ANTIMICROBIAL PEPTIDE, COMPOSITIONS, AND METHODS OF USE

This Application claims priority to US Provisional Application No. 60/952,059 filed July 26, 2007, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

The invention relates to antimicrobial peptides for use in pharmaceutical and cosmetic applications, to treat or prevent microbial infections, and/or prevent the recurrence of a microbial infection after treatment.

BACKGROUND OF THE INVENTION

Small, cationic antimicrobial peptides (AMPs) are naturally occurring antibiotics of the innate immune system. AMPs are widely distributed in animals and plants and are among the most ancient host defense factors. Their spectrum of activity may include Gram-positive and Gram-negative bacteria as well as fungi and certain viruses. As resistance of pathogenic microbes to conventional antibiotics increases, these endogenous antibiotics are an important source for designing new therapies against a variety of infectious diseases, including therapies for chronic microbial infections as well as for routine and/or prophylactic use. However, for many applications, such peptides must be, in addition to effective, sufficiently safe and stable so as to allow long term and/or routine use without negatively impacting the affected area.

SUMMARY OF THE INVENTION

The present invention provides antimicrobial peptides that exhibit a broad range of antimicrobial activity against gram positive and gram negative bacteria, as well as fungi,

mold, and certain viruses. The peptides of the invention are cationic, containing positively charged amino acids such as lysine, arginine, and histidine, along with other non-charged amino acids such as glycine or glutamine. The peptides of the invention are safe, effective, and sufficiently stable to allow long-term or routine use.

The present invention further provides antimicrobial compositions containing the antimicrobial peptide. The compositions are stable, and thus may be formulated in a variety of forms including for routine use. Such compositions are especially useful for topical application to the skin, hair, nail, vagina, urethra, ear, oral cavity, nasal passage, respiratory system, opthalmic region, various mucosal regions, and other affected areas, to treat, prevent, or prevent the reoccurrence of, a microbial infection. The compositions of the present invention, by reducing, inhibiting, or preventing microbial infection, improve the condition and/or appearance of the treated region, even with long term or routine use.

The present invention further provides kits for use in improving the condition or appearance of skin, nail, or other treated area. These kits may contain components that facilitate dispensing and applying the compositions of the invention, and may be designed for convenient application and long-term storage of the compositions.

DETAILED DESCRIPTION OF THE INVENTION

Peptide fragments of HIV-TAT were used in conjunction with botulinum toxin to produce more stable, more effective, and safer botulinum toxin compositions for therapeutic, aethetic, and/or cosmetic purposes. These botulinum toxin containing compositions are disclosed in a U.S. Provisional Application filed December 29, 2006, entitled "Compositions and Methods of Topical Application And Transdermal Delivery of Botulinum Toxins Stabilized With Polypeptide Fragments Derived From HIV-TAT," which is hereby

incorporated by reference in its entirety. The present inventors discovered that peptide fragments of HIV-TAT, and related peptides, have antimicrobial activity, and are useful as active ingredients in pharmaceutical and cosmetic compositions. Such peptides are safe and effective for long term or routine use (e.g., in the absence of botulinum toxin).

Thus, one aspect of the present invention is a cationic peptide that comprises an HIV-TAT sequence or reverse HIV-TAT sequence at the N- or C-terminus, or both the N-terminus and the C-terminus. For example, the antimicrobial peptide may have an HIV-TAT sequence, such as Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg (SEQ ID NO:1), or reverse HIV TAT sequence, such as Arg-Arg-Arg-Gln-Arg-Arg-Lys-Lys-Arg (SEQ ID NO:2), at the N- or C-terminus or both the N-terminus and the C-terminus.

In one embodiment, the cationic peptide comprises an N-terminal portion that is an HIV-TAT or reverse HIV-TAT sequence, a C-terminal portion that is an HIV-TAT or reverse HIV-TAT sequence, and one or more cationic residues (e.g., Lys or Arg) between the N-terminal portion and the C-terminal portion. For example, the peptide may have from 5 to 20 cationic residues such as Lys between the N-terminal portion and the C-terminal portion, such as about 12, about 15, or about 17 cationic residues.

