TOPICAL GELS CONTAINING ALPHA CONNEXIN C-TERMINAL (ACT) PEPTIDES

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Patent Application No. 61/605,528, filed March 1, 2012, which is incorporated herein by reference in its entirety.

DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

[0002] The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: FIRS_006_01WO_SeqList_ST25.txt, date recorded: March 1, 2013, file size 32 kilobytes).

TECHNICAL FIELD

[0003] The present invention relates to compositions for wound treatment comprising alpha connexin polypeptides and stabilizing agents. Topical formulations containing alpha connexin polypeptides and hydroxyethylcellulose are particularly stable. These compositions may be used to treat a variety of wounds including acute surgical wounds and chronic ulcers.

BACKGROUND

[0004] While human tissues damaged by mechanical wounding, disease processes and other causes are capable of healing, complex tissue structure and function is rarely, if ever wholly restored. Instead, recovery of nearly all tissues from injury in humans and other higher vertebrates is dominated by the formation of scar tissue. The most familiar example of this is the discolored and fibrotic scars that linger following the healing of a skin cut or graze. Less well appreciated is that formation of glial scar tissue following injury to the brain or spinal chord is one of the main

obstacles to restoration of neural function following damage to the central nervous system (Silver and Miller JH, 2004). There is currently no means of treating or preventing such scarring and promoting the regeneration of complex tissue structure and function following injury.

[0005] Once such treatments are discovered, there is also a need to provide formulations that stabilize the active pharmaceutical ingredients (API) which they contain. While there are a myriad of potential stabilizing agents, not all of them work equally well in stabilizing specific APIs.

SUMMARY OF THE INVENTION

[0006] The present invention is directed to a topical gel drug product preparation containing a composition comprising an isolated alpha connexin polypeptide. The topical formulation may further comprise hydroxyethylcellulose gel, which stabilizes the alpha connexin polypeptide during storage. In certain embodiments the alpha connexin polypeptide comprises a carboxy terminal amino acid sequence of an alpha connexin (hereinafter "ACT"). The alpha connexin polypeptides of the present invention may comprise or consist of the carboxy-terminal most 4 to 30 contiguous amino acids of an alpha connexin protein or conservative variant thereof, wherein said at least one alpha connexin polypeptide is linked at its amino terminus to a cellular internalization transporter.

[0007] It is an object of the present invention to prepare a formulation of a stable, elegant, and pourable topical gel carrier that contains ACT for aesthetic as well as therapeutic applications including the prevention of scaring and accelerated healing of wounds of a subject. The drug product may be administered to treat acute surgical wounds to reduce scarring or be applied chronically to difficult-to-heal wounds including, but not limited to, venous leg ulcers, diabetic foot ulcers, pressure ulcers, and the like. The drug product possesses physicochemical, biochemical, and rheological properties that enable its ability to provide a therapeutic and effective amount of ACT peptide when applied to all type of wounds.

[0008] In one aspect of the invention, one or more stabilizer is selected among many, and prepared with ACT peptide to stabilize the isolated polypeptide. Preferably the stabilizers are non-irritating, non-staining, and non-immunogenic. The stabilizers enable the long term (i.e., for 3 months, for 6 months, for 9 months, for 12 months, for 18 months, or for 24 months) storage of the drug product under a variety of temperature conditions (i.e., at about 5° C, at about 10° C, at about 15° C, at about 20° C, at about 25° C, at about 30° C, at about 35° C, or at about 40° C) and under a range of relative humidities (i.e., at 0% relative humidity, at 10% relative humidity, at 20% relative humidity, at 30% relative humidity, at 40% relative humidity, at 50% relative humidity, at 60% relative humidity, at 70% relative humidity, at 80% relative humidity, at 90% relative humidity, or at 100% relative humidity) .

[0009] In another aspect of the invention, one or more excipients are selected from the group consisting of water-soluble porogens, polymers, ionic compounds, non-ionic compounds, and solvents. In a further embodiment, excipients act as one or more of carriers, stabilizers, and gelling agents. In one embodiment, at least one excipient is prepared with ACT. Preferably the excipients are non-irritating, non-staining, and non-immunogenic. In one embodiment the formulation comprises at least one polymer. In a further embodiment, the formulation comprises a derivative of cellulose. In a yet further embodiment, the derivative of cellulose is hydroxyethylcellulose.

[0010] In yet another aspect of the invention, an optimal pH range for the topical gel is about pH 5 to about pH 7, and is maintained by the addition of one or more buffering agents. Preferably the buffering agents are non-irritating, non-staining, and non-immunogenic.

[0011] In yet another aspect of the invention, at least one excipient is a gelling agent. In one embodiment, a gelling agent is a carrier of ACT. In some embodiments, the gelling agent also acts as a stabilizer. In some embodiments, the

gelling agent may be combined with at least one of the following: one or more buffering agent, and one or more additional excipient. In one embodiment, the gelling agent is hydroxyethylcellulose.

[0012] In some embodiments, the present invention includes a method of wound treatment comprising administering to a subject in need thereof a topical formulation comprising at least one alpha connexin polypeptide and hydroxyethylcellulose gel.

[0013] In an exemplary embodiment, the steps are disclosed for manufacturing of the drug product.

DESCRIPTION OF DRAWINGS

[0014] For a fuller understanding of the nature and advantages of the present invention, reference should be had to the following detailed description taken in connection with the accompanying drawings, in which:

[0015] **Figure 1** illustrates the manufacturing process flow chart.

DETAILED DESCRIPTION

[0016] Before the present compositions and methods are described, it is to be understood that this invention is not limited to the particular processes, compositions, or methodologies described, as these may vary. The terminology used in the description is for the purpose of describing the particular versions or embodiments only, and it is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art. All publications mentioned herein are incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

[0017] It must also be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to an “element” is a reference to one or more elements and equivalents thereof known to those skilled in the art, and so forth.

[0018] The term "topical" refers to administration of inventive drug product at, or immediately beneath, the point of application. As used herein “topical application” refers to application onto one or more surfaces(s) including keratinous tissue, i.e., "topically applying." Topical application or "topically applying" may involve direct application to the area of the desired substrate. The topical preparation and/or composition may be applied by pouring, dropping, or spraying, if a liquid; rubbing on, if an ointment, lotion, cream, gel, or the like; dusting, if a powder; spraying, if a liquid or aerosol composition; or by any other appropriate means.

[0019] As used herein, the phrase "gelling agents" refers to agents that make a topical preparation denser or more viscous in consistency. Gelling agents may be water-based or oil-based. Gelling agents may also be referred to as “thickening agents.” “Excipients,” as used herein, are inactive ingredients that serve as carriers of the active pharmaceutical ingredient (API). Excipients may also serve as gelling or thickening agents, or stabilizers.

[0020] A “subject” or a “mammal” includes a human or a non-human mammal. Non-human mammals include, but not limited to, livestock and pets, such as ovine, bovine, porcine, canine, feline and murine mammals. In one embodiment, the subject or mammal is human.

[0021] “Administering” when used in conjunction with a therapeutic means to administer a therapeutic directly into or onto a target tissue or to administer a therapeutic to a patient whereby the therapeutic positively impacts the tissue to which it is targeted. Thus, as used herein, the term “administering”, when used in

conjunction with the drug product, can include, but is not limited to, providing the drug product and the ACT peptide into or onto the target tissue. "Administering" a composition may be accomplished by injection, topical administration, or by any method in combination with other known techniques.

[0022] Unless otherwise indicated, the term "skin" means that outer integument or covering of the body, consisting of the dermis and the epidermis and resting upon subcutaneous tissue.

[0023] As used herein, the term "therapeutic" means an agent utilized to treat, combat, ameliorate, prevent or improve an unwanted condition or disease of a patient. In part, embodiments of the present invention are directed to the treatment of scarring and delayed wound healing.

[0024] As used herein, the phrase "effective amount" or "therapeutically effective amount" refers to a nontoxic but sufficient amount of the ACT polypeptides used in the practice of the invention that is effective to achieve the desired effect, *i.e.*, to treat acute and chronic wounds. The activity contemplated by the present methods includes both medical therapeutic and/or prophylactic treatment, as appropriate, including, for example, a reduction and/or alleviation of the signs, symptoms, or causes of a disease or disorder, or any other desired alteration of a biological system. The specific dose of a compound administered according to this invention to obtain therapeutic and/or prophylactic effects will, of course, be determined by the particular circumstances surrounding the case, including, for example, the specific polypeptide administered and the condition being treated. A therapeutically effective amount of compound of this invention is typically an amount such that when it is administered in a physiologically tolerable excipient composition, it is sufficient to achieve an effective intracellular concentration and local concentration in the tissue.

[0025] Generally speaking, the term "tissue" refers to any aggregation of similarly specialized cells which are united in the performance of a particular function. As

used herein, “tissue”, unless otherwise indicated, refers to tissue which includes elastin as part of its necessary structure and/or function.

[0026] The terms "treat," "treated," or "treating" as used herein refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent, ameliorate the effects of or slow down (lessen) an undesired physiological condition, disorder or disease, or to obtain beneficial or desired clinical results. For the purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of the extent of the condition, disorder or disease; reducing the severity of a symptom of a condition, disease or disorder; reducing the frequency of a symptom of a condition, disease or disorder; stabilization (*i.e.*, not worsening) of the state of the condition, disorder or disease; delay in onset or slowing of the progression of the condition, disorder or disease; amelioration of the condition, disorder or disease state; and remission (whether partial or total), whether detectable or undetectable, or enhancement or improvement of the condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. In some embodiments, treatment may include preventing or reducing scarring and/or promoting the regeneration of complex tissue structure and function following injury.

