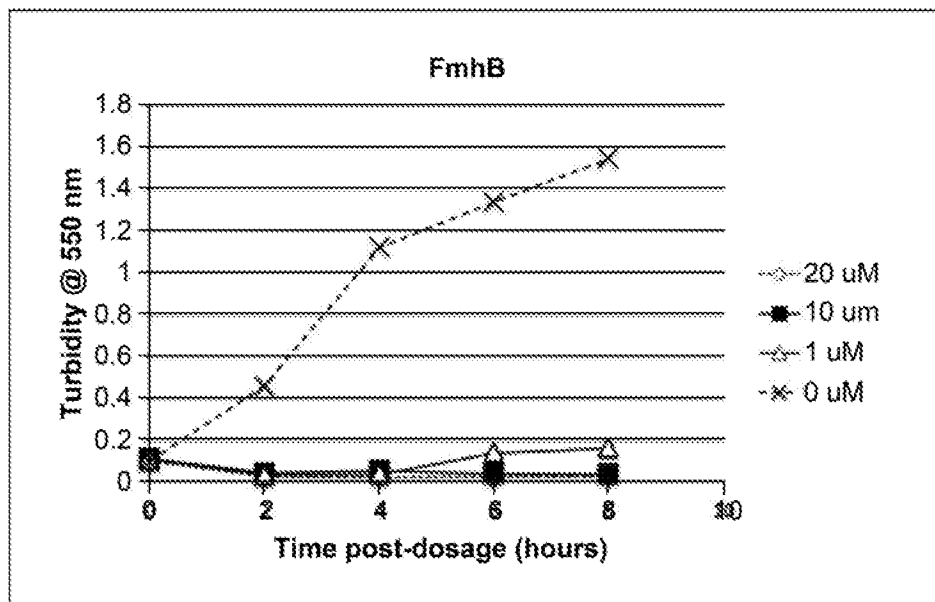


FIGURE 4A

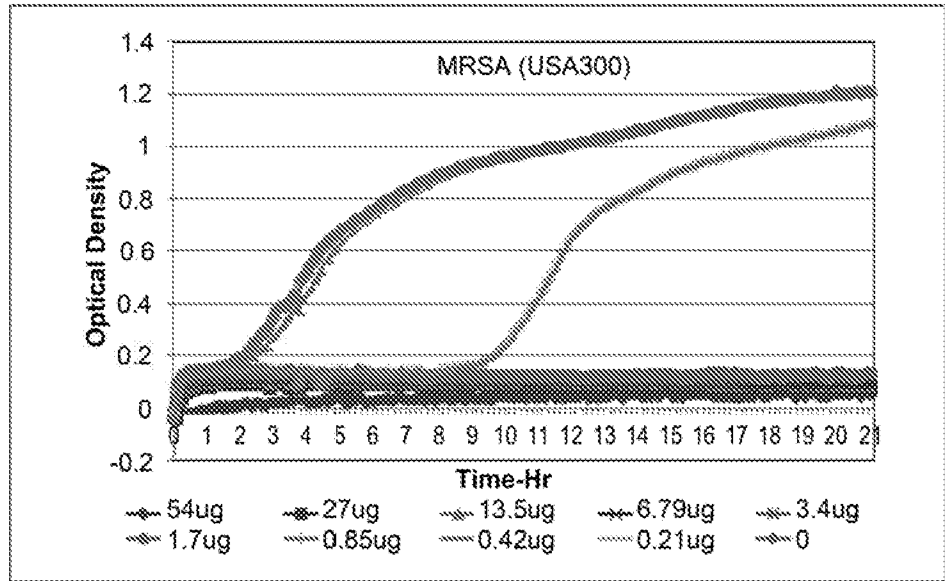


FIGURE 4B

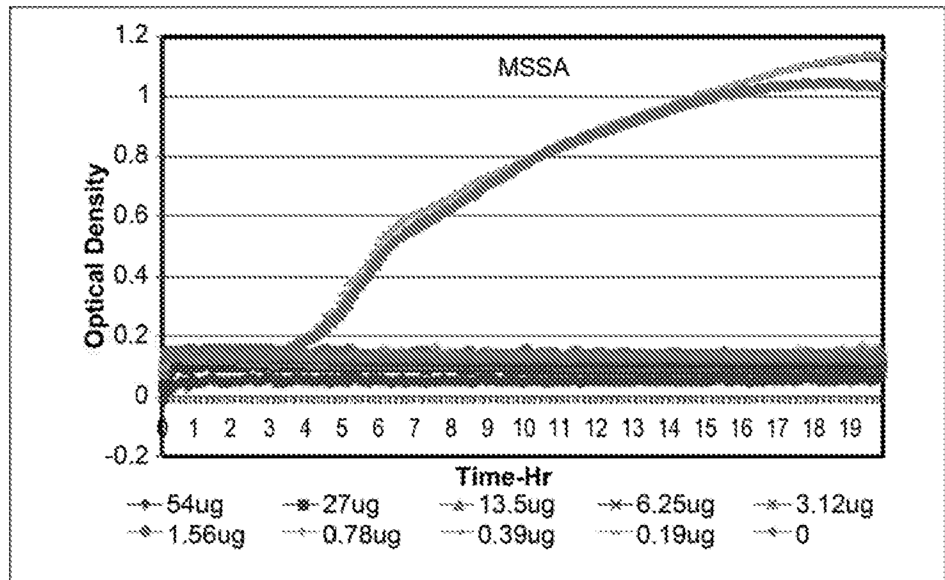


FIGURE 4C

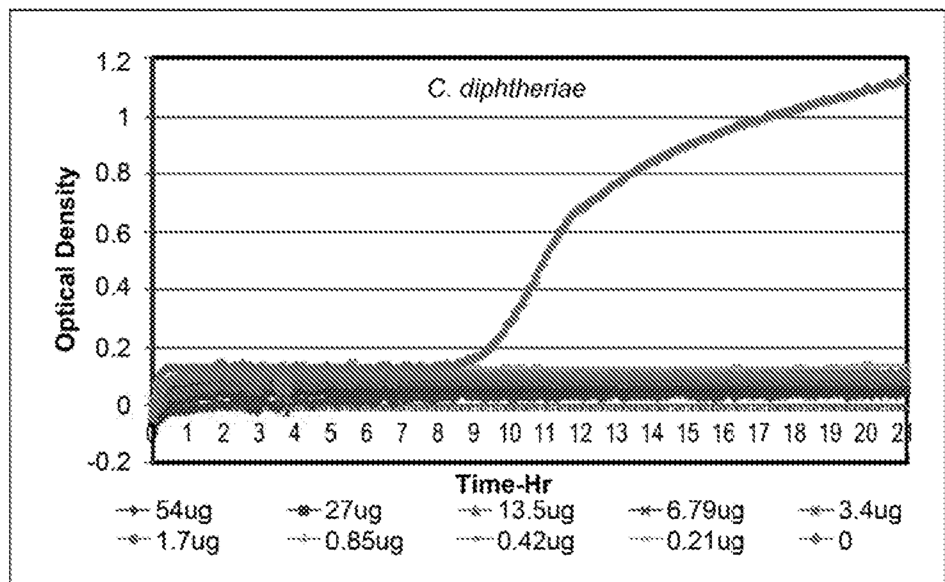


FIGURE 4D

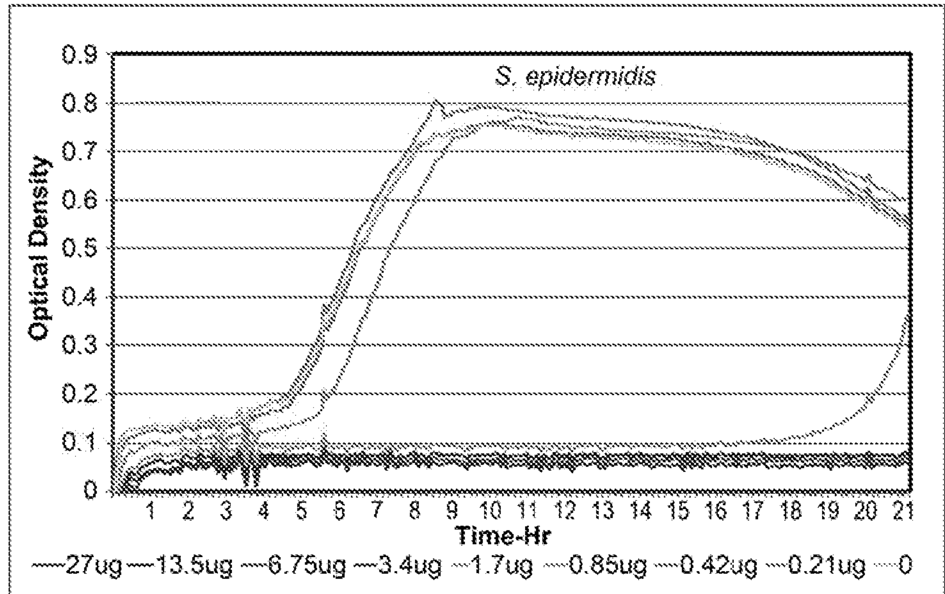
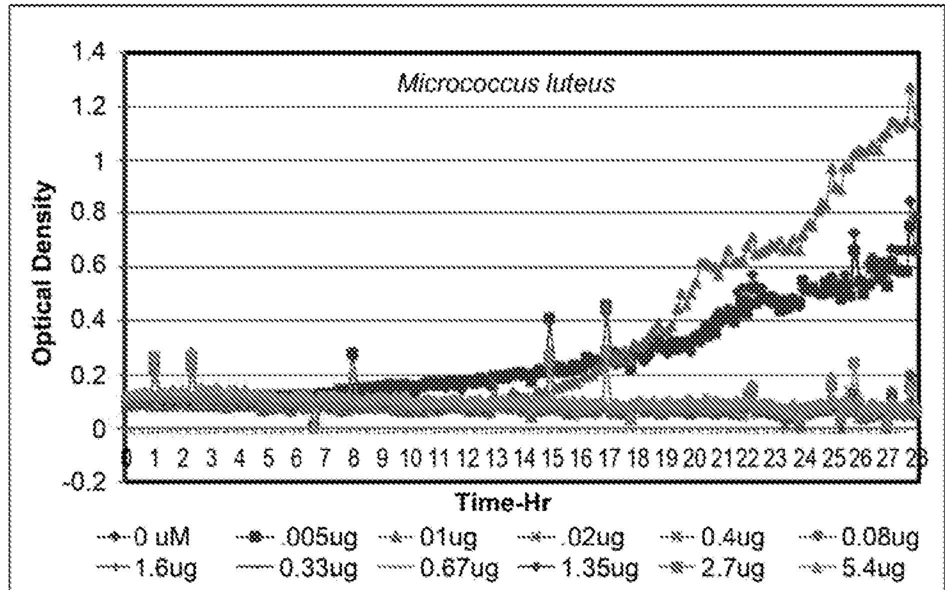
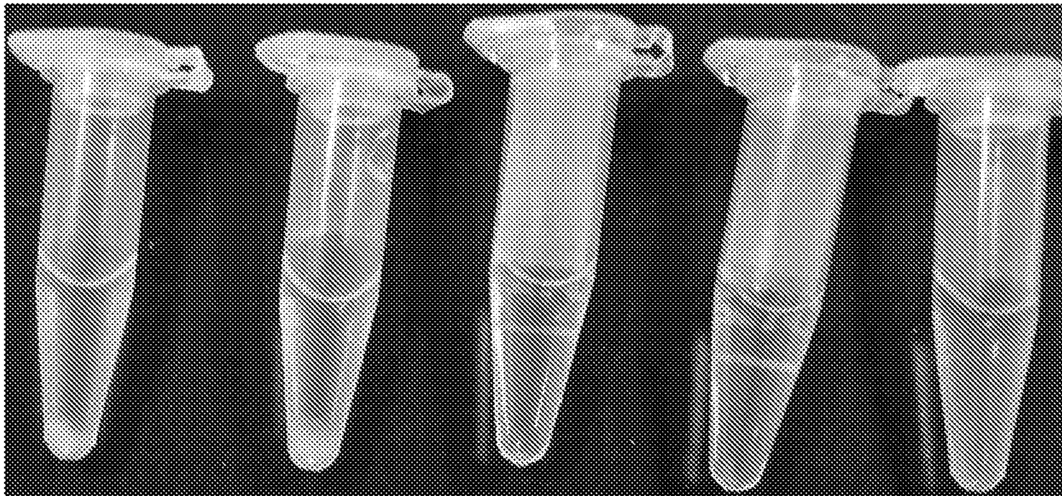


FIGURE 4E





0 0.43 0.85 1.70 3.40

Concentration (μg/ml)

FIGURE 5

Group #	N	Agent	Dose level (mg/kg)	Volume	Route of Admin.	Dosing Schedule	Terminal bleed (for serum) times	Tissue harvest
1	15	Vehicle (PBS)	N/A	0.2 ml	IV	qd x 1	3 mice/group: • 15 min. • 30 min. • 1 hr. • 4 hr. • 12 hr.	Harvest liver, kidney, lung, and spleen; snap freeze and store at -80°C
2	15	PNA-CPP	1	0.2 ml	IV	qd x 1		
3	15	PNA-CPP	3.3	0.2 ml	IV	qd x 1		
4	15	PNA-CPP	10	0.2 ml	IV	qd x 1		

Terminally bleed 3 mice/group/time point at 15 min, 30 min, 1 hr, 4 hr, and 12 hr.

FIGURE 6

Group #	N	Agent	Dose level (mg/kg)	Volume	Route of Admin.	Dosing Schedule
1	10	Vehicle (PBS)	N/A	0.2 ml	IV	qd x 7
2	10	PNA-CPP	10	0.2 ml	IV	qd x 7
3	10	PNA-CPP	10	0.2 ml	IV	q2d x 4
4	10	PNA-CPP	10	0.2 ml	IV	q3d x 3