In an exemplary embodiment, the invention is a cationic peptide having the following sequence: Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-(Lys)n-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg (SEQ ID NO:3), or Arg-Arg-Arg-Gln-Arg-Arg-Lys-Lys-Arg-Gly-(Lys)n-Gly-Arg-Arg-Arg-Gln-Arg-Arg-Lys-Lys-Arg (SEQ ID NO:4), where n is from about 5 to about 20, such as from about 10 to about 20.

In one embodiment of the invention, the N-terminal portion of the peptide is an HIV-TAT sequence and the C-terminal portion of the peptide is an HIV-TAT sequence. In

another embodiment, the N-terminal portion is a reverse HIV-TAT sequence and the C-terminal portion is a reverse HIV-TAT sequence. For example, the antimicrobial peptide may have the following amino acid sequence: Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-$(Lys)_{15}$-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg (SEQ ID NO:5).

In certain embodiments, the peptide contains a naturally occurring sequence of amino acids, such as an HIV-TAT sequence, but may alternatively contain improvements of the naturally occurring sequence to improve its potency. Antimicrobial peptides with improved potency may be identified using an assay as described or exemplified herein. In certain embodiments, the peptide of the invention is readily biodegradable.

Generally, antimicrobial derivatives of the HIV-TAT and reverse HIV-TAT sequence, which are contemplated by the instant invention, are characterized as having a particularly high content of Arg and Lys residues. For example, the peptide of the invention may contain at least about 50%, collectively, of Arg and Lys amino acid residues, but may contain at least about 75%, or at least about 80%, Arg and Lys residues. In these or other embodiments, such derivatives may have the amino acid sequence of SEQ ID NO: 3 or 4, with from 1 to 5 amino acid substitutions, insertions, or deletions (collectively), including 1, 2, 3, or 4 amino acid substitutions, insertions, or deletions with respect to SEQ ID NO: 3 or 4. In certain embodiments, such substitutions, insertions, or deletions, are located within the HIV-TAT or reverse HIV-TAT sequence.

The antimicrobial peptides of the invention have a length of from about 15 amino acids to about 100 amino acids. In certain embodiments, the cationic peptide is from about 25 to about 50, or from about 25 to about 40 amino acids in length. In an exemplary embodiment of the invention, the antimicrobial peptide is about 35 amino acids in length.

The present invention is effective for inhibiting, killing, and/or lysing various microbial organisms, and may have a broad antimicrobial spectrum. Exemplary bacteria for which the invention may be bacteriostatic or bactericidal include both gram-negative and gram-positive bacteria such as: *Staphylococcus aureus*, *Streptococcus pyogenes* (group A), *Streptococcus spp.* (viridans group), *Streptococcus agalactiae* (group B), *Streptococcus bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, *Streptococcus mutans*, *Enterococcus spp.*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Branhamella catarrhalis*, *Bacillus anthracis*, *Bacillus subtilis*, *Propionibacterium acnes*, *Corynebacterium diphtheriae*, *Listeria monocytogenes*, *Clostridium tetani*, *Clostridium difficile*, *Escherichia coli*, *Enterobacter spp.*, *Proteus mirablis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella spp.*, *Shigella spp.*, *Campylobacter jejuni*, *Actinobacillus actinomycetumcomitans*, *Porphyromonas gingivalis*, *Bacteriodes forcythus*, *Treponema denticola*, *Prevotella intermedia*, and *Eubacterium nodatum*.

Thus, the antimicrobial peptide of the invention may be useful for inhibiting such bacteria to ameliorate microbial infection(s), or reduce the likelihood of microbial infection(s), including: bacteremia, pneumonia, meningitis, osteomyelitis, endocarditis, dental caries, periodontal disease, sinusitis, rhinitis, pink eye, urinary tract infections, tetanus, gangrene, colitis, acute gastroenteritis, impetigo, acne, acne posacue, wound infections, burn infections, fascitis, bronchitis, and a variety of abscesses, nosocomial infections, and opportunistic infections.

