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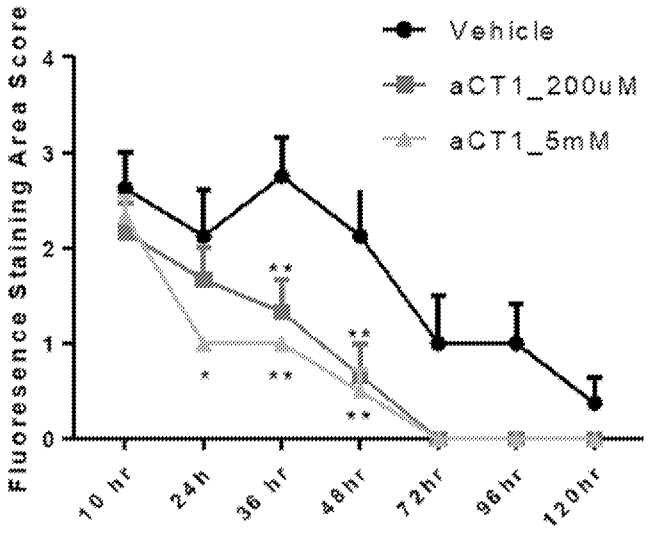
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(54) Title: PEPTIDE FORMULATIONS AND OPHTHALMIC USES THEREOF

FIG. 2A




(57) Abstract: This disclosure provides formulations suitable for topical ophthalmic delivery of peptide therapeutics. The disclosure also provides compositions and methods for treating ocular injury. The compositions and formulations may include therapeutically active alpha connexin peptides.

[Continued on next page]

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# PEPTIDE FORMULATIONS AND OPHTHALMIC USES THEREOF

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This reference claims the benefit of priority of U.S. Provisional Application No. 63/104,086, filed on October 22, 2020, the entire contents of which are hereby incorporated by reference.

## 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_012_01WO_SeqList.txt, date recorded: October 22, 2021, file size 34 kilobytes).

## BACKGROUND OF THE INVENTION

**[0003]** Corneal injuries and ocular trauma have the potential to instigate ocular morbidity, which can span in severity to include vision loss. Possible insults to the cornea are limitless, but significant efforts to address burn and blast injuries in combat soldiers along with the incidence of secondary corneal damage due to diseases, such as diabetes, exemplify the need for biotherapeutics that address the multifaceted and complex wound healing process of the eye. In order to maintain visual acuity, corneal injury treatment must promote rapid corneal reepithelization, mitigate injury progression/persistence, and, depending on the affected corneal cell types/tissue layers, also encourage regeneration of the other affected tissue layers. Significantly, if the corneal stroma is penetrated and damaged, the ocular treatment must allow for proper healing through the transformation of keratocytes to fibroblasts and myofibroblasts but preclude excessive actions by myofibroblasts that can cause corneal opacification and scarring. Importantly, inflammatory cell infiltrates also require calculated consideration as disproportionate inflammation can have detrimental effects. Suppressed immune actions can lead to infection, while excessive inflammation disrupts normal wound healing and regeneration. Therefore, an injury to the cornea, where distinct cellular layers and structural uniformity and composition of extracellular matrices are essential to proper corneal biomechanics and functionality, requires a biotherapeutic with specific biological effects on several different cell types present following tissue damage.

**[0004]** The current standard of care (SOC) for corneal injuries includes ocular irrigation, lubricants, artificial tears, antibiotics, bandage contact lenses, tarsorrhaphy, or construction of a conjunctival flap. These therapeutic approaches have two significant limitations. First, they do not address the fundamental biological and molecular processes in corneal wound healing, where therapeutic failure is associated with severe impairment or loss of vision. Second, as epitomized by corneal injury and trauma caused by explosive or incendiary devices in combat situations, these SOC treatments are either not possible or probable to occur in timely manner where medical facilities are limited and ocular wounds are treated secondarily.

**[0005]** In addition, there is a clear need for topical therapeutic formulations that have the characteristics necessary to provide safe and effective treatment of the sensitive tissues of the eye. In particular, the development of peptide containing formulations for ocular use presents unique challenges; the poor chemical and physical stability of peptides in solution limits formulation options. Therapeutics to be used for ophthalmic delivery must meet International Council on Harmonization (ICH) and United States Pharmacopeia (USP) guidelines governing formulation heterogeneity, stability, viscosity, and pH to ensure safety as well as effective delivery of the active pharmaceutical ingredient to the surface of the eye. Moreover, macromolecules such as proteins, antibodies, and small peptides exhibit poor bioavailability when delivered topically to the eye in traditional eye drop vehicles.