[0027] As used herein, the terms “peptide,” “polypeptide,” or “protein” are used interchangeably, and refer to a compound comprised of amino acid residues covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise the sequence of a protein or peptide. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used herein, the term refers to both short chains, which also commonly are referred to in the art as peptides, oligopeptides and oligomers, for example, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. “Polypeptides” include, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides,

homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs and fusion proteins, among others. The polypeptides include natural peptides, recombinant peptides, synthetic peptides or a combination thereof. A peptide that is not cyclic will have an N-terminal and a C-terminal. The N-terminal will have an amino group, which may be free (i.e., as a NH2 group) or appropriately protected (for example, with a BOC or aFmoc group). The C-terminal will have a carboxylic group, which may be free (i.e., as a COOH group) or appropriately protected (for example, as a benzyl or a methyl ester). A cyclic peptide does not have free N- or C-terminal, since the ends are covalently bonded through an amide bond to form the cyclic structure. Amino acids may be represented by their full names (for example, leucine), 3-letter abbreviations (for example, Leu) and 1-letter abbreviations (for example, L). The structure of amino acids and their abbreviations can be found in the chemical literature, such as in Stryer, "Biochemistry", 3rd Ed., W. H. Freeman and Co., New York, 1988.

[0028] Excipients for use in topical gels are well-known in the art and examples may be found in the Handbook of Pharmaceutical Excipients (Rowe, R.C. et al, APhA Publications; 5th ed., 2005). Exemplary excipients may include waxes, various sugars and types of starch, polymers, gels, emollients, thickening agents, rheology modifiers, humectants, glycerol, organic basic compounds, cellulose derivatives, gelatin, vegetable oils, polyethylene glycols and solvents. Examples of rheology modifiers include Carbopol, hydroxypropyl cellulose, C $_{26\text{-}28}$ alkyl dimethicone, C $_{26\text{-}28}$ alkyl methicone, polyphenylsisquioxane, trimethylsiloxysilicate, crosspolymers of cyclopentasiloxane and dimethicone/vinyltrimethylsiloxysilicate, fumed silica (e.g. Cab-O-Sil M5P), and mixtures thereof. Examples of emollients include glycerine, pentylene glycol, sodium pyrrolidone carboxylic acid, lanolin, saccharide isomerate, stearoxy dimethicone, stearyl dimethicone, and mixtures thereof. Emollients may be useful to prevent stratum corneum dehydration occurring due to the use of anhydrous solvents in the formulation. Examples of organic bases include 2-amino-2-methyl propanol, niacinamide, methanolamines, triethanolamines, Trisamino, AMP-95, AmP-Ultra PC 2000, triisopropanolamine, diisopropanolamine, Neutrol TE,

Ethomeen, and mixtures thereof. The organic base may render the pH of the medicament basic or neutral.

[0029] Other exemplary excipients include water-soluble porogens. A water- soluble porogen is an additive that may facilitate water uptake and diffusion into the gel. Any suitable porogen may be used, but in some embodiments, the porogen may include sodium chloride, potassium chloride, sucrose, glucose, lactose, sorbitol, xylitol, polyethylene glycol, polyvinylpyrrollidone, polyvinyl alcohol or mixtures thereof.

[0030] Polymers may also act as excipients in topical gels. Exemplary polymers include hydrophilic polyurethanes, hydrophilic polyacrylates, co-polymers of carboxymethylcellulose and acrylic acid, N-vinylpyrrolidone, poly(hydroxy acids), polyanhydrides, polyorthoesters, polyamides, polycarbonates, polyalkylenes (e.g., polyethylene and polypropylene), polyalkylene glycols (e.g., poly(ethylene glycol)), polyalkylene oxides (e.g., polyethylene oxide), polyalkylene terephthalates (e.g., polyethylene terephthalate), polyvinyl alcohols, polyvinyl ethers, polylvinyl esters, polyvinyl halides (e.g., poly(vinyl chloride)), polyvinylpyrrolidone, polysiloxanes, poly(vinyl acetates), polystyrenes, polyurethane copolymers, cellulose, derivatized celluloses (e.g., hydroxyethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, methylcellulose, ethylcellulose, carboxymethyl cellulose, or cellulose acetate), alginates, poly(acrylic acid), poly(acrylic acid) derivatives, acrylic acid copolymers, methacrylic acid, methacrylic acid derivatives, methacrylic acid copolymers, poly(butyric acid), poly(valeric acid), poly(lactide-co-caprolactone), copolymers thereof and blends thereof.

[0031] In some embodiments of the invention, the polymers may be superabsorbent polymers (SAPs). A polymer is considered superabsorbent, as defined per IUPAC, as a polymer that can absorb and retain extremely large amounts of water relative to its own mass. SAPs may absorb water up to 500 times their own weight and may swell up to 1000-times their original volume. Particular SAPs of interest include sodium polyacrylate, the polyurethane Tecophilic TG-2000, and polymers prepared by the use of polyacrylamide copolymer, ethylene maleic anhydride copolymer,

cross-linked carboxy-methyl-cellulose, polyvinyl alcohol copolymers, polyvinylpyrrolindone and cross-linked polyethylene oxide.

[0032] In some embodiments of the invention, polymers that are relatively hydrophobic may be used. Any suitable hydrophobic polymer may be used. However, exemplary polymers that are relatively hydrophobic include aromatic polyurethanes, silicone rubber, polysiloxanes, polycaprolactone, polycarbonate, polyvinylchloride, polyethylene, poly-L-lactide, poly-DL-glycolide, polyetheretherketone (PEEK), polyamide, polyimide and polyvinyl acetate. In addition, a hydrophobic gel-base and/or rheology modifier may be used.

[0033] In some embodiments of the invention, the polymers may act as thickening agents in the medicaments. Specifically, the polymeric portion of the gel may act as a visco-elastic substance and may retain the gel at the site of application, along with the alpha connexin polypeptides dispersed therein.

[0034] In some other embodiments, a gel that includes a polymer may have spreadability such that it forms a thin film when applied on the skin surface. This film may enable the application of the contained alpha connexin polypeptides over a wide area, and may serve to maintain the alpha connexin polypeptides on the affected area of the skin.

[0035] Other excipients may include various ionic or non-ionic compounds to maintain stability of the formulation, thereby protecting from the de-emulsification, settling, agglomeration or degradation of the formulation constituents that may reduce its therapeutic or aesthetic value.

[0036] Examples of ionic compounds may include salts such as sodium chloride, potassium chloride; cationic, anionic or zwitterionic surfactants such as sodium dodecyl sulfate (SDS), perfluorooctanoate (PFOA), perfluorooctanesulfonate (PFOS), ammonium lauryl sulfate (ALS), sodium lauryl ether sulfate (SLES), alkyl benzene sulfonate, cetyl trimethylammonium bromide (CTAB), cetylpyridinium chloride (CPC), polyethoxylated tallow amine (POEA), benzalkonium chloride

(BAC), benzethonium chloride, dodecyl betaine, cocamidopropyl betaine and cocoampho glycinate .

[0037] Examples of non-ionic compounds that may act as excipients include non-ionic surfactants such as Pluronic, Tween, AMP, and Brij family of surfactants; and surfactants derived from biological sources, e.g, natural or semi-synthetic surfactants, such as oleic acid, sorbitan trioleate, sorbitan monooleate, lecithin, cocamide MEA, cocamide DEA and cocamidopropyl betaine. Surfactants (both ionic and non-ionic) may reduce the interfacial surface energy and may facilitate spreading of the topical formulation over a wider area.

[0038] In some embodiments of the invention, solvent excipients may be used as a carrier vehicle for the alpha connexin polypeptides and other excipients. The polymer chains may interact with the solvent and undergo swelling to form a network that may impart visco-elastic properties to the topical formulation. In some embodiments of the topical formulation, the solvent may evaporate upon application, leaving a residual film of the polymer along with the entrapped alpha connexin polypeptides.

[0039] Exemplary solvent excipients that may be useful in hydrophilic formulations may include dimethyl isosorbide, propylene glycol, glycerol, isopropanol, ethanol, benzyl alcohol, ethylene glycol, polyethylene glycol, ethoxydiglycol or mixtures thereof. Exemplary solvent excipients that may be useful in hydrophobic formulations may include capric/caprylic triglycerides, isopropyl myristate, mineral oil, isododecane, isodecyl neopentanoate, butylene glycol, pentylene glycol, hexylene glycol, methoxypolyethyleneglycol, cyclopentasiloxane, cyclotetrasiloxane, dimethicone, caprylyl methicone or mixtures thereof.

[0040] In addition to the alpha connexin polypeptides and excipients, the topical formulation may also include at least one additional therapeutic agent such as antimicrobial agents, anti-acne agents, anti- inflammatory agents, analgesic agents, anesthetic agents, antihistamine agents, antiseptic agents, immunosuppressants, antihemorrhagic agents, vasodilators, wound healing agents, anti-biofilm agents and mixtures thereof.

[0041] Examples of antimicrobial agents include penicillins and related drugs, carbapenems, cephalosporins and related drugs, erythromycin, aminoglycosides, bacitracin, gramicidin, mupirocin, chloramphenicol, thiamphenicol, fusidate sodium, lincomycin, clindamycin, macrolides, novobiocin, polymyxins, rifamycins, spectinomysin, tetracyclines, vanomycin, teicoplanin, streptogramins, anti-folate agents including sulfonamides, trimethoprim and its combinations and pyrimethamine, synthetic antibacterials including nitrofurans, methenamine mandelate and methenamine hippurate, nitroimidazoles, quinolones, fluoroquinolones, isoniazid, ethambutol, pyrazinamide, para-aminosalicylic acid (PAS), cycloserine, capreomycin, ethionamide, prothionamide, thiacetazone, viomycin, eveminomycin, glycopeptide, glyclyclycline, ketolides, oxazolidinone; imipenen, amikacin, netilmicin, fosfomycin, gentamycin, ceftriaxone, Ziracin, Linezolid, Synercid, Aztreonam, and Metronidazole, Epiroprim, Sanfetrinem sodium, , Biapenem, Dynemicin, Cefluprenam, Cefoselis, Sanfetrinem celexetil, Cefpirome, Mersacidin, Rifalazil, Kosan, Lenapenem, Veneprim, Sulopenem, ritipenam acoxyl, Cyclothialidine, micacocidin A, carumonam, Cefozopran and Cefetamet pivoxil.