FIGURE 7

FIGURE 8A

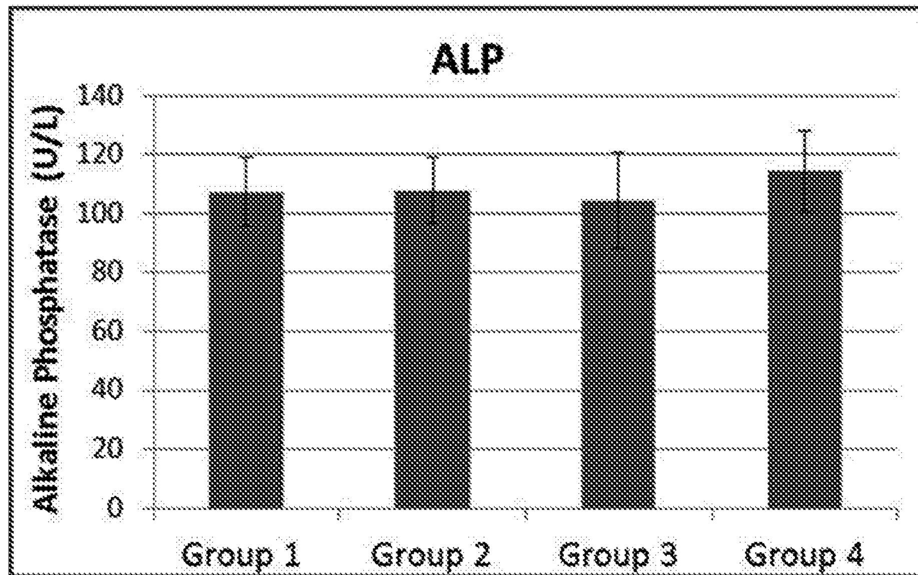


FIGURE 8B

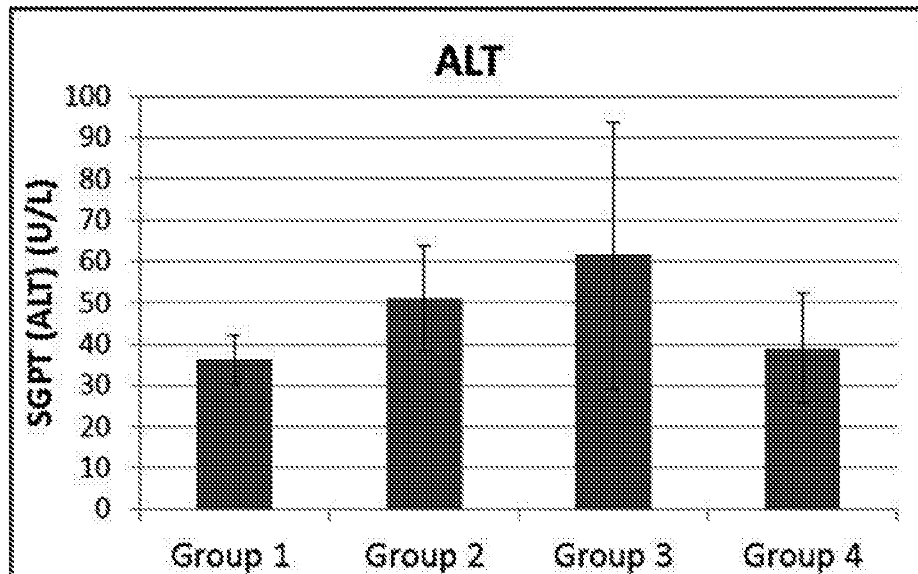


FIGURE 8C

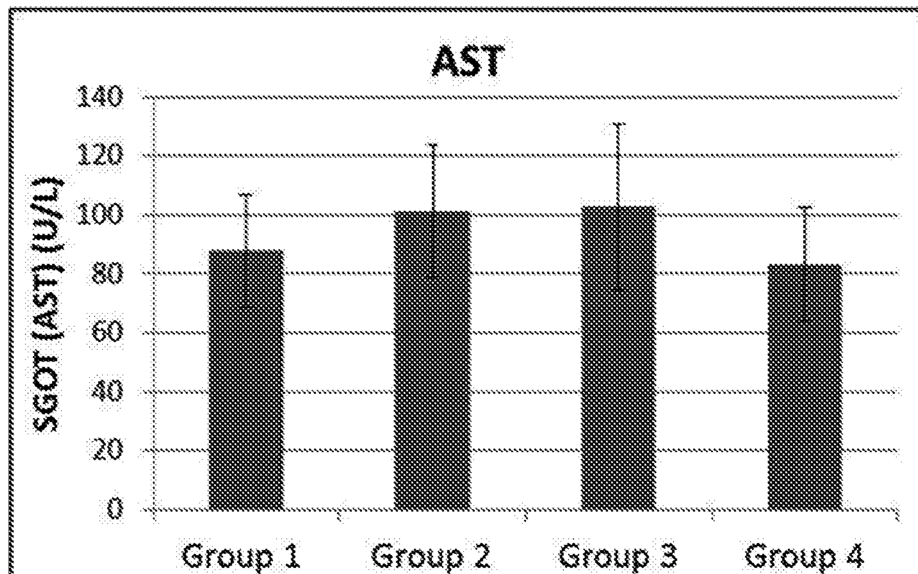


FIGURE 8D

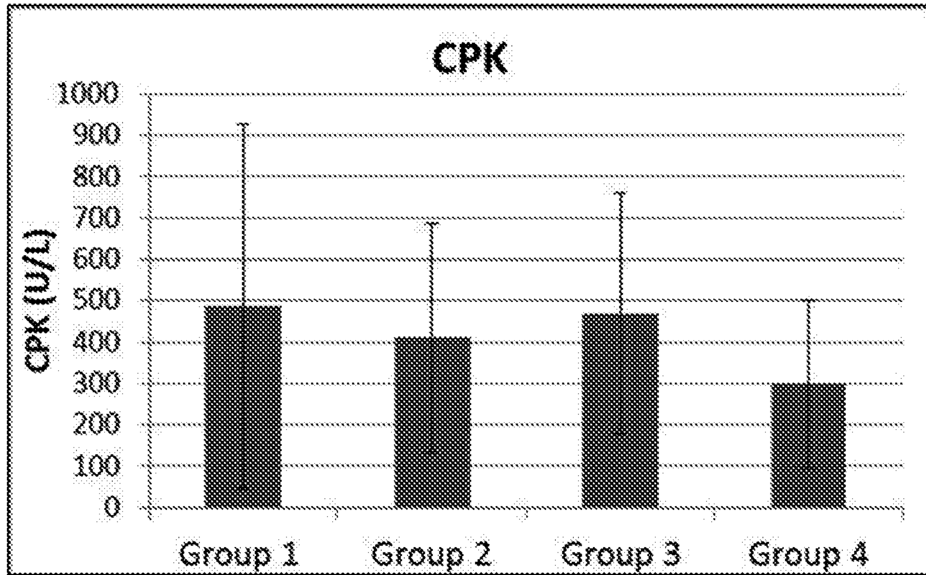


FIGURE 8E

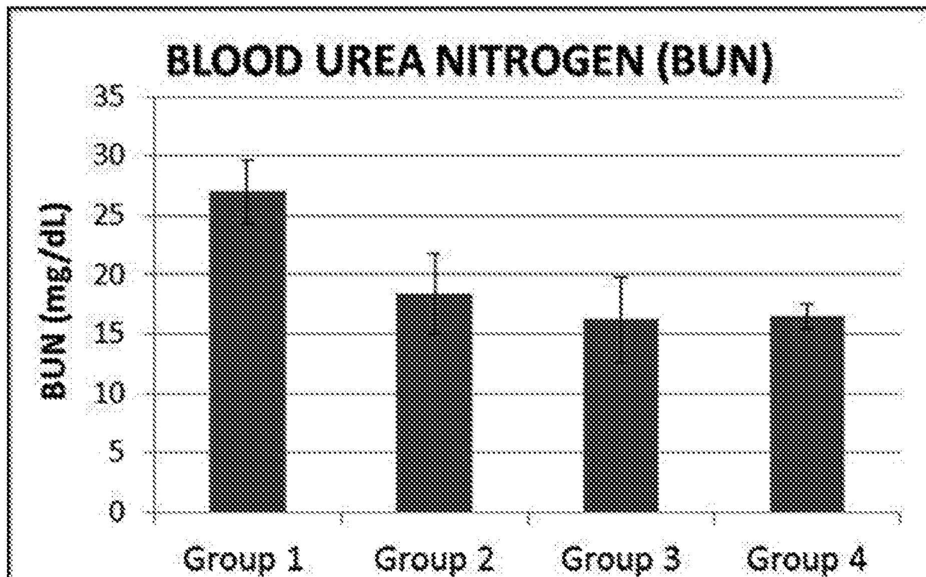
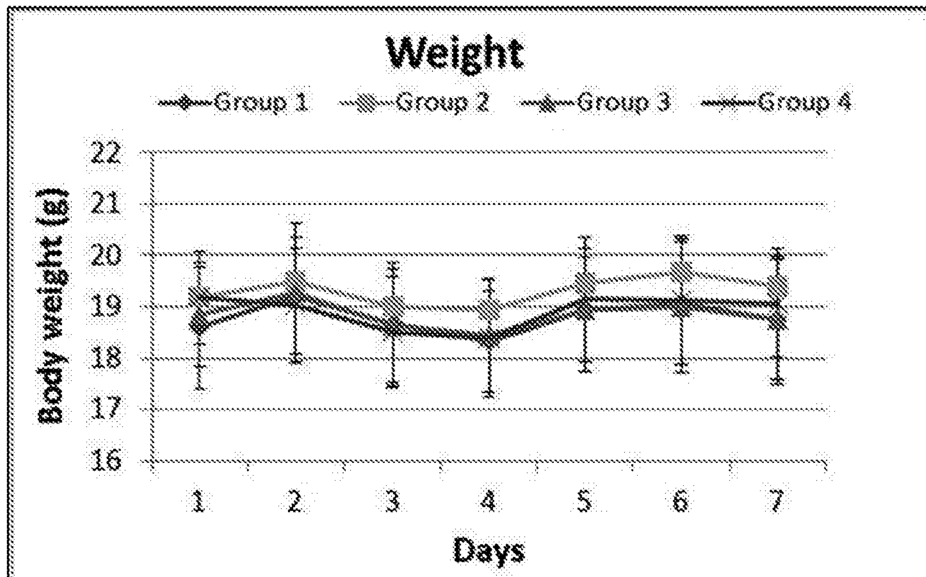


FIGURE 8F



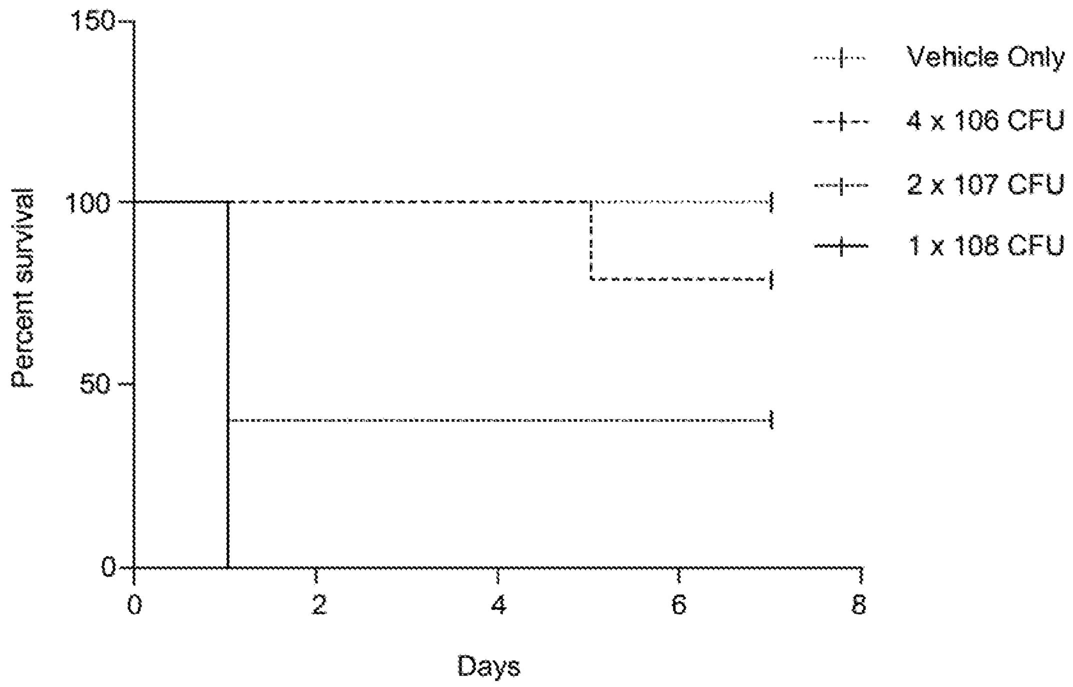


FIGURE 9

Group #	N	MRSA			Treatment				
		Dose (2×10^7 CFU/mL)	Volume (mL)	Route	Agent	Dose (mg/kg)	Volume (mL)	Route	Dose Schedule
1	10	4×10^6	0.2	IV	Vehicle (PBS)	10	0.2	IV	b.i.d X 4
2	10	4×10^6	0.2	IV	PNA-CPP	10	0.2	IV	b.i.d. X 4
3	10	4×10^6	0.2	IV	Vancomycin	2	0.2	IV	b.i.d. X 4