The antimicrobial peptide of the invention is also effective for inhibiting the growth and survival of fungal organisms such as dermatophytes (e.g., *Microsporium spp.* such as *Microsporum canis*, *Microsporum audouinii*, and *Microsporum gypseum*; and *Trichophyton spp.*, such as *T. rubrum*, *T. mentagrophytes*, *T. Trichophyton*, *T. schoenleinii*, and *T.*

tonsurans), *Acremonium spp.*, *Fusarium oxysporum*, *Scopulariopsis brevicaulis*, *Onychocola canadensis*, *Scytalidium dimidiatum*; yeasts (e.g., *Candida albicans*, *C. Tropicalis*, or other Candida species, and *Saccharomyces cerevisiae*), *Torulopsis glabrata*, *Epidermophyton floccosum*, *Malassezia furfur*, *Pityropsporon orbiculare* or *ovale*, *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus niger* and other *Aspergillus spp.*, *Zygomycetes* (e.g., *Rhizopus* and *Mucor*), *Paracoccidioides brasiliensis*, *Blastomyces dermatitides*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenckii*.

The antimicrobial peptide is therefore useful for inhibiting fungal organisms to treat or prevent infections such as: Aspergillosis, Blastomycosis, Candidiasis, Coccidioidomycosis, Cryptococcosis, Histoplasmosis, Paracoccidiomycosis, Sporotrichosis, Zygomycosis. In certain embodiments of the invention, the antimicrobial peptide is effective for treating or preventing Tinea pedis, Tinea versicolor, and Onychomycosis. Other fungal infections for which the antimicrobial peptide of the invention is effective, include: Tinea barbae, Lobomycosis, Mycetoma, Piedra, Pityriasis versicolor, Tinea capitis, Tinea corporis, Tinea cruris, Tinea favosa, Tinea nigra, Otomycosis, Phaeohyphomycosis, and Rhinosporidiosis.

The present invention is also effective against certain viruses, such as HIV, herpes simplex viruses, cytomegalovirus, and human papillomavirus. Thus, the antimicrobial peptide of the invention is also effective for preventing and treating infections such as cold sores, genital herpes, and warts, and effective for preventing HIV infection.

The present invention provides a pharmaceutical or cosmetic composition containing the antimicrobial peptide of the invention, and a pharmaceutically or cosmetically acceptable carrier and/or diluent. Generally, the compositions of the invention do not contain botulinum

toxin, as disclosed in the U.S. Provisional Application filed December 29, 2006, entitled "Compositions and Methods of Topical Application And Transdermal Delivery of Botulinum Toxins Stabilized With Polypeptide Fragments Derived From HIV-TAT," and that is incorporated by reference herein.

The compositions of the invention may comprise, consist of, or consist essentially of, the antimicrobial peptide of the invention in an amount effective for antimicrobial activity, in combination with a carrier and/or diluent. In this respect, the term "consists essentially of" means that the composition contains only the antimicrobial peptide with pharmaceutically or cosmetically acceptable carrier(s) and/or diluent(s), and may optionally contain an additional antimicrobial agent(s), such as an antibiotic, or an anti-inflammatory agent (e.g., NSAID). For example, when formulated for topical administration, the composition of the invention may optionally contain Benzoyl peroxide, Clindamycin, Erythromycin, Tetracycline, Bacitracin, Neomycin, Mupirocin, Polymyxin B, Miconazole, and/or Clotrimazole. In certain embodiments, the carrier and/or diluent is an aqueous carrier or diluent, such as buffered saline.

The pharmaceutical or cosmetic composition of the invention may be formulated for topical administration, which can be a welcome alternative to systemic therapy for treating or preventing many microbial infections. In certain embodiments, topical therapy with the composition of the invention is administered alongside a systemic or other topical therapy to provide either additive or synergistic results. For example, the antimicrobial peptide of the invention may act synergistically with one or more of Benzoyl peroxide, Clindamycin, Erythromycin, Tetracycline, Bacitracin, Neomycin, Mupirocin, Polymyxin B, Miconazole, Clotrimazole and/or equivalent antimicrobial agents.

Treatment with the composition of the invention may not only clear various microbial infections, but is also effective for preventing the occurrence of such infections in a first instance, and for preventing recurrence of such infections. In this latter aspect, the present composition is administered to the affected region after treatment with topical or systemic therapy. To prevent recurrence of an effectively treated infection, or to prevent occurrence of an infection in a first instance at a susceptible region, the composition may be administered routinely (e.g., daily) for a long period of time, including for days, weeks, or even years.