[0042] Examples of topical anti-acne agents include adapalene, azelaic acid, benzoyl peroxide, clindamycin and clindamycin phosphate, doxycycline, erythromycin, keratolytics such as salicylic acid and retinoic acid (Retin-A"), norgestimate, organic peroxides, retinoids such as isotretinoin and tretinoin, sulfacetamide sodium, and tazarotene. Particular anti-acne agents include adapalene, azelaic acid, benzoyl peroxide, clindamycin {e.g., clindamycin phosphate), doxycycline {e.g., doxycycline monohydrate), erythromycin, isotretinoin, norgestimate, sulfacetamide sodium, tazarotene, etretinate and acetretin.

[0043] Examples of antihistamine agents include diphenhydramine hydrochloride, diphenhydramine salicylate, diphenhydramine, chlorpheniramine hydrochloride, chlorpheniramine maleate isothipendyl hydrochloride, tripelennamine hydrochloride, promethazine hydrochloride, methdilazine hydrochloride, and the like. Examples of local anesthetic agents include dibucaine hydrochloride, dibucaine, lidocaine hydrochloride, lidocaine, benzocaine, p-buthylaminobenzoic acid 2-(die- ethylamino) ethyl ester hydrochloride, procaine hydrochloride, tetracaine, tetracaine

hydrochloride, chloroprocaine hydrochloride, oxyprocaine hydrochloride, mepivacaine, cocaine hydrochloride, piperocaine hydrochloride, dyclonine and dyclonine hydrochloride.

[0044] Examples of antiseptic agents include alcohols, quaternary ammonium compounds, boric acid, chlorhexidine and chlorhexidine derivatives, iodine, phenols, terpenes, bactericides, disinfectants including thimerosal, phenol, thymol, benzalkonium chloride, benzethonium chloride, chlorhexidine, povidone iode, cetylpyridinium chloride, eugenol and trimethylammonium bromide.

[0045] Examples of anti-inflammatory agents include nonsteroidal antiinflammatory agents (NSAIDs); propionic acid derivatives such as ibuprofen and naproxen; acetic acid derivatives such as indomethacin; enolic acid derivatives such as meloxicam, acetaminophen; methyl salicylate; monoglycol salicylate; aspirin; mefenamic acid; flufenamic acid; indomethacin; diclofenac; alclofenac; diclofenac sodium; ibuprofen; ketoprofen; naproxen; pranoprofen; fenoprofen; sulindac; fenclofenac; clidanac; flurbiprofen; fentiazac; bufexamac; piroxicam; phenylbutazone; oxyphenbutazone; clofezone; pentazocine; mepirizole; tiaramide hydrochloride; steroids such as clobetasol propionate, bethamethasone dipropionate, halbetasol proprionate, diflorasone diacetate, fluocinonide, halcinonide, amcinonide, desoximetasone, triamcinolone acetonide, mometasone furoate, fluticasone proprionate, betamethasone diproprionate, triamcinolone acetonide, fluticasone propionate, desonide, fluocinolone acetonide, hydrocortisone vlaerate, prednicarbate, triamcinolone acetonide, fluocinolone acetonide, hydrocortisone and others known in the art, predonisolone, dexamethasone, fluocinolone acetonide, hydrocortisone acetate, predonisolone acetate, methylpredonisolone, dexamethasone acetate, betamethasone, betamethasone valerate, flumetasone, fluorometholone, beclomethasone diproprionate, fluocinonide, topical corticosteroids, and may be one of the lower potency corticosteroids such as hydrocortisone, hydrocortisone-21 - monoesters (e.g., hydrocortisone-21 -acetate, hydrocortisone-21-butyrate, hydrocortisone-21 -propionate, hydrocortisone-21 -valerate, etc.), hydrocortisone-17,21-diesters (e.g., hydrocortisone- 17,21 -diacetate, hydrocortisone- 17-acetate-21- butyrate, hydrocortisone- 17,21 -dibutyrate, etc.), alclometasone,

dexamethasone, flumethasone, prednisolone, or methylprednisolone, or may be a higher potency corticosteroid such as clobetasol propionate, betamethasone benzoate, betamethasone dipropionate, diflorasone diacetate, fluocinonide, mometasone furoate, triamcinolone acetonide.

[0046] Examples of analgesic agents include alfentanil, benzocaine, buprenorphine, butorphanol, butamben, capsaicin, clonidine, codeine, dibucaine, enkephalin, fentanyl, hydrocodone, hydromorphone, indomethacin, lidocaine, levorphanol, meperidine, methadone, morphine, nicomorphine, opium, oxybuprocaine, oxycodone, oxymorphone, pentazocine, pramoxine, proparacaine, propoxyphene, proxymetacaine, sufentanil, tetracaine and tramadol.

[0047] Examples of anesthetic agents include alcohols such as phenol; benzyl benzoate; calamine; chloroxylenol; dyclonine; ketamine; menthol; pramoxine; resorcinol; troclosan; procaine drugs such as benzocaine, bupivacaine, chloroprocaine; cinchocaine; cocaine; dexivacaine; diamocaine; dibucaine; etidocaine; hexylcaine; levobupivacaine; lidocaine; mepivacaine; oxethazaine; prilocaine; procaine; proparacaine; propoxycaine; pyrrocaine; risocaine; rodocaine; ropivacaine; tetracaine; and derivatives, such as pharmaceutically acceptable salts and esters including bupivacaine HCl, chloroprocaine HCl, diamocaine cyclamate, dibucaine HCl, dyclonine HCl, etidocaine HCl, levobupivacaine HCl, lidocaine HCl, mepivacaine HCl, pramoxine HCl, prilocaine HCl, procaine HCl, proparacaine HCl, propoxycaine HCl, ropivacaine HCl, and tetracaine HCl.

[0048] Examples of antihemorrhagic agents include thrombin, phytonadione, protamine sulfate, aminocaproic acid, tranexamic acid, carbazochrome, carbaxochrome sodium sulfanate, rutin and hesperidin.

[0049] Beside the bioactive polypeptide component, the instant invention may also contain other active agents such as niacinamide, phytantriol, farnesol, bisabolol and salicylic acid. It is expected that certain additional active agents will act synergistically with the bioactive peptide component, or will enhance the shelf-life of the formulation.

[0050] Examples of wound treatments that may be used together with the drug product of the present invention include fibrinolytic enzymes, such as fibrinolysin, deoxyribonuclease, streptokinase, and streptodornase, necrotomy tissue agents containing lysozyme chloride, antimicrobial agents containing gentamicin sulfate, sulfadiazine silver, bacitracin, and fradiomycin sulfate, incarnant agents containing trafermin, bucladesine sodium, tretinoin tocoferil (tocoretinate), alprostadil alfadex, solcoseryl (extract from hemolysed blood of young cattle), and alcloxa, iodine preparations containing white soft sugar, povidone iodine, and iodine, and preparations containing bendazac, dimethyl isopropylazulene (guaiazulene), and epinephrine as active ingredients.

[0051] In addition to the alpha connexin polypeptides, excipients, and other therapeutic agents, the gels may also include other compounds that improve the organoleptic properties of the topical formulation.

[0052] Examples of such compounds include perfumes, dyes and colorants; chelating agents including but not limited to edetate disodium (EDTA), EGTA, CP94, citric acid; preservatives including but not limited to quaternary ammonium compounds, such as benzalkonium chloride, benzethonium chloride, cetrimide, dequalinium chloride, and cetylpyridinium chloride; mercurial agents, such as phenylmercuric nitrate, phenylmercuric acetate, and thimerosal; alcoholic agents, for example, chlorobutanol, phenylethyl alcohol, and benzyl alcohol; antibacterial esters, for example, esters of parahydroxybenzoic acid; and other anti-microbial agents such as chlorhexidine, chlorocresol, benzoic acid and polymyxin.

[0053] Alpha connexin polypeptides that may be used in the formulation of the present invention are described in U.S. Patent No. 7,786,074, which is hereby incorporated by reference. In some embodiments, the alpha connexin polypeptide is a full length alpha connexin protein such as connexin 37, connexin 40, connexin 43, or connexin 45. In some embodiments, the polypeptide does not comprise the full-length connexin protein.

[0054] Peptides useful in the formulations of the present invention include alpha connexin polypeptides. The alpha connexin polypeptides useful in the formulations

of the present invention may comprise or consist of the carboxy-terminal most 4 to 30 contiguous amino acids of an alpha connexin protein or conservative variant thereof. Thus, the polypeptides useful in the formulations can comprise the c-terminal-most 4 to 30 amino acids of the alpha Connexin, including the c-terminal most 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 25 22, 23, 24, 25, 26, 27, 28, 29, 30 amino acids ofthe alpha Connexin. In some aspects, the provided polypeptide further comprises a deletion of one or more amino acids of the c-terminal-most 4 to 30 amino acids of the alpha Connexin, including a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the c-terminal-most 4 to 30 amino acids of the alpha Connexin. For example, in some aspects, the polypeptides useful in the formulations of the present invention do not comprise the c-terminal-most 1, 2, or 3 amino acids of the alpha Connexin. For example, the polypeptides can consist essentially of the amino acid sequence SEQ ID NO:92, or a carboxy terminal fragment thereof of at least 4, 5, 6, 7, 8, 9, 10 amino acids in length.

[0055] In certain embodiments, the alpha connexin polypeptide of the present invention is linked at its amino or carboxy terminus to a cellular internalization transporter. Separate embodiments include the alpha connexin polypeptide without any cellular internalization transporter attached. The cellular internalization transporter linked to the alpha connexin polypeptides may be any internalization sequence known or newly discovered in the art, or conservative variants thereof. Non-limiting examples of cellular internalization transporters and sequences include Antennapedia sequences, TAT, HIV-Tat, Penetratin, Antp-3A (Antp mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB1, Pep-7, HN-1, BGSC (Bis-Guanidinium-Spermidine-Cholesterol, and BGTC (BisGuanidinium-Tren-Cholesterol).