FIGURE 10A

FIGURE 10B

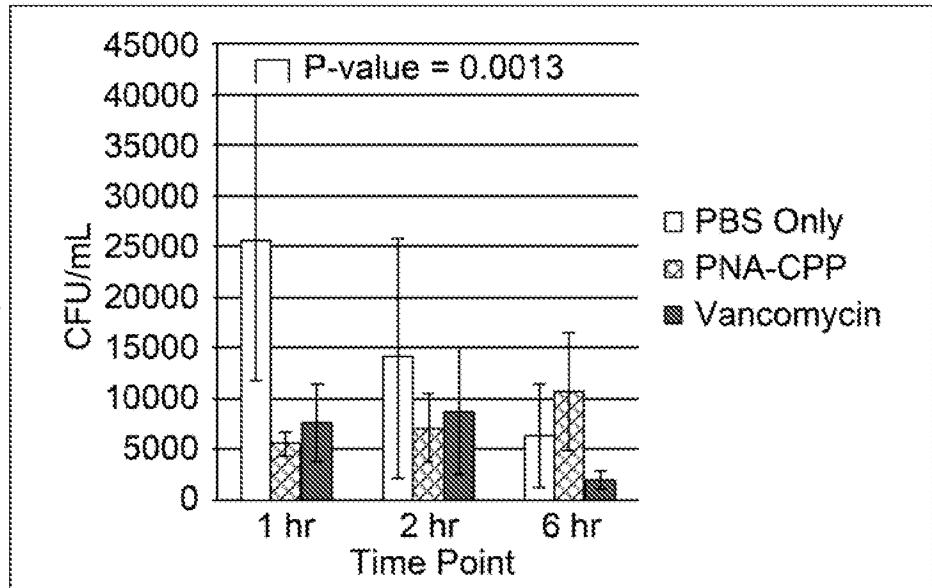
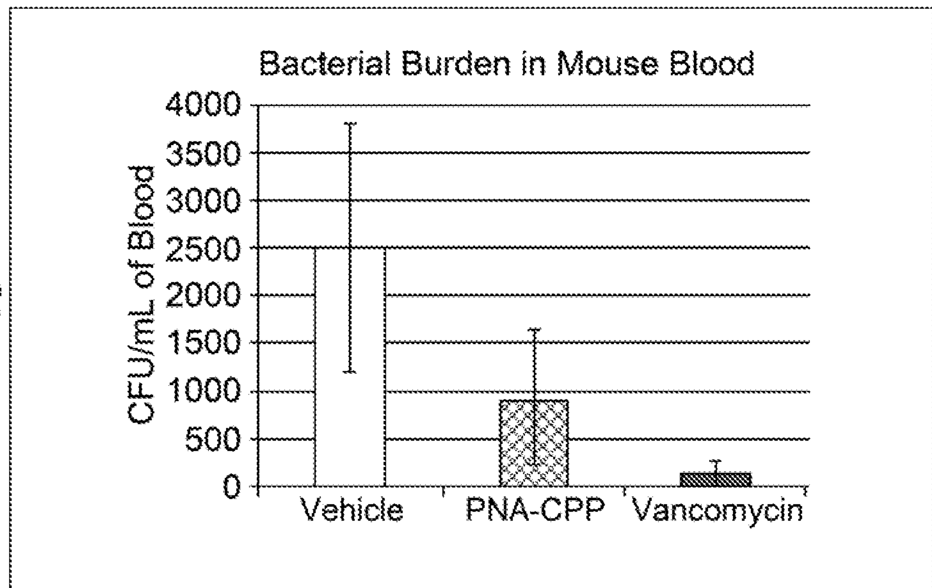


FIGURE 10C



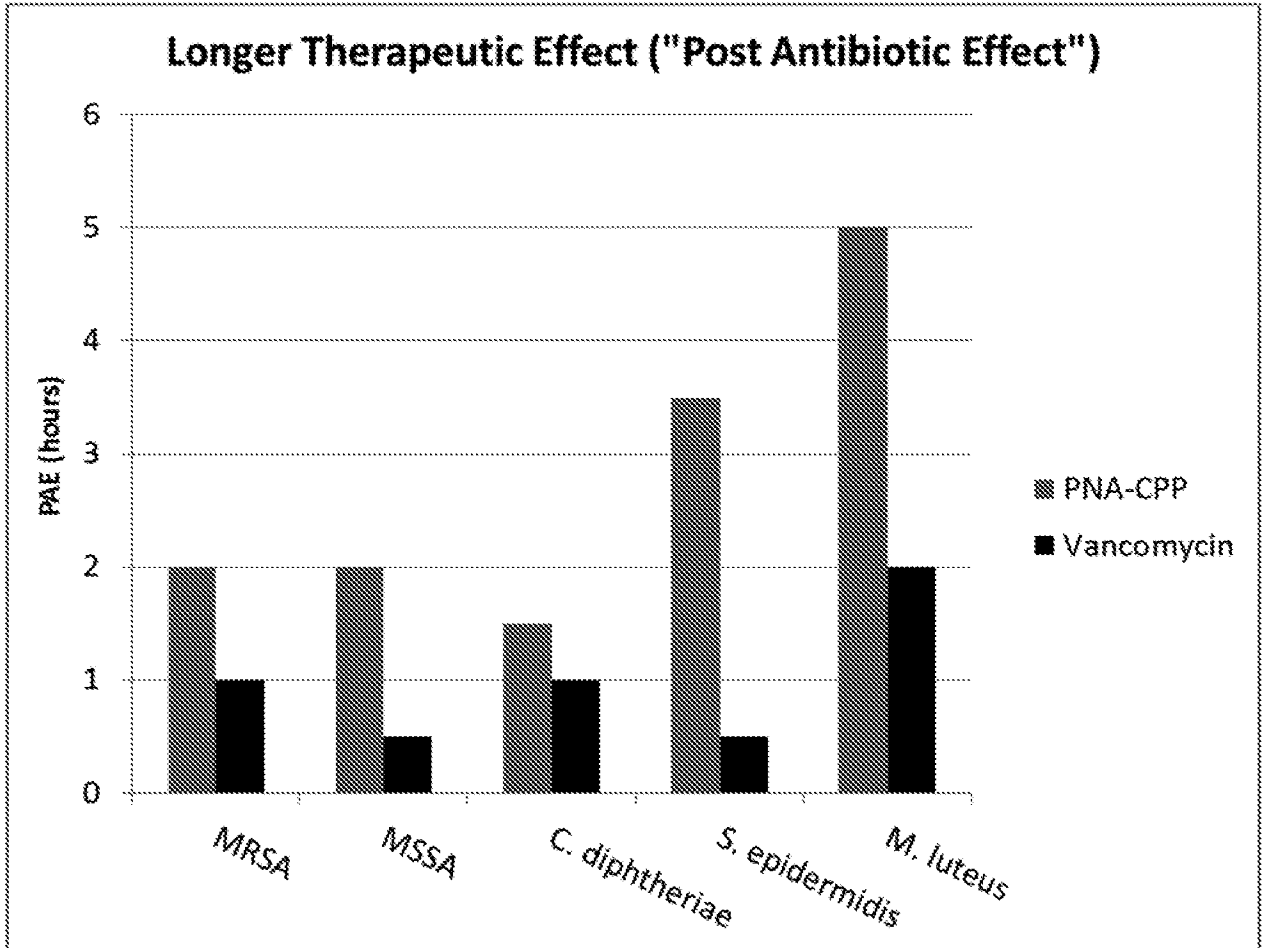


FIGURE 11

PAE Sub MIC Effect for PNA-CPP					
Strain	**PAE**	**0.2 x MIC**	**0.4 x MIC**	**0.6 x MIC**	**0.8 x MIC**
MRSA	1	> 24 hrs	> 24 hrs	> 24 hrs	> 24 hrs
MSSA	1	> 24 hrs	> 24 hrs	> 24 hrs	> 24 hrs

FIGURE 12

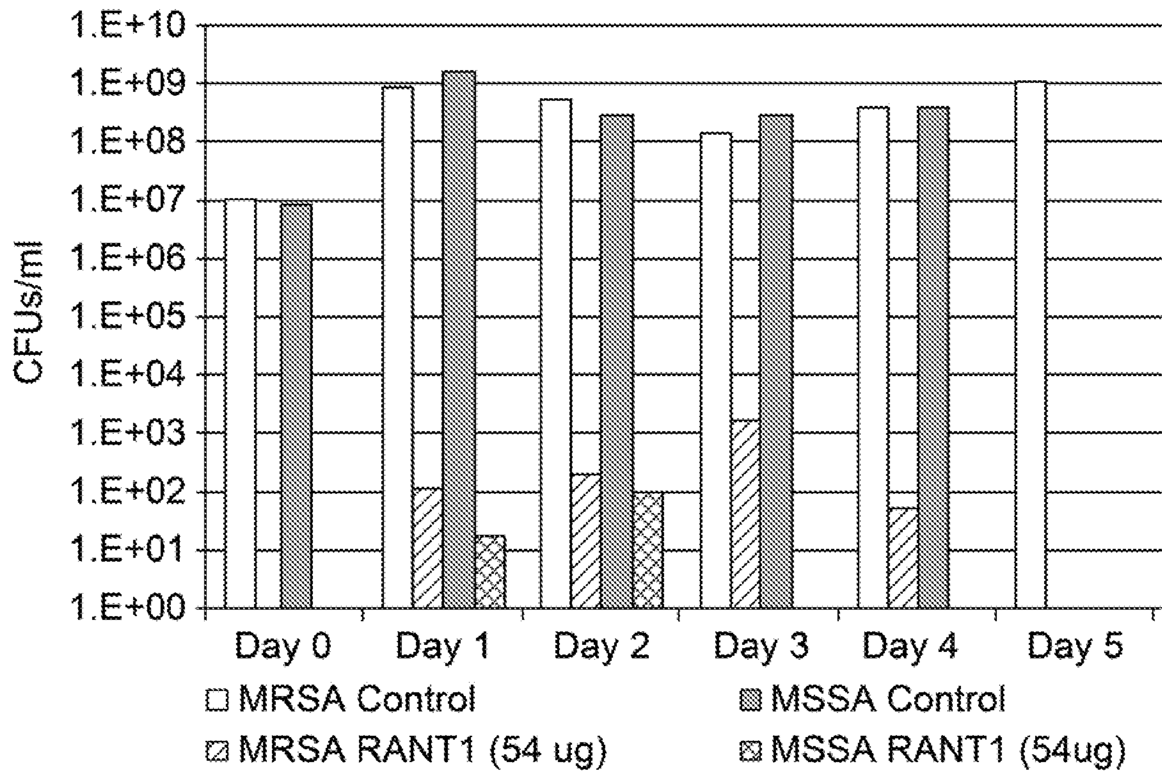


FIGURE 13

Clinical Isolate of MRSA #	MIC of PNA-CPP (µg)	MIC of Vancomycin (µg)	MBC of PNA-CPP (µg)
MRSA-1	0.68	5	1.36
MRSA-3	0.68	10	2.72
MRSA-6	0.68	10	2.72
MRSA-15	0.68	5	5.43
MRSA-28	1.36	10	5.43
MRSA-31	1.36	10	2.72
MRSA-37	1.36	5	2.72
MRSA-39	0.68	5	10.86
MRSA-42	0.68	5	10.86
MRSA-43	1.36	20	10.86
ATCC-1556 USA 300	2.72	10	43.44