When formulated for topical administration, the composition of the present invention may contain ingredients typical in topical pharmaceutical or cosmetic compositions, such as a carrier, vehicle or medium. Specifically, the carrier, vehicle, or medium is compatible with the tissues to which it will be applied, such as the skin, hair, nail, vagina, urethra, ear, oral cavity, nasal passage, respiratory system, opthalmic region, and/or mucosa. The compositions and components of the invention are suitable for contacting infected tissues or for use in patients in general without undue toxicity, incompatibility, instability, allergic response, and the like. As appropriate, compositions of the invention may comprise any ingredient conventionally used in the fields under consideration.

In terms of their form, compositions of this invention may include solutions, emulsions (including microemulsions), suspensions, creams, lotions, gels, powders, or other typical solid or liquid compositions used for application to skin and other tissues where the compositions may be used. Such compositions may contain: additional antimicrobials, moisturizers and hydration agents, penetration agents, preservatives, emulsifiers, natural or synthetic oils, solvents, surfactants, detergents, gelling agents, emollients, antioxidants, fragrances, fillers, thickeners, waxes, odor absorbers, dyestuffs, coloring agents, powders, viscosity-controlling agents and water, and optionally including anesthetics, anti-itch actives,

botanical extracts, conditioning agents, darkening or lightening agents, glitter, humectants, mica, minerals, polyphenols, silicones or derivatives thereof, sunblocks, vitamins, and phytomedicinals. In certain embodiments, the composition of the invention is formulated with the above ingredients so as to be stable for a long period of time, as may be beneficial where continual or long-term treatment is intended.

The compositions of the invention may be in the form of controlled-release or sustained-release compositions, wherein the antimicrobial peptide along with additional active agents are encapsulated or otherwise contained within a material, such that they are released onto the skin or affected area in a controlled manner over time. The compositions of the invention may be contained within or on matrixes, liposomes, vesicles, microcapsules, microspheres and the like, or within or on a solid particulate material.

Administration of the composition of the invention may be to any affected or susceptible region, for example, to the legs, shoulders, back (including lower back), axilla, palms, feet, neck, groin, dorsa or the hands or feet, elbows, upper arms, knees, upper legs, buttocks, torso, pelvis, or any other part of the body for which treatment or prevention of infection may be desired. Such treatment is also contemplated for treating and/or dressing wounds, such as cuts, scrapes, and burns to the skin, so as to treat or prevent infection of the wounded area.

The compositions of the invention are suitable for use in physiologic environments with a pH ranging from about 4.5 to about 6.3, and thus, the compositions may be formulated at a similar or equivalent pH. The compositions according to this invention may be stored either at room temperature or under refrigerated conditions.

The composition of the invention contains an amount of antimicrobial peptide effective for antimicrobial action. Generally, the composition contains from about 0.01% (wt./vol.) to about 20% antimicrobial peptide. In certain embodiments, the composition contains from about 0.5% to about 10% antimicrobial peptide, such as about 0.5%, about 1%, about 5%, or about 10% antimicrobial peptide.

In one embodiment, the pharmaceutical composition of the invention is a cleanser or moisturizer. Cleansers and moisturizers of the invention may be particularly useful for conditioning the skin while treating and/or preventing a microbial infection, such as acne including propionicbacterium acne. This aspect of the invention is also effective for preventing the reoccurrence of acne after the acne has effectively dissipated. In this aspect, the cleanser or moisturizer may be applied to the effected area routinely, e.g., approximately daily, and on a continual or routine basis. For example, treatment may be continual for days, weeks, months, or even years.

In another embodiment, the composition of the invention is a topical solution effective against Athlete's feet (Tinea pedis), and/or onychomycosis. In this aspect, the composition may be an aqueous solution for soaking or painting the affected region. In this embodiment, the invention is also effective for preventing the recurrence of the condition, by continual daily treatment. Further, when used for the treatment of onychomycosis, the composition of the invention may be used alongside systemic therapy, such as, for example, Lamisil®, to provide more effective results and to improve the condition and/or appearance of the nails.