[0056] In one embodiment of the present invention, the amino acid sequence of the alpha connexin polypeptide is selected from the group consisting of SEQ ID NOs: 1, 2, 3, 4, and 5, or a conservative variant thereof. In a further embodiment of the present invention, the alpha connexin polypeptide comprises the amino acid sequence of SEQ ID NO: 2. In another embodiment of the present invention, the polypeptide comprises an alpha connexin polypeptide and a cellular internalization

transporter. In a further embodiment, the alpha connexin polypeptide is linked at is amino terminus to the cellular internalization transporter. In a yet further embodiment, the amino acid sequence of the alpha connexin polypeptide linked to the cellular internalization transporter is selected from the group consisting of SEQ ID NO: 8, 9, 10, 11, and 12, or a conservative variant thereof. In one embodiment, the polypeptide consists of or comprises the amino acid sequence of SEQ ID NO: 9, namely, RQPKIWFPNRRKPWKKRPRPDDLEI.

[0057] When specific proteins are referred to herein, variants, derivatives, and fragments are contemplated. Protein variants and derivatives are well understood to those of skill in the art and can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. These variants ordinarily are prepared by site specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known and include, for example, M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues. Deletions or insertions may be made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure unless such a change in secondary structure of the mRNA is desired. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are referred to as conservative

substitutions. For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions Conservatively substituted variations of each explicitly disclosed sequence are included within the polypeptides provided herein. Typically, conservative substitutions have little to no impact on the biological activity of a resulting polypeptide. In a particular example, a conservative substitution is an amino acid substitution in a peptide that does not substantially affect the biological function of the peptide. A peptide can include one or more amino acid substitutions, for example 2-10 conservative substitutions, 2-5 conservative substitutions, 4-9 conservative substitutions, such as 2, 5 or 10 conservative substitutions. A polypeptide can be produced to contain one or more conservative substitutions by manipulating the nucleotide sequence that encodes that polypeptide using, for example, standard procedures such as site-directed mutagenesis or PCR. Alternatively, a polypeptide can be produced to contain one or more conservative substitutions by using standard peptide synthesis methods. An alanine scan can be used to identify which amino acid residues in a protein can tolerate an amino acid substitution. In one example, the biological activity of the protein is not decreased by more than 25%, for example not more than 20%, for example not more than 10%, when an alanine, or other conservative amino acid (such as those listed below), is substituted for one or more native amino acids. Further information about conservative substitutions can be found in, among other locations, in Ben-Bassat *et al., (J. Bacterial.* 169:751-7, 1987), O'Regan *et al., (Gene* 77:237-51, 1989), Sahin-Toth *et al.,* (Protein Sci. 3:240-7, 1994), Hochuli *et al., (Bio/Technology* 6:1321-5, 1988) and in standard textbooks of genetics and molecular biology.

[0058] As used herein, “analytical methods” includes the following techniques that are useful for determining the identity and quantity of the ACT peptide in a particular formulation: NMR, HPLC, Amino Acid Composition and Molecular Weight Determinations, and Specific Optical Rotation.

[0059] The present invention includes a topical formulation comprising at least one alpha connexin polypeptide and hydroxyethylcellulose gel, wherein the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide. In certain embodiments, the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide so that after 3 months of storage at 5°C at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% of the alpha connexin polypeptide is detectable by analytical methods. In some embodiments, the alpha connexin polypeptide is present in the formulation at a concentration of about 0.0025% (w/w), of about 0.005% (w/w), of about 0.0075% (w/w), of about 0.010% (w/w), of about 0.015% (w/w), of about 0.020% (w/w), of about 0.025% (w/w), of about 0.030% (w/w), of about 0.035% (w/w), of about 0.040% (w/w), of about 0.045% (w/w), of about 0.050% (w/w), of about 0.055% (w/w), of about 0.060% (w/w), of about 0.065% (w/w), of about 0.070% (w/w), of about 0.075% (w/w), of about 0.080% (w/w), of about 0.085% (w/w), of about 0.090% (w/w), of about 0.095% (w/w), of about 0.100% (w/w), of about 0.150% (w/w), of about 0.200% (w/w), of about 0.250% (w/w), of about 0.500% (w/w), of about 0.750% (w/w), of about 1.00% (w/w), of about 1.50% (w/w), of about 2.00% (w/w), of about 2.50% (w/w), or of about 5.00 (w/w). In one embodiment, the alpha connexin polypeptide is present in the formulation at a concentration of between about 0.005% (w/w) and about 1.00% (w/w).

[0060] In other embodiments, the drug product of the invention is a clear colorless gel which contains 0.0072% (w/w) (20μM) of the ACT peptide, 0.018% (w/w) (50μM) of the ACT peptide, 0.036% (w/w) (100μM) of the ACT peptide, or 0.072% (w/w) (200μM) of the ACT peptide. The ACT peptide may be dissolved in a semisolid dosage form that contains >0% water, >10% water, >20% water, >30% water, >40% water, >50% water, >60% water, >70% water, >80% water, or > 90% water and 0.25% gelling agent (polymer), 0.55% gelling agent (polymer), 0.75% gelling agent (polymer), 1.00% gelling agent (polymer), 1.25% gelling agent (polymer), 1.50% gelling agent (polymer), 1.75% gelling agent (polymer), 2.00% gelling agent (polymer), 2.25% gelling agent (polymer), or 2.50% gelling agent (polymer). The ACT peptide may be well preserved and adequately buffered to pH

6. In one embodiment of the topical formulation, the qualitative and quantitative composition is that listed in Table 1.

Table 1: Drug Product Gel Qualitative & Quantitative Composition

Ingredients	Grade	Function	Concentration (% w/w)
Peptide 328967 (ACT peptide)	--	Active	0.0072; 0.018 0.036; 0.072
Methylparaben	NF	Preservative	0.17
Propylparaben	NF	Preservative	0.02
Glycerin	USP	Solvent	5.0
Sodium Phosphate Monobasic	USP	Buffer Agent	0.263
Sodium Phosphate Dibasic	USP	Buffer Agent	0.044
Propylene Glycol	USP	Solvent	3.0
Edetate Disodium (EDTA)	USP	Chelating Agent	0.05
D-Mannitol	USP	Stabilizer	0.05
Hydroxyethylcellulose, 250HHX	NF	Gelling Agent	1.25%
Purified Water, qsad	USP	Solvent	100%

[0061] The ACT1 peptide sequence is listed in Table 2 below in which Ahx refers to L-2-aminohexanoic acid, also known as 6-aminohexanoic acid:

Table 2: Peptide 328967 (Antp/ACT1) sequence.

Biotin-Ahx-Arg-Gln-Pro-Lys-Ile-Trp-Phe-Pro-Asn-Arg-Arg-Lys-Pro-Trp-Lys-Lys-Arg-Pro-Arg-Pro-Asp-Asp-Leu-Glu-Ile-OH

[0062] The general properties of the Peptide 328967 are listed in Table 3.

Table 3: General Physical Properties of Peptide 328967

Physical Appearance	White to off-white powder
Molecular Weight	3597.33 ± 2.0 amu
Counter-Ion	AcOH
Solubility	Soluble in water at room temperature

[0063] In one aspect of the invention, the excipients used in the drug product topical preparation are selected from the group consisting of or are one or more of the following:

[0064] Methylparaben

[0065] Propylparaben

[0066] Glycerin

[0067] Sodium Phosphate Monobasic

[0068] Sodium Phosphate Dibasic

[0069] Propylene Glycol

[0070] Edetate Disodium (EDTA)

[0071] D-Mannitol

[0072] Hydroxyethylcellulose, 250 HHX

[0073] Purified Water

[0074] In one embodiment, the drug product topical preparation comprises a peptide, D-mannitol, hydroxyethylcellulose, and purified water. Said preparation may further cmoprise one or more of methylparaben, propylparaben, glycerin, sodium phosphate monobasic, sodium phosphate dibasic, propylene glycol, and edetate disodium (EDTA). In a further embodiment, the hydroxyethylcellulose is 250 HHX. In a further embodiment, the peptide is an alpha connexin polypeptide.

[0075] In such embodiments, the drug product topical preparation comprises a peptide at a concentration between about 0.001 % (w/w) and about 0.5% (w/w) (for example, at about 0.0072%, 0.018%, 0.036%, or 0.072% (w/w)); methylparaben at a concentration between about 0.10% (w/w) and about 0.25% (w/w) (for example, about 0.17% (w/w)); propylparaben at a concentration between about 0.01% (w/w) and about 0.03% (w/w) (for example, about 0.02% (w/w)); glycerin at a concentration between about 1% (w/w) and about 10% w/w) (for example, about 5% (w/w)); sodium phosphate monobasic at a concentration between about 0.1% (w/w) and about 0.5% (w/w) (for example, about 0.263% (w/w)); sodium phosphate dibasic at a concentration between about 0.02% and about 0.06% (for example, about 0.044% (w/w)); propylene glycol at a concentration between about 1% (w/w) and about 5% (w/w) (for example, about 3% (w/w)); EDTA at a concentration between about 0.01% and about 0.1% (for example, about 0.05% (w/w)); D-mannitol at a concentration between about 0.01% (w/w) and about 0.1% (w/w) (for example, about 0.05% (w/w)); hydroxyethylcellulose at a concentration between about 0.5% and about 2.5% (for example, about 1.25% (w/w)), and purified water at a concentration of about 0.1% to about 10% (for example, about 1%). In a further embodiment, the peptide is an alpha connexin polypeptide. In a yet further embodiment, the peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, 2, 3, 4, 5, 8, 9, 10, 11, and 12. In one embodiment, the peptide comprises an amino acid sequence according to SEQ ID NO: 9.