FIGURE 14A

Clinical Isolate of MSSA #	MIC of PNA-CPP (µg)	MIC of Vancomycin (µg)	MBC of PNA-CPP (µg)
MSSA-7	1.36	10	10.68
MSSA-16	0.68	10	10.68
MSSA-25	1.36	10	5.43
MSSA-27	0.68	10	21.72
MSSA-34	1.36	10	10.68
MSSA-38	1.36	10	10.68
MSSA-49	1.36	10	10.68
MSSA-57	2.72	20	10.68
MSSA-60	1.26	10	10.68
MSSA-61	0.68	5	5.43
ATCC-BAA1721	5.43	10	54.30

FIGURE 14B

Isolate	MIC of PNA-CPP (μg)	MIC of Vancomycin (μg)	MBC of PNA-CPP (μg)
MRSA	2.72	10	43.44
MSSA	1.36	10	10.86
C. dipetheriae	0.68	0.039	2.73
S. epidermidis	2.72	20	21.72
M. luteus	5.43	20	21.72
P. acnes	0.85	Not tested	13.59

FIGURE 15A

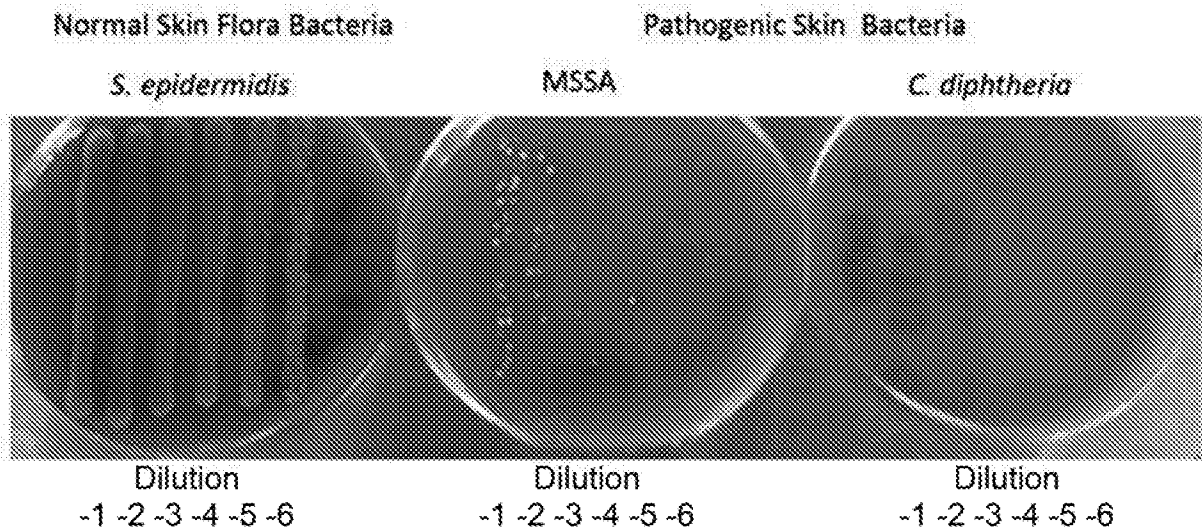


FIGURE 15B

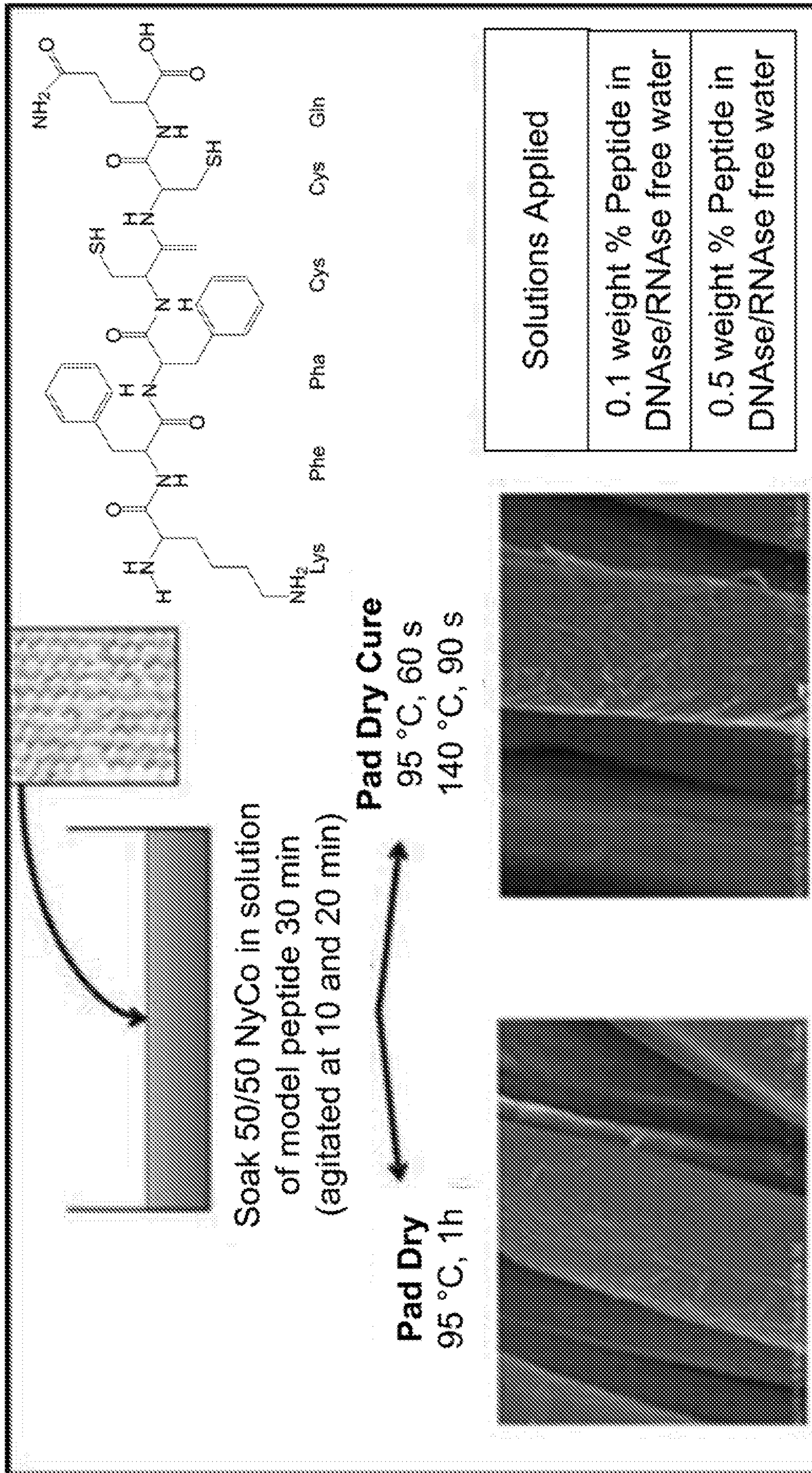


FIGURE 16A

XPS of treated fibers indicates sulfur present on surface

Sample	C1s	N1s	O1s	Si2p	S2p	Cl2p
0.1 wt % Pad Dry	76.68	2.91	18.26	2.16	0	0
0.1 wt % Pad Dry Cure	80.24	4.2	14.06	0.86	0.41	0.22
0.5 wt % Pad Dry	74.01	5.4	17.43	2.14	0.73	0.3
0.5 wt % Pad Dry Cure	79.85	4.36	13.35	1.52	0.71	0.2

Sulfur Present from model peptide

Sample	C1s	N1s	O1s	Si2p	S2p	Cl2p
0.1 wt % Pad Dry (Autoclaved)	73.24	2.05	21.52	3.18	0	0
0.1 wt % Pad Dry Cure (Autoclaved)	72.59	2.7	21.46	3.24	0	0
0.5 wt % Pad Dry (Autoclaved)	79.85	4.36	13.35	2.69	0	0
0.5 wt % Pad Dry Cure (Autoclaved)	69.79	1.24	23.83	5.15	0	0