In other embodiments, the composition of the invention is formulated as a mouth wash, oral spray, or oral gel. In this aspect, the mouthwash or oral spray of the invention is

effective for the prevention of dental caries, for the treatment and/or prevention of periodontal disease, for the treatment of sore throat, or to treat or prevent halitosis that might be caused by the presence or activity of microbes. When formulated as an oral gel, the composition of the invention is effective for the treatment and prevention of sores around the mouth, such as cold sores and canker sores. When formulated as a mouthwash, oral spray, or oral gel, the composition generally contains from about 0.01% to about 10% of the antimicrobial peptide, such as about 1%, about 2%, about 3%, or about 5% antimicrobial peptide.

In yet another embodiment, the invention is formulated as a cream or lotion. Such formulations are particularly suitable for the treatment and/or prevention of herpes simplex virus infection or outbreak. Thus, in certain embodiments, the invention is suitable for treatment genital herpes, cold sores, or chicken pox.

The composition of the invention may also be formulated as a nasal spray effective against rhinitis, or formulated as an eye drop effective against pink eye.

In another aspect, the invention provides a method for treating or preventing a condition associated with a microbial infection. The method of the invention comprises administering the pharmaceutical or cosmetic composition of the invention, containing the antimicrobial peptide, to a patient having or suspected of having a microbial infection. In certain embodiments, the composition is administered to the patient after treatment of the infection, either with the composition of the invention or with an alternative therapy, to prevent further recurrence. The patient may be any veterinary or human patient.

The compositions of the invention are useful for the treatment and/or prevention of local and systemic bacterial, fungal, and viral infection to the skin, the hair, the nail, the

vagina, the urethra, the ear, the oral cavity, the respiratory system, the opthalmic region, the nasal, and various mucosal regions via topical route or systemic infection by various routes, such as intravenously or subdermally. Such diseases can be, for example, acne including propionicbacterium acne, onychomycosis, athelete's feet, genital herpes, caries, peridontal disease, rhinitis, and pink eye. In exemplary embodiments, the infection is an infection of *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Aspergillus niger*, and/or a herpes simplex virus.

In order to treat, prevent, or prevent recurrence of, microbial infection, the antimicrobial peptide may be administered at least once a day for at least about one week. Alternatively, the composition is administered at least twice a day for at least two days. In certain embodiments, the composition is administered approximately daily, at least daily, twice a week, weekly, or for about one month. In certain embodiments, the composition of the invention is administered for several months, such as at least two months, six months, or about one year or longer. The invention is further suited for long-term use, which may be particularly beneficial for preventing recurring infection, or for preventing infection or conditions in at-risk or susceptible patients, including immune compromised patients. Such long-term use may involve treatment for at least two years, three years, four years, or even five or more years.

In another aspect, the composition of the invention is a kit, which contains the peptide or compositions of the invention packaged to facilitate dispensing and/or applying the composition to affected or susceptible regions. The packaging or dispenser may include a bottle, tube, spray bottle, or other dispenser. In certain embodiments of the invention, the composition is packaged in a concentrated form, and diluted to a desired concentration upon

use by the end user. In this or other aspect, the composition is formulated and packaged in a manner suitable for long-term storage to maintain efficacy of the composition.

The kit may further include additional components to facilitate application of the composition to the affected area, such as, for example, a brush, sponge, cotton swab, or the like.

EXAMPLES

EXAMPLE 1. Effect of Peptide on Conditions Characterized by Microbial Infection

The following experiments were designed to test the effects of a diluent formulation; however, surprisingly, it was found that the cationic peptide is itself a potent antimicrobial. It was discovered that the peptide of the invention was not only safe for long-term use, but effective for treating and preventing, including preventing recurrence of, various conditions associated with microbial infection. In light of these results, the effect of the peptide on microbial growth was tested in Example 2.

Example 1.1

A 15-year-old male with a two-year history of acne was using topical 5% peptide (SEQ ID NO:5) formulation twice a day for 4 weeks. The cream formulation was composed of the excipients cetyl alcohol, isopropyl myristate, stearyl alcohol, butylated hydrosyxtoluene, polyoxyethylene stearyl ether, polyoxyethylene stearyl ether, edetate disodium, and purified water. The acne disappeared in two weeks with use of the topical formulation. With continued use of the formulation for two years, no new breakouts or adverse events were observed.