[0076] Derived through in vitro and in vivo studies, these stabilizers and excipients are incorporated into the drug product since they are non-irritating, non-staining, and non-immunogenic. Stability studies showed that the ACT1 peptide is more stable in the gelling agent Hydroxyethylcellulose, 250 HHX (1.25%) compared to Pluronic gels. The ACT peptide within the drug product containing 1.25% hydroxyethylcellulose only dropped to 98% of the label claim (i.e., initial concentration) when stored at 5°C for three months and to 84% of the label claim when stored at 25° C for the same duration. In one aspect of the invention, edetate disodium (EDTA) and Mannitol are incorporated within the drug product to provide stability to the ACT1 peptide. In some embodiments of the invention, the Mannitol is

present in the formulation at 0.01% (w/w) to 1.6% (w/w), 0.01% (w/w) to 1.5% (w/w), 0.01% (w/w) to 1.4% (w/w), 0.01% (w/w) to 1.3% (w/w), 0.01% (w/w) to 1.2% (w/w), 0.01% (w/w) to 1.1% (w/w), 0.01% (w/w) to 1.0% (w/w), 0.01% (w/w) to 0.9% (w/w), 0.01% (w/w) to 0.8% (w/w), 0.01% (w/w) to 0.7% (w/w), 0.01% (w/w) to 0.6% (w/w), 0.01% (w/w) to 0.5% (w/w), 0.01% (w/w) to 0.4% (w/w), 0.01% (w/w) to 0.3% (w/w), 0.01% (w/w) to 0.2% (w/w), 0.01% (w/w) to 0.1% (w/w), or 0.01% (w/w) to 0.0.05% (w/w). In one embodiment, the Mannitol is present in the formulation at about 0.05% (w/w).

[0077] In another aspect of the present invention, a buffering agent is included in the topical formulation to maintain the pH in a certain range. Suitable buffering agents can include, but are not limited to, acetate buffers, citrate buffers, phosphate buffers, lactic acid buffers, malic acid buffers, succinic acid buffers, borate buffers, sodium hydroxide, potassium hydroxide, and ammonium hydroxide. Phosphate salts such as monosodium phosphate (NaH_2PO_4; also known as monobasic sodium phosphate), disodium hydrogen phosphate (Na_2HPO_4; also known as dibasic sodium phosphate), monopotassium phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), and mixtures thereof can also be used. In one embodiment, phosphate buffer provides superior stability compared to citrate buffer. A buffer capacity at 25 mM was found to be adequate for the drug product. Buffer is needed to control pH of the gel system and to maintain stability of the peptide drug. The pH range of the topical formulation may be pH 2 to pH 12, pH 4 to pH 10, or pH 6 to pH 8. In one embodiment, the proper pH range is in the range of 5 to 7. In some embodiments, the pH of the topical formulation of the present invention is 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, or 8.0. In yet another aspect, propylene glycol in the amount of 3% provides better solubilization of parabens in the aqueous system.

[0078] In one embodiment, the formulation with an ACT peptide incorporated into hydroxyethylcellulose enables large scale manufacturing of a product with the characteristics that make it practical for clinical treatments as well as meeting desired storage and stability requirements. While a formulation for ACT1 using Pluronic gel may require a relatively long incorporation time of approximately 2.5

hours and only yield 50 gram batches, a formulation with hydroxyethylcellulose provides allows for significantly faster incorporation of the ACT peptide into the gel and yields much larger batches. For example, when using Pluronic F 127 gel in the topical formulation it takes over an hour to incorporate the polymer, and the formulation needs to be placed in a water bath to help with incorporation. In contrast, hydroxyethlycellulose (*e.g.*, HEC 250 HHX) is easily incorporated at room temperature and hydrates within 30 minutes. Thus, the use of hydroxyethylcellulose may facilitate large scale manufacturing. The manufacturing process with Pluronic gel may require a cold bath to bring its viscosity into a desired range and require more energy than the manufacturing process with hydroxyethylcellulose. In addition, the final formulation in Pluronic gel may be very thin at a storage condition of 5 degrees C.

[0079] It has been discovered that hydroxyethylcellulose (HEC) is a suitable gelling agent and acceptable carrier of the drug product of the present invention. In one embodiment, the gelling agent is Hydroxyethylcellulose (HEC), 250 HHX. In one embodiment, the percent (w/w) of HEC isin the range of 1-5%. In a further embodiment, the percent (w/w) of HEC is 1.25%. In the manufacture of HEC, a purified cellulose is reacted with sodium hydroxide to produce a swollen alkali cellulose. The alkali-treated cellulose is more chemically reactive than cellulose. By reacting the alkali cellulose with ethylene oxide, a series of hydroxyethylcellulose ethers is produced. In this reaction, the hydrogen atoms in the hydroxyl groups of cellulose are replaced by hydroxyethyl groups, which confer water solubility to the gel. It is contemplated in this invention that a single HEC ether may be used, or a mixture of HEC ethers of difference molecular weight and structure may be used. Suitable grades of HEC for pharmaceutical purposes are well known and full described in the pharmaceutical literature. Suitable commercially available brands of HEC include but are not limited to Fuji HEC-HP;Fuji HEC-AG 15;NATRO-SOL 250HR;NATROSOL 250MH;NATROSOL 250G;CELLOSIZE QP 30000;TYLOSE H SERIES;NATROSOL 180L;NATROSOL 300H;TYLOSE P-X;NATROSOL 250M;CELLOSIZE WP 4400;CELLOSIZE UT 40;NATROSOL 250H4R;Tylose H 20P;NATROSOL LR;TYLOSE MHB;NATROSOL 250HHP;HERCULES N

100;CELLOSIZE WP 300;TYLOSE P-Z SERIES;NATROSOL 250H;TYLOSE PS-X;Cellobond HEC 400;CELLOSIZE QP;CELLOSIZE QP 1500;NATRO-SOL 250;HYDROXYETHYL CELLULOSE ETHER;HESPAN;TYLOSE MHB-Y;NATROSOL 240JR;HYDROXYETHYL STARCH;CELLOSIZE WP;CELLOSIZE WP 300H;2-HYDROXYETHYL CELLULOSE ETHER;BL 15;CELLOSIZE QP 4400;CELLOSIZE QP3;TYLOSE MB;CELLULOSE HYDROXY-ETHYLATE;CELLOSIZE WPO 9H17;CELLOSIZE 4400H16;CELLULOSE HYDROXYETHYL ETHER;Hydroxyethyl Cellulose;Hydroxyl Ethyl Cellulose(HEC);Hydroxyethyl Cellulose 100H (celocell 100h);TYLOSE MH-XP;NATROSOL 250HX;Natrosol;Daicel EP 500;HEC-Unicel;HEC (Hydroxyethyl cellulose);Cellosize;HEC-Al 5000;Fuji HEC-AL 15;HEC-Unicel QP 09L;Cellulose,ethers,2-hydroxyethyl ether;Unicel QP 52000H;HEC-QP 4400;SP 250 (cellulose);Hetastarch;Cellulose,ethers,2-hydroxyethyl ether;Glutofix 600;FL 52;Fuji HEC-AX 15F;Tylose H 300P;HEC-Unicel QP 300H;Tylose H 300;Daicel SP 550;Daicel SE 600;Unicel QP 15000;HEC-QP 100MH;HEC-QP 9H;OETs;Daicel EP 850;H. E. Cellulose;Cellobond 25T;Unicel QP 100MH;Tylose H 4000;SE 850K;Tylomer H 20;Daicel SE 850K;Tylose H 30000YP;Unicel QP 4400;SP 407;Tylose H 100000;Daicel SP 200;Culminal HEC 5000PR;Tylopur H 300;Daicel SP 750;Sanhec;BL 15 (cellulose derivative);Unicel QP 300H;Tylomer H 200;J 164;Tylose H 10;Tylose H 20;AH 15;Daicel SP 600;Daicel SE 900;HEC-Unicel QP 4400H;AX 15;Daicel SP 800;Fuji HEC-AW 15F;HEC-SE 850;HEC-A 5-25CF;Metolose 90SEW;AW 15 (polysaccharide);Cellobond HEC 5000;HEC-QP 100M;Cellobond HEC 15A;Tylose H 15000YP2;Walocel HT 6.000PFV;2-Hydroxyethyl cellulose (Natrosol Type 250HRCS);Fuji HEC-BL 20;Fuji HEC-SY 25F;Telhec;HEC-SP 200;HEC-AH 15;HEC-Unicel QP 30000H; see;HEC 10A;Daicel SP 400;Admiral 3089FS;Fuji HEC-A 5000F;HEC-SP 400;Hydroxyethyl Methyl Cellulose（HEMC）;HYDROXYETHYL CELLULOSE (HEC);Hydroxyethyl Starch(CAS No:9004-62-0);Hydroxy Ethyl Cellulose;“Natrosol” [Aqualon];HEC;2-HYDROXYETHYL CELLULOSE;NATROSOL 150L;TYLOSE MHB-YP;HYDROXYETHYL ETHER CELLULOSE;NATROSOL 250L;CELLOSIZE WP 400H;TYLOSE P;CELLULOSE, 2-HYDROXYETHYL ETHER;TYLOSE MH-K;NATROSOL 250HHR.

[0080] In another embodiment, the drug product of the present invention is packaged in 20 mL vial (USP Type I, Borosilicate clear scintillation glass with poly-seal cone urea screw cap). A mixture of methylparaben at 0.17% (w/w) and propylparaben 0.02% (w/w) is used as a preservative.

[0081] In some embodiments, the present invention includes a method of wound treatment comprising administering to a subject in need thereof a topical formulation comprising at least one alpha connexin polypeptide and hydroxyethylcellulose gel, wherein the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide. The wound treated may be an acute surgical wound or a chronic, non-infected, full-thickness lower extremity ulcer.