Sulfur Present after autoclaving samples

FIGURE 16B

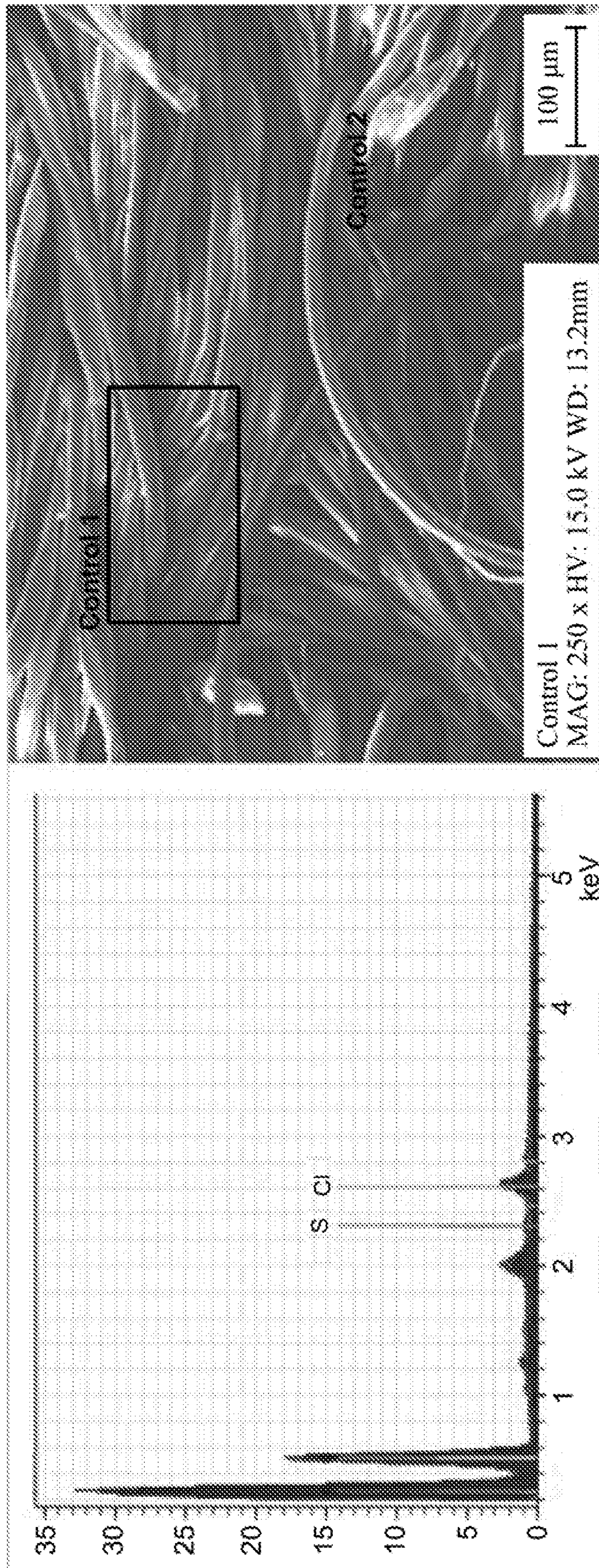


FIGURE 17A

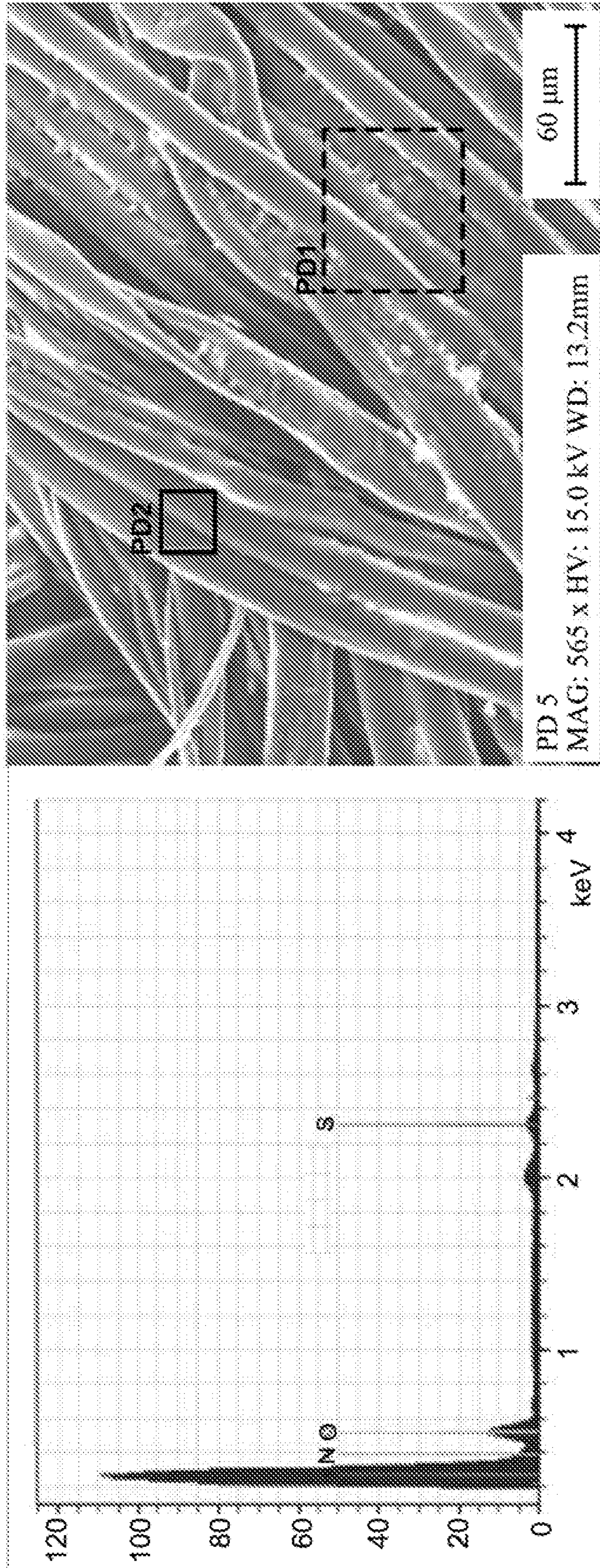


FIGURE 17B

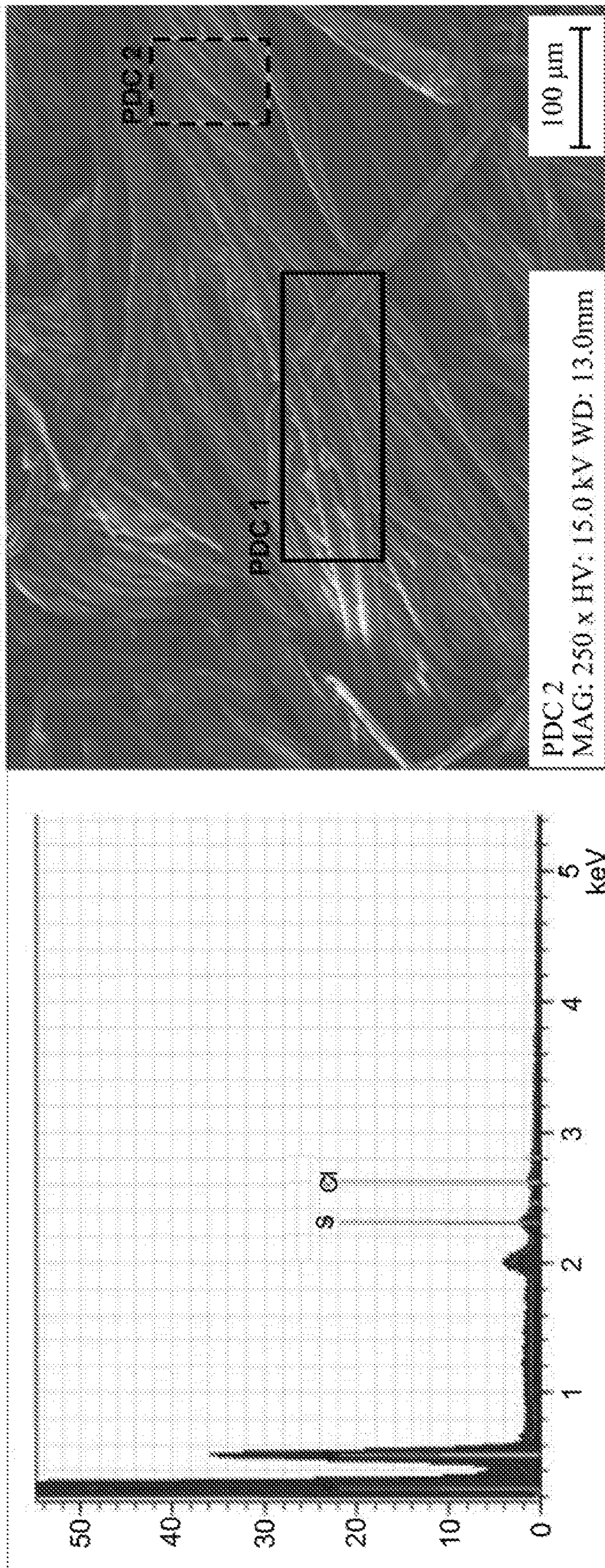


FIGURE 17C

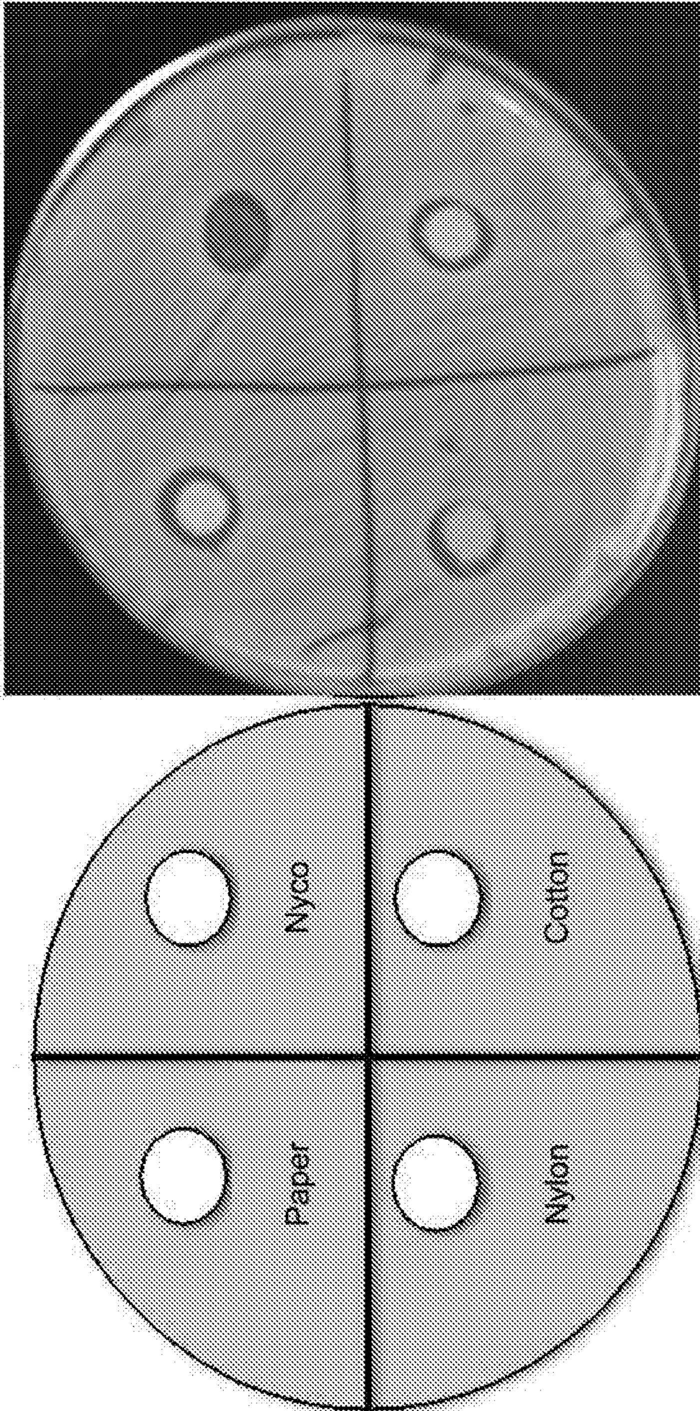


FIGURE 18A

	Fabric (zone of inhibition in mm)					
Bacteria	**Cotton**		**Nylon**		**Nyco**	
	PNA-CPP	**Vanc.**	**PNA-CPP**	**Vanc.**	**PNA-CPP**	**Vanc.**
MRSA	8	10	11	10	9	10
MSSA	11	11	11	10	11	9
C. diphtheria	9	10	11	10	9	9
M. luteus	15	14	15	14	16	9
S. epidermidis	12	10	11	9	10	12

FIGURE 18B

	Paper		**Theragauze®**		
Bacteria	**Zone of inhib. (mm)**		**Zone of inhib. (mm)**		
	PNA-CPP	**Vanc.**	**PNA-CPP**	**Vanc.**	**control**
MRSA	11	11	11	11	0
MSSA	11	10	8	10	0
C. diphtheria	11	9	10	8	0
M. luteus	17	11	15	12	0
S. epidermidis	11	10	11	8	0