Example 1.2

A 36 year-old female who had Tinea Pedis (Athlete's feet) as exhibited by an irritating, itchy rash and oozing between her toes, had soaked her feet in a 5% peptide (SEQ ID NO:5) formulation for 10 minutes before bedtime and applied topical 1% peptide formulation twice a day on the infected area and the rest of her feet. The cream formulation was composed of the excipients cetyl alcohol, isopropyl myristate, stearyl alcohol, butylated hydrosyxtoluene, polyoxyethylene stearyl ether, polyoxyethylene stearyl ether, edetate disodium, and purified water. At week 1, the itchiness reduced and the oozing stopped. By week 4, the crusty patches of skin were gone and there was no evidence of abnormal skin on her feet. She continued to use 0.05% peptide formulation for 5 years with no further breakout or adverse event.

Example 1.3

A 78 year-old male was diagnosed with onychomycosis after laboratory confirmation of fungal invasion based on microscopic examination and culture of nail scrapings or clippings. He started painting his finger nails and surrounding nail-bed with 5% peptide (SEQ ID NO:5) formulation twice a day. The cream formulation was composed of the excipients cetyl alcohol, isopropyl myristate, stearyl alcohol, butylated hydrosyxtoluene, polyoxyethylene stearyl ether, polyoxyethylene stearyl ether, edetate disodium, and purified water. 12 weeks later, the finger nails that grew in all had normal texture and color. He continued using the peptide formulation for 2 years and his nails were all free of fungal infection.

Example 1.4

A 65 year-old male was diagnosed with onychomycosis after laboratory confirmation of fungal invasion based on microscopic examination and culture of nail scrapings or

clippings. He started with oral therapy for 1 month. Afterward, he started to paint his fingers nails and surrounding nail-bed with 10% peptide (SEQ ID NO:5) formulation daily. The cream formulation was composed of the excipients cetyl alcohol, isopropyl myristate, stearyl alcohol, butylated hydrosyxtoluene, polyoxyethylene stearyl ether, polyoxyethylene stearyl ether, edetate disodium, and purified water. 12 weeks later, the finger nails that grew in all had normal texture and color. He continued using peptide formulation for 2 years and his nails were all free of fungi infection and there was no relapse of additional fungal infection in his toe nails.

Example 1.5

A 10 year-old boy who had a two-year history of dental caries used peptide (SEQ ID NO:5) mouth wash for two weeks. The formulation consisted of normal saline and 1-5% peptide. His symptoms were significantly relieved. He continued using the peptide mouth wash for two years with no new tooth-cavity observed.

Example 1.6

A 36 year-old woman who had a cold sore outbreak in her mouth applied a 1% peptide (SEQ ID NO: 5) formulation three times a day. The hydrogel formulation was composed of 30% poloxamer. All core sores disappeared within two days.

Example 1.7

A 24-year-old women who had genital herpes applied 1% peptide (SEQ ID NO:5) twice a day for a week to the affected area. The cream formulation was composed of the excipients cetyl alcohol, isopropyl myristate, stearyl alcohol, butylated hydrosyxtoluene, polyoxyethylene stearyl ether, polyoxyethylene stearyl ether, edetate disodium, and purified water. All symptoms disappeared at the end of the treatment period.

Example 1.8

An 18-year-old female with a three-year history of rhinitis used nasal spray containing the peptide of the invention (SEQ ID NO:5) three times a day for a week. The formulation consisted of normal saline and 1-5% peptide. She felt immediate relieve of symptoms such as itching, sneeze, and nasal congestion.

Example 1.9

A 8 year-old boy who had pink eyes, applied the peptide (SEQ ID NO:5) formulation consisting of normal saline and 1-5% peptide, and two days later, the symptoms disappeared.