[0082] In a certain embodiment, the drug product of the present invention may be used to mitigate excessive scar formation associated with acute surgical wounds. In this embodiment, the drug product of the present invention may be applied at the time of surgical incision closure, 1 hour after surgical incision closure, 2 hours after surgical incision closure, 3 hours after surgical incision closure, 4 hours after surgical incision closure, 5 hours after surgical incision closure, 6 hours after surgical incision closure, 7 hours after surgical incision closure, 8 hours after surgical incision closure, 9 hours after surgical incision closure, 10 hours after surgical incision closure, 11 hours after surgical incision closure, 12 hours after surgical incision closure, 13 hours after surgical incision closure, 14 hours after surgical incision closure, 15 hours after surgical incision closure, 16 hours after surgical incision closure, 17 hours after surgical incision closure, 18 hours after surgical incision closure, 19 hours after surgical incision closure, 20 hours after surgical incision closure, 21 hours after surgical incision closure, 22 hours after surgical incision closure, 23 hours after surgical incision closure, 24 hours after surgical incision closure, 48 hours after surgical incision closure, 72 hours after surgical incision closure, or thereafter.

[0083] In another embodiment, the drug product of the present invention may be used to treat chronic ulcers. For example, ulcers may include diabetic foot ulcers,

venous leg ulcers, and pressure ulcers. These ulcers may be chronic, non-infected, full-thickness lower extremity ulcers. In one embodiment, the drug product of the present invention may be applied to a chronic ulcer in a daily regimen, a regimen of every other day, a regimen of once a week, or in various other regimens until healing of the chronic ulcer is apparent. In another embodiment, the drug product of the present invention may be applied to a chronic ulcer in a regimen at day 0, 3, 7, 14, 21, and 28. In another embodiment, the drug product of the present invention may be applied to a chronic ulcer in a regimen at day 0, day 3, week 1, week 2, week 3, week 4, week 5, week 6, week 7, week 8, week 9, week 10, week 11, and week 12. In another aspect of the present invention, the drug product is manufactured with the following steps:

[0084] Step 1: In a suitable size of beaker, add propylene glycol, glycerin, methylparaben and propylparaben. Mix with a propeller until the parabens are completely dissolved.

[0085] Step 2: In a manufacturing vessel, add purified water (part I), EDTA, monobasic sodium phosphate, dibasic sodium phosphate and D-mannitol. Mix with a propeller until a clear solution is obtained.

[0086] Step 3: Add the solution from step 1 to the manufacturing vessel. Rinse the beaker with purified water (part II, divided into approximately 3 equal portions) and add the rinse back to the vessel. Continue with propeller mixing until the solution is visually homogeneous.

[0087] Step 4: With homogenization mixing, add hydroxyethyl cellulose into the manufacturing vessel from Step 3. Mix until the polymer is fully dispersed.

[0088] Step 5: In a separate beaker, add purified water (part III) and an alpha connexin polypeptide (e.g., Peptide 328967 ACT1 peptide). Mix with a stir bar or propeller mixer until the peptide is completely dissolved and a gel is formed.

[0089] Step 6: With continuous propeller mixing, add the drug solution from step 5 to the manufacturing vessel. Rinse the beaker with purified water (part IV, divided into approximately 3 equal portions) and add the rinse back to the vessel. Mix until the gel is homogeneous.

[0090] The manufacturing process flow chart is provided in **Figure 1** Manufacturing Process Flow Chart.

[0091] Sequences

SEQ ID NO:1 (ACT 2)
PSSRASSRASSRPRPDDLEI

SEQ ID NO:2 (ACT 1)
RPRPDDLEI

SEQ ID NO:3 (ACT 3)
RPRPDDLEV

SEQ ID NO:4 (ACT 4)
RPRPDDVPV

SEQ ID NO:5 (ACT 5)
KARSDDLSV

SEQ ID NO:6
aga cct cgg cct gat gac ctg gag att

SEQ ID NO:7 (Antp)
RQPKIWFPNRRKPWKK

SEQ ID NO:8 (Antp/ ACT 2)
RQPKIWFPNRRKPWKKPSSRASSRASSRPRPDDLEI

SEQ ID NO:9 (Antp/ ACT 1)
RQPKIWFPNRRKPWKKRPRPDDLEI

SEQ ID NO:10 (Antp/ ACT 3)
RQPKIWFPNRRKPWKKRPRPDDLEV

SEQ ID NO:11 (Antp/ ACT 4)
RQPKIWFPNRRKPWKKRPRPDDVPV

SEQ ID NO:12 (Antp/ ACT 5)
RQPKIWFPNRRKPWKKKARSDDLSV

SEQ ID NO:13 (encodes polypeptide of SEQ ID NO 9)
cgg cag ccc aag atc tgg ttc ccc aac cgg cgg aag ccc tgg aag aag cgg ccc ggc ccg acg acc tgg aga tc

SEQ ID NO:14 (HIV-Tat)
GRKKRRQRPPQ

SEQ ID NO:15 (Penetratin)
RQIKIWFQNRRMKWKK

SEQ ID NO:16 (Antp-3A)
RQIAIWFQNRRMKWAA

SEQ ID NO:17 (Tat)
RKKRRQRRR

SEQ ID NO:18 (Buforin II)
TRSSRAGLQFPVGRVHRLLRK

SEQ ID NO:19 (Transportan)
GWTLNSAGYLLGKINKALAALAKKIL

SEQ ID NO:20 (model amphipathic peptide)
KLALKLALKALKAALKLA

SEQ ID NO:21 (K-FGF)
AAVALLPAVLLALLAP

SEQ ID NO:22 (Ku70)
VPMLK- PMLKE

SEQ ID NO:23 (Prion)
MANLGYWLLALFVTMWTDVGLCKKRPKP

SEQ ID NO:24 (pVEC)
LLIILRRRIRKQAHAHSK

SEQ ID NO:25 (Pep-1)
KETWWETWWTEWSQPKKKRKV

SEQ ID NO:26 (SynB1)
RGGRLSYSRRRFSTSTGR

SEQ ID NO:27 (Pep-7)
SDLWEMMMVSLACQY

SEQ ID NO:28 (HN-1)
TSPLNIHNGQKL

SEQ ID NO:29 (Chick alpha Cx43 ACT)
PSRASSRASSRPRPDDLEI

SEQ ID NO:30 (Human alpha Cx45)
GSNKSTASSKSPDPKNSVWI

SEQ ID NO:31 (Chick alpha Cx45)
GSNKSSASSKSGDGKNSVWI

SEQ ID: 32 (Human alpha Cx46)
GRASKASRASSGRARPEDLAI

SEQ ID: 33 (Human alpha Cx46.6)
GSASSRDGKTVWI

SEQ ID NO:34 (Chimp alpha Cx36)
PRVSVPNFGRTQSSDSAYV

SEQ ID NO:35 (Chick alpha Cx36)
PRMSMPNFGRTQSSDSAYV

SEQ ID NO:36 (Human alpha Cx47)
PRAGSEKGSASSRDGKTTVWI

SEQ ID NO:37 (Human alpha Cx40)
GYHSDKRRLSKASSKARSDDLSV

SEQ ID NO:38 (Human alpha Cx50)
PLSRLSKASSRARSDDLTV

SEQ ID NO:39 (Human alpha Cx59)
PNHVVSLTNNLIGRRVPTDLQI

SEQ ID NO:40 (Rat alpha Cx33)
PSCVSSSAVLTTICSSDQVVPVGLSSFYM

SEQ ID NO:41 (Sheep alpha Cx44)
GRSSKASKSSGGRARAADLAI

SEQ ID NO:42 (Human beta Cx26)
LCYLLIRYCSGKSKKPV

SEQ ID: 43 (Human alpha Cx37)
GQKPPSRPSSSASKKQ*YV

SEQ ID 44: (conservative Cx43 variant)
SSRASSRASSRPRPDDLEV

SEQ ID 45: (conservative Cx43 variant)
RPKPDDLEI

SEQ ID 46: (conservative Cx43 variant)
SSRASSRASSRPKPDDLEI,

SEQ ID 47: (conservative Cx43 variant)
RPKPDDLDI

SEQ ID 48: (conservative Cx43 variant)
SSRASSRASSRPRPDDLDI

SEQ ID 49: (conservative Cx43 variant)
SSRASTRASSRPRPDDLEI

SEQ ID 50: (conservative Cx43 variant)
RPRPEDLEI

SEQ ID 51: (conservative Cx43 variant)
SSRASSRASSRPRPEDLEI,

SEQ ID 52: (conservative Cx45 variant)
GDGKNSVWV

SEQ ID 53: (conservative Cx45 variant)
SKAGSNKSTASSKSGDGKNSVWV

SEQ ID 54: (conservative Cx37 variant)
GQKPPSRPSSSASKKLYV

SEQ ID NO: 55 (non-active control peptide)
RQPKIWFPNRRKPWKIELDDPRPR

SEQ ID NO:56 (HIV-Tat/ ACT 1)
GRKKRRQRPPQ RPRPDDLEI

SEQ ID NO:57 (Penetratin/ ACT 1)
RQIKIWFQNRRMKWKK RPRPDDLEI

SEQ ID NO:58 (Antp-3A/ ACT 1)
RQIAIWFQNRRMKWAA RPRPDDLEI

SEQ ID NO:59 (Tat/ ACT 1)
RKKRRQRRR RPRPDDLEI

SEQ ID NO:60 (Buforin II/ ACT 1)
TRSSRAGLQFPVGRVHRLLRK RPRPDDLEI

SEQ ID NO:61 (Transportan/ ACT 1)
GWTLNSAGYLLGKINKALAALAKKIL RPRPDDLEI

SEQ ID NO:62 (MAP/ ACT 1)
KLALKLALKALKAALKLARPRPDDLEI

SEQ ID NO:63 (K-FGF/ ACT 1)
AAVALLPAVLLALLAP RPRPDDLEI

SEQ ID NO:64 (Ku70/ ACT 1)
VPMLKPMLKERPRPDDLEI

SEQ ID NO:65(Prion/ ACT 1)
MANLGYWLLALFVTMWTDVGLCKKRPKPRPRPDDLEI

SEQ ID NO:66 (pVEC/ ACT 1)
LLIILRRRIRKQAHAHSK RPRPDDLEI

SEQ ID NO:67 (Pep-1/ ACT 1)
KETWWETWWTEWSQPKKKRKV RPRPDDLEI

SEQ ID NO:68 (SynB1/ ACT 1)
RGGRLSYSRRRFSTSTGR RPRPDDLEI

SEQ ID NO:69 (Pep-7/ ACT 1)
SDLWEMMMVSLACQY RPRPDDLEI

SEQ ID NO:70 (HN-1/ ACT 1)
TSPLNIHNGQKL RPRPDDLEI

SEQ ID NO: 71 (20 to 120 residues flanking amino acid 363 of human Cx43)
KGKSDPYHATSGALSPAKDCGSQKYAYFNGCSSPTAPLSPMSPPGYKLVTGDRN
NSSCRNYNKQASEQNWANYSAEQNRMGQAGSTISNSHAQPFDFPDDNQNSKKL
AAGHELQPLAIVDQR