FIGURE 18C

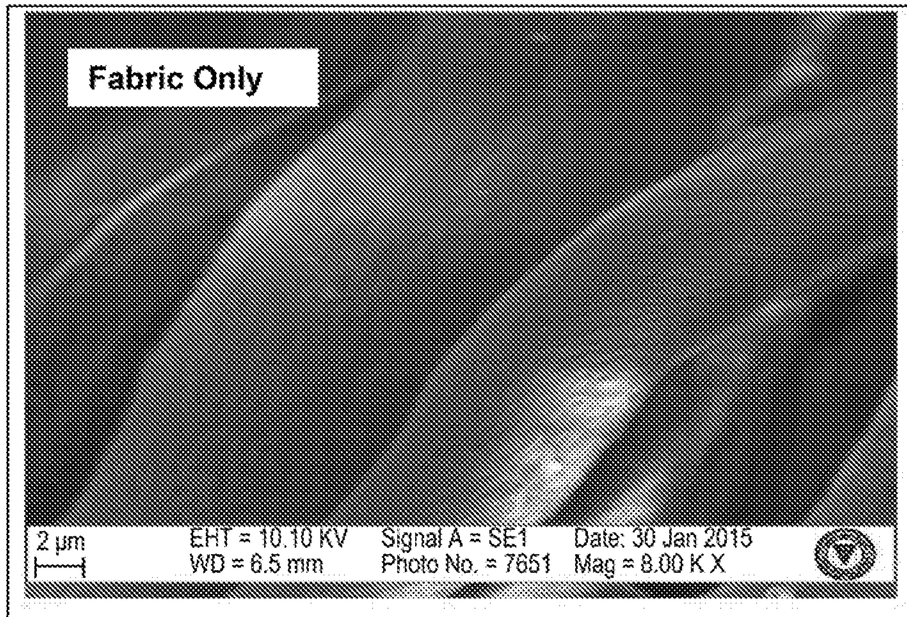


FIGURE 19A

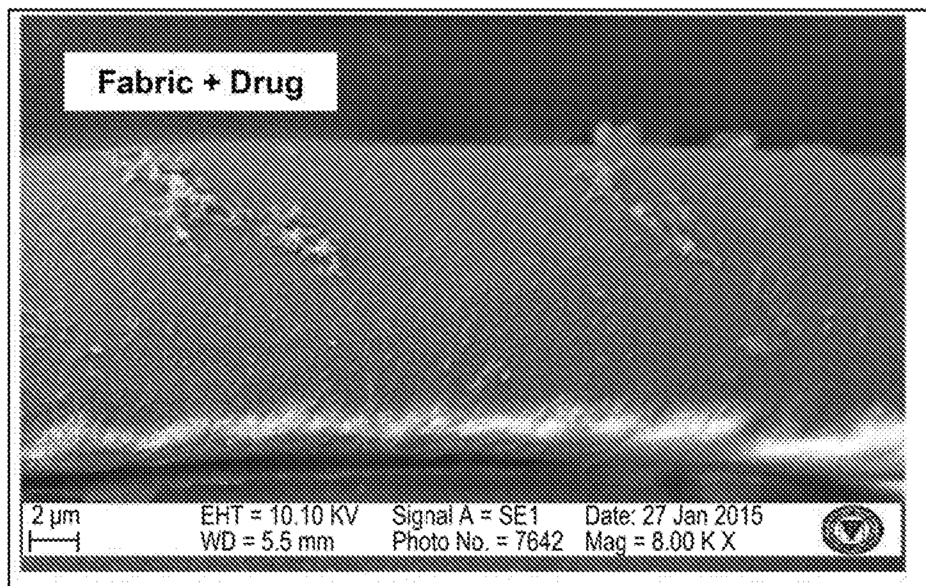


FIGURE 19B

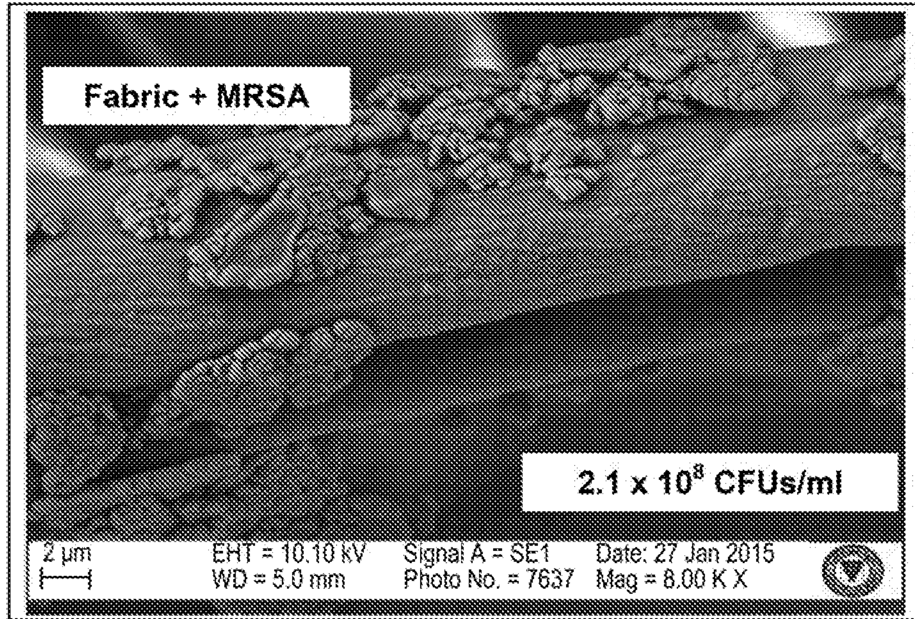


FIGURE 19C

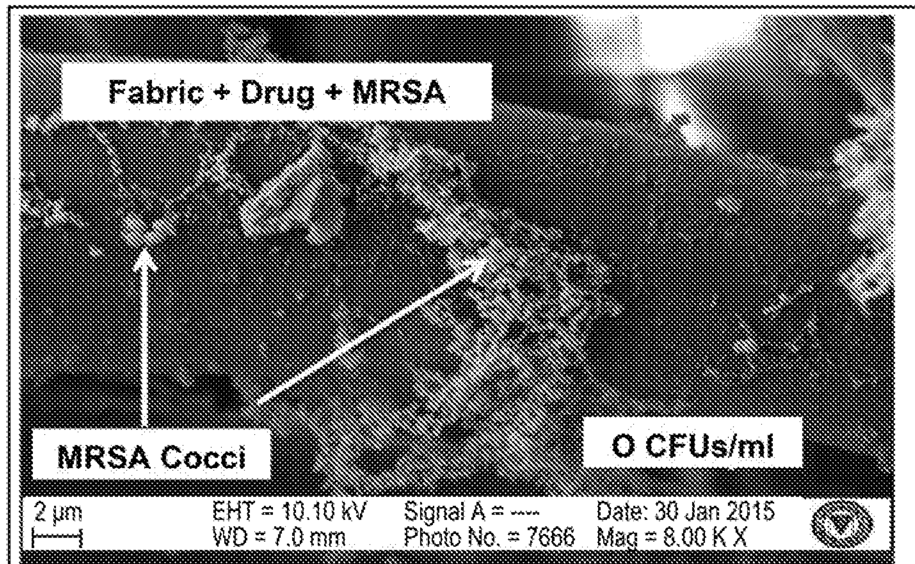


FIGURE 19D

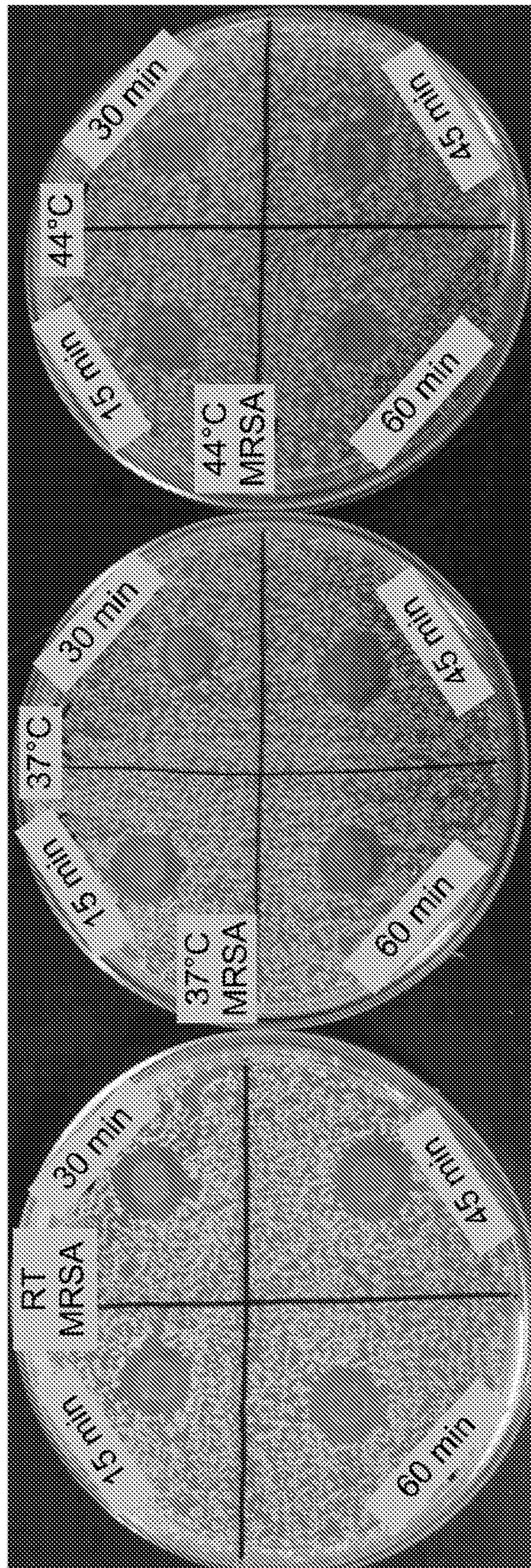


FIGURE 20A

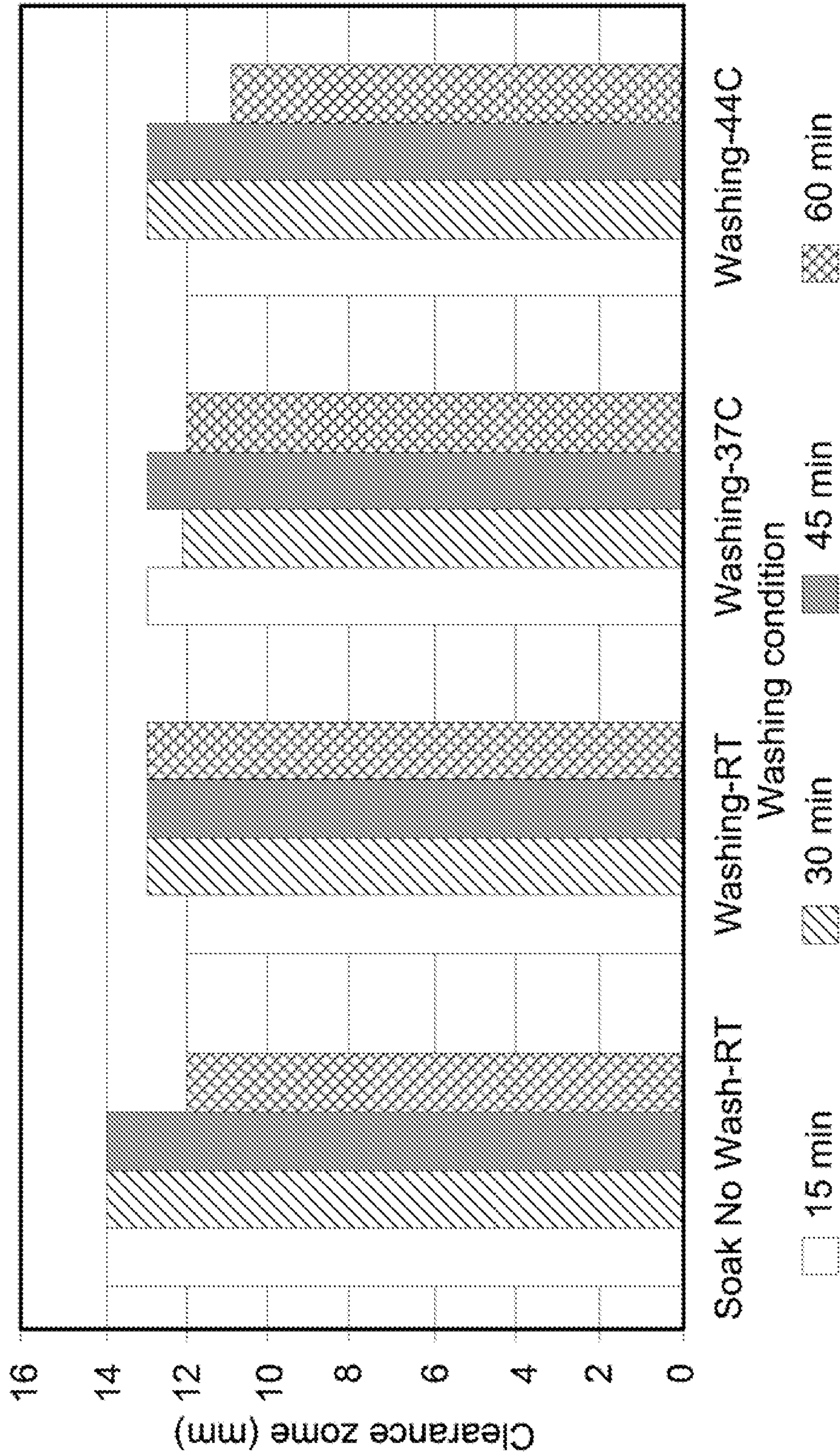


FIGURE 20B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/36430

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C07D 487/00, A01N 43/90, A61K 31/519 (2016.01)
CPC - C07D 487/04
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8) - C07D 487/00, A01N 43/90, A61K 31/519 (2016.01)
CPC - C07D 487/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/259.5, 544/281

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patent, Google Web
Search terms used - peptide nucleic acid gram positive CGTCATACGCGGTCC KFFKFFKFFK PNA-CPP cationic block copolymer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2014/144442 A2 (TECHULON INC.) 18 September 2014 (18.09.2014); para [0004], [0016], [0049], [0058], [0069]-[0070]	1-7, 11-13
A	Nekhotiaeva et al. "Inhibition of Staphylococcus aureus Gene Expression and Growth Using Antisense Peptide Nucleic Acids" Molecular Therapy. 20 August 2004 (20.08.2004) vol 10, pg. 652-659; pg. 656, Table 2	1-7, 11-13
A	US 6,548,651 B1 (Nielsen et al.) 15 April 2003 (15.04.2003); entire document	1-7, 11-13
A	US 2014/0235828 A1 (Beaumont et al.) 21 August 2014 (21.08.2014); entire document	1-7, 11-13

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
30 August 2016 (30.08.2016)	30 SEP 2016

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/36430

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 8-10, 14-25
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.