EXAMPLE 2. *In Vitro* Antimicrobial Activity

The antimicrobial activity was tested using an exemplary peptide of the invention in a panel of gram-negative and gram-positive bacteria, and fungus. The exemplary peptide sequence was Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-Gly-$(Lys)_{15}$-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg (SEQ ID NO:5) and it had been formulated in 15% Poloxamer 0.9% saline. The peptide concentrations were 3.3 mg/ml, 11 μg/ml, and 11 ng/ml, respectively. The antimicrobial activities are shown in Tables 1-3. The peptide demonstrated strong antimicrobial activity in a dose-dependent manner. Notably, *Pseudomonas aeruginosa*, a gram-negative bacterium that is noted for its resistance to antibiotics, can be inhibited with 0.33% peptide of the invention (Table 1).

Table 1. Anti-microbial Activity at 3.3 mg/ml (0.33% peptide)

Organism	Initial Count	14 Days (CFU/ml)	28 Days (CFU/ml)
Staphylococcus aureus ATCC 6538	7.4×10^5	125 3.8 log reduction	< 10 No increase
Pseudomonas aeruginosa ATCC 9027	1.1×10^6	< 100 > 4.0 log reduction	<100 No increase
Escherichia coli ATCC 8739	5.5×10^5	<10 >4.7 log reduction	<10 No increase
Candida albicans ATCC 10231	1.0×10^5	<10 No increase	<10 No increase
Aspergillus niger ATCC 16404	3.0×10^5	3.1×10^5 No increase	3.0×10^5 No increase

Table 2. Anti-microbial Activity at 11 µg/ml (0.0011% peptide)

Organism	Initial Count	14 Days (CFU/ml)	28 Days (CFU/ml)
Staphylococcus aureus ATCC 6538	7.0×10^5	< 10 >4.8 log reduction	< 10 No increase
Pseudomonas aeruginosa ATCC 9027	1.2×10^6	4.3×10^6 0.6 log increase	1.7×10^7 0.6 log increase
Escherichia coli ATCC 8739	6.9×10^5	1.7×10^3 2.6 log reduction	<10 No increase
Candida albicans ATCC 10231	1.6×10^5	2.1×10^3 No increase	1.0×10^3 No increase
Aspergillus niger ATCC 16404	4.6×10^5	2.5×10^5 No increase	2.2×10^5 No increase

Table 3. Anti-microbial Activity at 11 ng/ml (0.0000011% peptide)

Organism	Initial Count	14 Days (CFU/ml)	28 Days (CFU/ml)
Staphylococcus aureus ATCC 6538	7.0×10^5	< 10 >4.8 log reduction	< 10 No increase
Pseudomonas aeruginosa ATCC 9027	1.2×10^6	3.9×10^6 0.5 log increase	5.6×10^6 No increase
Escherichia coli ATCC 8739	6.9×10^5	4.0×10^4 1.2 log reduction	860 No increase
Candida albicans ATCC 10231	1.6×10^5	3.8×10^4 No increase	3.1×10^4 No increase
Aspergillus niger ATCC 16404	4.6×10^5	2.9×10^5 No increase	2.6×10^5 No increase

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What is claimed is:

1. A composition consisting essentially of a cationic peptide in an amount effective for antimicrobial activity, and a pharmaceutically or cosmetically acceptable carrier and/or diluent,

wherein said cationic peptide comprises at least 50% Arg and Lys residues.

2. The composition of claim 1, wherein said cationic peptide contains an HIV-TAT, reverse HIV-TAT, or derivative thereof.

3. The composition of claim 1 or 2, wherein the composition is formulated for topical administration.

4. The composition of any one of claims 1 to 3, wherein the composition contains from about 0.01% to about 20% cationic peptide.

5. The composition of any one of claims 1 to 3, wherein the composition contains from about 0.5% to about 10% cationic peptide.

6. The composition of any one of claims 1 to 3, wherein the composition has about 0.5%, about 1%, about 5%, or about 10% cationic peptide.

7. The composition of any one of claims 1 to 6, wherein the composition is a cleanser or moisturizer effective against acne, including acne caused by propionicbacterium.

8. The composition of any one of claims 1 to 6, wherein the composition is effective against Athlete's feet.

9. The composition of any one of claims 1 to 6, wherein the composition is effective against Onychomycosis.

10. The composition of any one of claims 1 or 2, wherein the composition is formulated as a mouth wash or oral gel.

11. The composition of claim 10, wherein the composition is effective to prevent caries and/or ameliorate periodontal disease, and/or treat cold sores.