SEQ ID NO: 72 (20 to 120 residues flanking amino acid 362 of chick Cx43)
KTDPYSHSGTMSPSKDCGSPKYAYYNGCSSPTAPLSPMSPPGYKLVTGDRNNSS
CRNYNKQASEQNWANYSAEQNRMGQAGSTISNSHAQPFDFADEHQNTKKLASG
HELQPLTIVDQRP

SEQ ID NO: 73 (20 to 120 residues flanking amino acid 377 of human Cx45)
LGFGTIRDSLNSKRRELEDPGAYNYPFTWNTPSAPPGYNIAVKPDQIQYTELSNAK
IAYKQNKANTAQEQQYGSHEENLPADLEALQREIRMAQERLDLAVQAYSHQNNPH
GPREKKAKV

SEQ ID NO: 74 (20 to 120 residues flanking amino acid 375 of chick Cx45)
GFGTIRDTLNNKRKELEDSGTYNYPFTWNTPSAPPGYNIAVKPDQMQYTELSNAK
MAYKQNKANIAQEQQYGSNEENIPADLENLQREIKVAQERLDMAIQAYNNQNNPG
SSSREKKSKA.

SEQ ID NO: 75 (20 to 120 residues flanking amino acid 313 of human Cx37)
PYLVDCFVSRPTEKTIFIIFMLVVGLISLVLNLLELVHLLCRCLSRGMRARQGQDAPP
TQGTSSDPYTDQVFFYLPVGQGPSSPPCPTYNGLSSSEQNWANLTTEERLASSR
PPLFLDPP

SEQ ID NO: 76 (20 to 120 residues flanking amino acid 258 of rat Cx33)
CGSKEHGNRKMRGRLLLTYMASIFFKSVFEVAFLLIQWYLYGFTLSAVYICEQSPC
PHRVDCFLSRPTEKTIFILFMLVVSMVSFVLNVIELFYVLFKAIKNHLGNEKEEVYCN
PVELQK.

SEQ ID NO: 77 (enhanced green fluorescent protein)
MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPV
PWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTR
AEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFK
IRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLE
FVTAAGITLGMDELYK

SEQ ID NO:78 (ACT 2)
CCCTCCTCCCGGGCCTCCTCCCGGGCCTCCTCCCGGCCCCGGCCCGACGAC
CTGGAGATC

SEQ ID NO:79 (ACT 1)
CGGCCCCGGCCCGACGACCTGGAGATC

SEQ ID NO:80 (ACT 3)
CGGCCCCGGCCCGACGACCTGGAGGTG

SEQ ID NO:81 (ACT 4)
CGGCCCCGGCCCGACGACGTGCCCGTG

SEQ ID NO:82 (ACT 5)
AAGGCCCGGTCCGACGACCTGTCCGTG

SEQ ID NO:83 (Antp)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAG AAG

SEQ ID NO:84 (Antp/ ACT 2)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCCCT
CCTCCCGGGCCTCCTCCCGGGCCTCCTCCCGGCCCCGGCCCGACGACCTGG
AGATC

SEQ ID NO:85 (Antp/ ACT 1)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGG
CCCCGGCCCGACGACCTGGAGATC

SEQ ID NO:86 (Antp/ ACT 3)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGG
CCCCGGCCCGACGACCTGGAGGTG

SEQ ID NO:87 (Antp/ ACT 4)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGCGG
CCCCGGCCCGACGACGTGCCCGTG

SEQ ID NO:88 (Antp/ ACT 5)
CGGCAGCCCAAGATCTGGTTCCCCAACCGGCGGAAGCCCTGGAAGAAGAAGG
CCCGGTCCGACGACCTGTCCGTG

SEQ ID NO:89 (Zebrafish alpha Cx43)
PCSRASSRMSSRARPDDLDV

SEQ ID NO:90 (Chick alpha Cx36)
PRVSVPNFGRTQSSDSAYV

SEQ ID NO:91 (Zebrafish alpha Cx36)
PRMSMPNFGRTQSSDSAYV

SEQ ID NO:92 (Cx43 isoleucine deletion)
RQPKIWFPNRRKPWKKRASSRASSRPRPDDLE

EXAMPLES

Example 1

[0092] The objective was to develop a stable, elegant and pourable gel in which the Active Pharmaceutical Ingredient (API) is in solution for wound healing purposes.

[0093] The major challenges encountered during formulation development were the selection of suitable stabilizers for the API (ACT1), and other excipients that are non-irritating for wound healing application. Studies were conducted to evaluate prototype formulations.

[0094] R&D Study per Protocol #1062

[0095] The first study was conducted per protocol #1062. During this study, 6 prototype formulations were evaluated as outlined in Table 4. The purposes of the study were to select a proper pH range for the product and also to select a proper gelling agent. The API concentration used for this study was 0.072% which was the initial targeted product concentration. Phosphate buffers at pH 5, 6, and 7 and two gelling agents, Pluronic and Hydroxyethylcellulose (HEC) were evaluated. The quantity of API required for the formulations was calculated as follows:

[0096] Batch size for each formulation = 12 × 5g = 60 g or 70 g

[0097] API quantity for 6 formulations = 6 × 70 × 0.072% / 82.4% = 0.37 g

[0098] API purity value = 82.4%

[0099] Five formulations, formulae 2414-10 to -14, were gelled with Pluronic 127, which was assumed to be the preferred gelling agent. Only one formulation, 2414-15, was gelled with HEC as a back-up. The available stability data indicated the following conclusions.

[00100] Buffer was needed to control the pH of the gel system and to maintain stability of the peptide drug. Proper pH range seemed to be between 5 and 7. Phosphate buffer was adequate.

[00101] Surprisingly, the API, ACT1, was more stable in HEC gel than Pluronic gels. With Pluronic gels, the API dropped to an average of 85% of the label claim (LC) from initial when gels were stored at 5° C for one month; and to 79% when stored at 25°C for one month. With HEC gel, the API dropped to 98% of label claim from initial when stored at 5°C for 3 months and 84% when stored at 25°C for three months.

[00102] HEC appeared to be a better choice of gelling agent than Pluronic when comparing formula 2414-14 to 2414-15. Both formulations were exactly same except that formula 2414-14 was gelled with Pluronic and formula 2414-15 was gelled with HEC. The API in formula 2414-14 dropped to 68% of the label claim from the initial concentration when stored at 5°C for one month while the API in formula 2414-15 only dropped to 98% of the label claim from the initial concentration when stored at 5°C for 3 months.

[00103] EDTA and Mannitol appear to provide some stability to the peptide drug.

[00104] The formulations did not appear to be stable at 25°C. The final product might require refrigeration, which is typical for products containing peptides and proteins.

[00105] Formula 2414-15 with HEC as the gelling agent is the most stable formulation compared to the rest of the formulations tested. It was selected for further development. Stability data of formulations 2414-14 and 2414-15 are summarized in Tables 5 and 6, respectively.

Table 4. Wound Healing Gel Formulation Compositions for Stability Testing per Study #1062

Components (% W/W)	**Formula #: 2414-10**	**Formula #: 2414-11**	**Formula #: 2414-12**	**Formula #: 2414-13**	**Formula #: 2414-14**	**Formula #: 2414-15**
API	0.072	0.072	0.072	0.072	0.072	0.072
Propylene glycol	1.0	1.0	1.0	1.0	1.0	1.0
Glycerin	5.0	5.0	5.0	5.0	5.0	5.0
Monobasic Sodium Phosphate	-	0.300	0.263	0.120	0.263	0.263
Dibasic Sodium Phosphate	-	0.0036	0.044	0.217	0.044	0.044
Methylparaben	0.17	0.17	0.17	0.17	0.17	0.17
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02
Pluronic 127 (Poloxmer 407)	20.0	20.0	20.0	20.0	20.0	-
EDTA Disodium	-	-	-	-	0.05	0.05
Mannitol	-	-	-	-	0.05	0.05
Hydroxyethylcellulose 250HHX	-	-	-	-	-	1.25
Purified water	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100	q.s. 100
	No Buffer	Buffer pH 5	Buffer pH 6	Buffer pH 7	Buffer pH 6	Buffer pH 6

Table 5. Stability Results of Formula 2414-14 Containing Pluronic 127 per Study #1062

Storage Condition	Time Point (Month)	Physical Properties and Appearance	Chemical Assay (%, LC)		
			API	Methylparaben	Propylparaben
	Initial	Clear and colorless gel	98.8	92.0	96.6
Freeze/Thaw		Conforms	89.8	88.6	94.0
5° C	1	Conforms	84.6	93.0	97.9
25° C	1/2	Conforms	90.6	93.6	98.6
	1	Conforms	82.8	88.8	94.0

Table 6. Stability Results of Formula 2414-15 Containing HEC per Study #1062

Storage Condition	Time Point (Month)	Physical Properties and Appearance	Chemical Assay (%, LC)		
			API	Methylparaben	Propylparaben
	Initial	Clear colorless gel	101.9	97	103.1
Freeze/Thaw		Conforms	98.8	97.1	103.9
5°C	1	Conforms	96	98.1	104.6
	3	Conforms	99.7	98.3	101.8
25°C	½	Conforms	91.6	99.7	106.7
	1	Conforms	93.4	96.8	103
	3	Conforms	85.7	92.8	96.5

Example 2

[00106] R&D Study per Protocol #1074

[00107] Based on the results from study #1062, additional prototype formulations based on formula 2414-15 were designed and evaluated per protocol #1074. During this study, 7 prototype formulations were prepared. The purposes of the study were to select a proper buffering system and antioxidants for the formulation. Five (5) antioxidants were evaluated: Mannitol, Methionine, Acetylcysteine, Lysine and Histidine. Two buffer systems were used: phosphate buffer at pH 6 and citrate buffer at pH 5. Glycerin (5%) was introduced into the new prototype formulations to provide some humectant property to the wound treatment topical gel. PEG 200 was also evaluated for its potential stabilizer effect. The compositions of the prototypes evaluated per study #1074 are listed in Table 7.