**[0006]** Thus, there is a significant need for eye drop biotherapeutics that expedite wound healing while mitigating the dysregulated biological processes that cause corneal opacity and vision loss. This disclosure addresses this and other needs.

## BRIEF SUMMARY OF THE INVENTION

**[0007]** In an aspect, the present disclosure provides formulations comprising one or more peptides, wherein the formulations are suitable for topical administration to the eye. For example, the provided formulations are eye drop formulations. In an aspect, the present disclosure provides formulations for use in treating corneal injuries.

**[0008]** In embodiments, the present disclosure provides a formulation comprising an active peptide having a molecular weight of about 1.0 kDa to about 10.0 kDa and hydroxypropyl methylcellulose (HPMC), wherein the formulation is suitable for topical ocular delivery. In embodiments, the HPMC is present in the formulation at a concentration of about 0.01% (w/w)

to about 2.0% (w/w), or at a concentration of about 0.1% (w/w) to about 0.19% (w/w). In embodiments, the HPMC is present in the formulation at a concentration of about 0.1% or about 0.2% or about 0.3% or about 0.5 % or about 1.0% (w/w). In embodiments, the formulation further comprises sodium chloride (NaCl). In embodiments, the NaCl is present at a concentration from about 0.5% to about 2.0%, or about 0.7% to about 1.5%. In embodiments, the NaCl is present at a concentration from about 0.25% to about 0.9%. In embodiments, the NaCl is present at a concentration of about 0.9% (w/w).

**[0009]** In embodiments, the formulation further comprises a tonicity modifier. For example, in embodiments, the formulation further comprises dextrose, glycerin, mannitol, potassium chloride, or magnesium chloride.

**[0010]** In embodiments, the active peptide is present in the composition at a concentration of about 0.005% (w/w) to about 5% (w/w), or about 0.035% (w/w) to about 3.5% (w/w). In embodiments, the active peptide is present in the composition at a concentration of about 0.035% (w/w) to about 3.0% (w/w). In embodiments, the active peptide is present in the composition at a concentration of about 0.05% (w/w) to about 2.5 % (w/w). In embodiments, the active peptide is present in the composition at a concentration of about 0.1% (w/w) to about 2.0 % (w/w). In embodiments, the active peptide is present in the composition at a concentration of about 0.5% (w/w) to about 1.5 % (w/w). In embodiments, the formulation has a viscosity between about 18 mPaS and about 28 mPaS. In embodiments, the formulation has a viscosity of about 18 mPaS, about 19 mPaS, about 20 mPaS, about 21 mPaS, about 22 mPaS, about 23 mPaS, about 24 mPaS, about 25 mPaS, about 26 mPaS, about 27 mPaS, or about 28 mPaS. In embodiments, the formulation has a pH of about 5 to about 8, or about 5 to about 7, or about 5, about 6, about 7, or about 8. In embodiments, the formulation has a pH of about 6.5. In embodiments, the formulation has a pH of between about 6.5 and about 7.5. In embodiments, the formulation has a pH of between about 6.5 and about 7.0. In embodiments, the formulation has an osmolality of about 200 to about 350 mOsm/kg, e.g., about 280 to about 350 mOsm/kg, e.g., about 288 mOsm/kg. In embodiments, the formulation has a density of about 0.5 g/mL to about 2.0 g/mL. In embodiments, the formulation has a density of about 0.5 g/mL, about 0.6 g/mL, about 0.7 g/mL, about 0.8 g/mL, about 0.9 g/mL, about 1.0 g/mL, about 1.1 g/mL, about 1.2 g/mL, about 1.3 g/mL, about 1.4 g/mL, about 1.5 g/mL, about 1.6 g/mL,

about 1.7 g/mL, about 1.8 g/mL, about 1.9 g/mL, or about 2.0 g/mL. For example, in embodiments, the formulation has a density of about 0.99 g/mL.

**[0011]** In embodiments, the active agent in the formulations provided herein is an alpha connexin peptide, or an active fragment thereof. For example, in embodiments, the polypeptide comprises the carboxy terminal-most 4 to 30 contiguous amino acids of the alpha Connexin. In embodiments, the polypeptide consists of the carboxy terminal-most 4 to 30 contiguous amino acids of an alpha connexin. In embodiments, the alpha Connexin is Connexin 37, Connexin 40, Connexin 43, or Connexin 45. In embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5. In embodiments, the polypeptide comprises the amino sequence of SEQ ID NO: 2. In embodiments, the polypeptide further comprises a cellular internalization sequence. In embodiments, the cellular internalization sequence comprises an amino acid sequence of a protein selected from a group consisting of Antennapedia, TAT, HIV-Tat, Penetratin, Antp-3A (Antp mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB 1, Pep-7, HN-1, BGSC (Bis-Guanidinium-Spermidine-Cholesterol) and BGTC (Bis-Guanidinium-Tren-Cholesterol). In embodiments, the cellular internalization sequence is Antennapedia, and wherein the sequence comprises the amino acid sequence of SEQ ID NO: 7. In embodiments, the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, and SEQ ID NO: 12. In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO: 9.