12. The composition of claim 10 or 11, wherein the composition contains from about 0.01% to about 20% cationic peptide.

13. The composition of any one of claims 1 to 6, wherein the formulation is effective against genital herpes.

13. The composition of claim 1 or 2, wherein the composition is formulated as a nasal spray.

14. The composition of claim 13, wherein the composition is effective against rhinitis.

15. The composition of claim 13 or 14, wherein the composition contains from about 0.01% to about 20% cationic peptide.

16. The composition of claim 1 or 2, wherein the composition is formulated as an eye drop.

17. The composition of claim 15, wherein the composition is effective against pink eye.

18. The composition claims 16 or 17, wherein the composition contains from about 0.01% to about 20% cationic peptide.

19. The composition of claim 1 or 2, wherein the cationic peptide comprises at least about 75% Arg and Lys residues.

20. The composition of claim 1 or 2, wherein the cationic peptide comprises at least about 80% Arg and Lys residues.

21. The composition of claim 1 or 2, wherein the cationic peptide is from about 20 to about 50 amino acids in length.

22. The composition of claim 1 or 2, wherein the cationic peptide is from about 25 to about 40 amino acids in length.

23. The composition of claim 1 or 2, wherein the cationic peptide is about 35 amino acids in length.

24. The composition of claim 1 or 2, wherein the cationic peptide comprises an HIV-TAT or reverse HIV-TAT sequence.

25. The composition of claim 24, wherein the HIV-TAT sequence is the amino acid sequence of SEQ ID NO:1 and the reverse HIV-TAT sequence has the amino acid sequence of SEQ ID NO:2

26. The composition of claim 1 or 2, wherein the cationic peptide comprises: an N-terminal portion that is an HIV-TAT or reverse HIV-TAT sequence, a C-terminal portion that is an HIV-TAT or reverse HIV-TAT sequence, and one or more lysine residues between said N-terminal portion and said C-terminal portion.

27. The composition of 26, wherein said peptide has from 5 to 20 lysine residues between said N-terminal portion and said C-terminal portion.

28. The composition of claim 26 or 27, wherein said N-terminal portion is an HIV-TAT sequence and said C-terminal portion is an HIV-TAT sequence.

29. The composition of claim 26 or 27, wherein said N-terminal portion is a reverse HIV-TAT sequence and said C-terminal portion is a reverse HIV-TAT sequence.

30. The composition of any one of claims 26 to 29, wherein said peptide has about 15 lysine residues between said N-terminal and C-terminal portions.

31. The composition of claim 26, wherein said peptide has the amino acid sequence of SEQ ID NO: 5.

32. The composition of any one of claims 1 to 31, wherein the composition consists of said cationic peptide and a pharmaceutically acceptable carrier and/or diluent.

33. A method for treating or preventing a condition associated with a microbial infection comprising administering the composition of any one of claims 1 to 32 to the affected or susceptible region of a patient having or suspected of having a microbial infection.

34. The method of claim 33, wherein said microbial infection includes an infection of Propionicbacterium, *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans, Aspergillus niger*, or a herpes simplex virus.

35. The method of claim 33, wherein said condition is acne, athlete's feet, onychomycosis, caries, periodontal disease, cold sore, genital herpes, rhinitis, and/or pink eye.

36. The method of any one of claims 33 to 35, wherein the composition is administered at least once a day for at least one week.

37. The method of any one of claims 33 to 35, wherein the composition is administered at least twice a day for at least two days.

38. The method of any one of claims 33 to 35, wherein the composition is administered for about one year, or greater than one year.

39. A method for preventing recurrence of a microbial infection, comprising, administering the composition of any one of claims 1 to 32 to the affected or susceptible region of a patient, said patient having been treated for a microbial infection.

40. A kit comprising the composition of any one of claims 1 to 32, and a dispenser and/or applicator.

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<120> Antimicrobial Peptide, Compositions, and Methods of Use

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<150> US 60/952,059
<151> 2007-07-26

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