Table 7. Composition of Prototype Formulations per Study #1074

Component (%w/w)	Formula # 2414-						
	18	19	20	21	22	23	26
Peptide 328967 (ACT1)	0.036	0.036	0.036	0.036	0.036	0.036	0.036
Propylene Glycol	1.0	1.0	1.0	1.0	1.0	-	-
PEG 200	-	-	-	-	-	5.0	5.0
Glycerin	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Monobasic Sodium Phosphate	0.263	0.263	0.263	0.263	0.526	-	-
Dibasic Sodium Phosphate	0.044	0.044	0.044	0.044	0.088	-	-
Citric Acid, anhydrous	-	-	-	-	-	0.60	0.60
Sodium Citrate, anhydrous	-	-	-	-	-	1.73	1.73
Methylparaben	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Propylparaben	0.02	0.02	0.02	0.02	0.02	0.02	0.02
EDTA disodium	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Mannitol	0.05	-	-	-	-	0.05	-
Methionine	-	0.5	0.5	0.5	0.5	-	-
Acetylcysteine	-	-	-	-	0.2	-	-
Lysine	-	-	0.1	-	-	-	0.1
Histidine	-	-	-	0.1	-	-	-
Hydroxyethylcellulose 250 HHX	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Purified Water, qsad	100	100	100	100	100	100	100
	25 mM Buffer	25 mM Buffer	25 mM Buffer	25 mM Buffer	50 mM Buffer	100 mM Buffer	100 mM

	pH 6	pH 6	pH 6	pH 6	pH 6	pH 5	Buffer pH 5

[00108] Due to API availability limitations, 0.036% of API concentration was used for this study. Formula 2414-18 is identical to 2414-15 except that the API concentration was reduced from 0.072% to 0.036%. The available stability data from study #1074 found in Table 8 indicated the following conclusions.

[00109] Phosphate buffer seemed to provide better stability to the peptide when compared to the citrate buffer. The buffer capacity at 25 mM is adequate for the system.

[00110] Mannitol seemed to provide adequate stability to the peptide.

[00111] The effect of other antioxidants was not clear due to the variability in the chemical data.

[00112] Based on the study results mainly from study #1062 and partially from #1074, a final formulation was selected for further development. The final formulation contained 25 mM phosphate buffer to maintain the pH of the formulation, mannitol as a stabilizer for the API, and HEC as a gelling agent. The amount of propylene glycol was increased from 1% to 3% in prototype formulations to allow for better solubilization of the parabens in the aqueous system. Four potential API concentrations were selected: 0.0072%, 0.018%, 0.036% and 0.072%. The final formula is summarized in Table 9. The stability of the final selected formula containing different levels of API was also evaluated with analytical methods. In addition, the overall stability was assessed by visual inspection for white powder or precipitate and determination of water content.

Table 8. Stability Results of Formulas 2414-18, 2414-19, 2414-20, 2414-21, 2414-22, 2414-23, and 2414-26 from Study #1074

Sample	Storage Condition	Time Point (Month)	Physical Properties	Chemical Assay (%, LC)		
			Appearance	API	Methylparaben	Propylparaben
Formula 2414-18		Initial	Clear and Colorless Gel	106.4	94.6	102.2
	5° C	1	Conforms	90.1	83.4	88.2
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	94.3	90.4	95.5
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
Formula 2414-19		Initial	Clear and Colorless Gel	109.3	94.2	119.7
	5° C	1	Conforms	101.1	90.4	112.6
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	103.3	94.6	117.4
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
Formula 2414-20		Initial	Clear and Colorless Gel	107.0	96.9	99.9
	5° C	1	Conforms	106.8	96.7	98.4
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	97.9	94.2	95.6
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA

Formula 2414-21		Initial	Clear and Colorless Gel	104.3	95.1	110.4
	5° C	1	Conforms	96.7	91.5	104.2
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	96.4	95.0	108.4
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
Formula 2414-22		Initial	Clear and Colorless Gel	112.2	96.1	109.4
	5° C	1	Conforms	111.4	94.9	106.1
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	96.5	89.5	100.0
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
Formula 2414-23		Initial	Clear and Colorless Gel	87.8	94.7	101.6
	5° C	1	Conforms	88.3	92.9	97.0
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
	25° C	1	Conforms	74.4	84.4	88.4
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA
Formula 2414-26		Initial	Clear and Colorless Gel	83.0	95.5	105.9
	5° C	1	Conforms	78.5	91.0	98.5
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA

	25° C	1	Conforms	77.4	93.3	100.7
		2	Conforms	NA	NA	NA
		3	Conforms[1]	NA	NA	NA

[1] Particles at the bottom of vial; NA = data not available

[00113] In one embodiment, a batch of the drug product may be prepared by following the steps of the manufacturing process flow chart and using the composition of Table 9.

[00114] Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods and materials are described herein. All publications cited herein are incorporated herein by reference for the purpose of disclosing and describing specific aspects of the invention for which the publication is cited.

[00115] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

[00116] While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

Table 9. Wound Healing Gel Qualitative and Quantitative Composition

Ingredients	Grade	Function	Concentration (% w/w)	Grams/kg Batch
Peptide 328967 (ACT1)	--	Active	0.0072; 0.018 0.036; 0.072	0.072; 0.18 0.36; 0.72
Methylparaben	NF	Preservative	0.17	1.7
Propylparaben	NF	Preservative	0.02	0.2
Glycerin	USP	Solvent	5	50
Sodium Phosphate Monobasic	USP	Buffer Agent	0.263	2.63
Sodium Phosphate Dibasic	USP	Buffer Agent	0.044	0.44
Propylene Glycol	USP	Solvent	3	30
Edetate Disodium (EDTA)	USP	Chelating Agent	0.05	0.5
D-Mannitol	USP	Stabilizer	0.05	0.5
Hydroxyethylcellulose, 250HHX	NF	Gelling Agent	1.25	12.5
Purified Water, qsad	USP	Solvent	1	1 kg

CLAIMS

1. A topical formulation comprising at least one alpha connexin polypeptide and hydroxyethylcellulose gel, wherein the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide.

2. The topical formulation of claim 1, wherein the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide so that after 3 months of storage at 5°C at least 98% of the alpha connexin polypeptide is detectable by analytical methods.

3. The topical formulation of claim 1, wherein the hydroxyethylcellulose gel stabilizes the alpha connexin polypeptide so that after 3 months of storage at 5°C at least 95% of the alpha connexin polypeptide is detectable by analytical methods.

4. The topical formulation of claim 1, wherein the hydroxyethylcellulose is present at a concentration of about 1.25% (w/w).

5. The topical formulation of claim 1, wherein the at least one alpha connexin polypeptide is present at a concentration of between about 0.005% (w/w) and about 1.00% (w/w).

6. The topical formulation of claim 1 further comprising a buffering agent that maintains the pH of the topical formulation between about 5 and about 7.

7. The topical formulation of claim 6, wherein the buffering agent is a phosphate buffer.

8. The topical formulation of claim 1, wherein the at least one alpha connexin polypeptide comprises the carboxy-terminal most 4 to 30 contiguous amino acids of an alpha connexin protein or conservative variant thereof.

9. The topical formulation of claim 8, wherein said at least one alpha connexin polypeptide is linked at its amino terminus to a cellular internalization transporter.

10. The topical formulation of claim 9, wherein the cellular internalization transporter is an antennapedia sequence.

11. The topical formulation of claim 8, wherein the at least one alpha connexin polypeptidecomprises the amino acid sequence of SEQ ID NO: 9.

12. The topical formulation of claim 8, wherein the alpha connexin polypeptide is connexin 37, connexin 40, connexin 43, or connexin 45.

13. A method of wound treatment comprising administering the topical formulation of claim 1 to a subject in need thereof.

14. The method of claim 13, wherein the wound is an acute surgical wound.

15. The method of claim 13, wherein the wound is a chronic, non-infected, full-thickness lower extremity ulcer.

16. A method of manufacturing a topical formulation comprising:

a) mixing propylene glycol, glycerin, methylparaben and propylparaben until the parabens are completely dissolved;

b) separately mixing purified water, EDTA, monobasic sodium phosphate, dibasic sodium phosphate and D-mannitol until a clear solution is obtained;

c) adding the solution from a) to the solution from b), rinsing the container of the solution from a) with purified water, adding the rinse to the combined solutions, and mixing until the combined solutions are visually homogeneous;

d) with homogenization mixing, adding hydroxyethyl cellulose into the combined

solutions of c) and mixing until the polymer is fully dispersed;

e) separately mixing purified water with an alpha connexin polypeptide until the peptide is completely dissolved;

f) adding the solution from e) to the solution of d), rinsing the container of the solution from e) with purified water, adding the rinse to the combined solutions, and mixing until the combined solution are homogeneous.