**[0012]** In embodiments, the formulations provided herein are suitable for topical ocular administration. In embodiments, the administration is via eye drop administration.

**[0013]** In embodiments, the present disclosure provides methods of treating or preventing an ocular injury in a subject in need thereof, comprising topically administering a formulation provided herein. In embodiments, the present disclosure provides formulations and methods for accelerating corneal reepithelialization following an ocular injury in a subject, the method comprising topically administering a formulation provided herein to the eye of the subject. In embodiments, the formulation is administered to the eye immediately after the event that caused the ocular injury. In embodiments, the polypeptide is administered to the subject within about 1 hour, within about 2 hours, within about 5 hours, or within about 12 hours of the event

that caused the ocular injury. In embodiments, the polypeptide is administered to the subject at least about 2 hours following the event that caused the ocular injury. In embodiments, the polypeptide is administered to the eye of the subject twice per day, or about every 8 hours, or about every 12 hours, until ocular healing is observed. In embodiments, the ocular injury is a corneal injury. In embodiments, the ocular injury is a retinal injury. In embodiments, the ocular injury is caused by a burn, explosion, or laceration. In embodiments, the ocular injury is a chemical or thermal burn injury. In embodiments, the ocular injury is caused by contact of the eye with a vesicating agent, such as mustard gas or the like. In embodiments, the ocular injury is caused by a chronic disease. In embodiments, the chronic disease is diabetes or diabetic keratopathy. In embodiments, the chronic disease is retinal disease. In embodiments, the subject has dry eye disease. In embodiments, the subject has a persistent corneal epithelial defect, such as may be caused by dry eye disease. In embodiments, the injury is secondary to an ocular surgery, a chemical or thermal burn injury, or a corneal laceration injury.

**[0014]** In embodiments, the present disclosure provides formulations for use in treating or preventing an ocular injury in a subject in need thereof, and/or formulations for use in accelerating corneal reepithelialization following an ocular injury in a subject.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0015]** FIG. 1 shows corneal staining using 1% fluorescein at 10, 24, 36, 48, 72, 96, or 120 hrs following corneal chemical burn, and with eye drop administration of aCT1 (200 μM or 5 mM) or vehicle control twice daily for two days following chemical injury.

**[0016]** FIG. 2A is a bar graph showing the quantified fluorescence staining of FIG. 1 (n=6 per treatment group; *$p<0.05$, **$p<0.01$ ***$p<0.001$; SEM).

**[0017]** FIG. 2B shows central corneal thickness (μm) in rabbit eyes pre-dose and at day 1, day 2, and day 3 following corneal chemical burn, with eye drop administration of aCT1 peptide (200 μM or 5 mM) or vehicle control.

**[0018]** FIG. 3 shows 1% fluorescein staining of rabbit eyes after bilateral central transepithelial phototherapeutic keratectomy (PTK) surgery and treatment with aCT1 peptide (150 μM) or vehicle control.

**[0019]** FIGS. 4A-4B show that treatment with aCT1 peptide decreases corneal thickening that occurs following corneal exposure to nitrogen mustard (NM). FIG. 4A shows corneal

thickness and FIG. 4B provides quantification of the same in the indicated groups. n = 3 per treatment group.

**[0020]** FIGS. 5A-5D show that treatment with aCT1 peptide decreases inflammatory responses in nitrogen mustard (NM)-exposed cornea. FIG. 5A shows H&E staining and FIG. 5B shows inflammatory cell infiltration was significantly reduced in aCT1 treated groups. FIG. 5C shows pro-inflammatory enzyme COX2 staining and FIG. 5D provides quantification of COX2 in corneal tissues. (n = 3 per treatment group; one- way ANOVA, **$p<0.01$; ***$p<0.001$; SD).

**[0021]** FIGS. 6A-6D show that aCT1 treatment of NM-exposed corneas may protect corneal fibroblasts and keratocytes. FIG. 6A provides H&E staining and FIG. 6B shows the corneal fibroblasts cell counts in treated corneas in the indicated groups. FIG. 6C shows IHC staining for matrix metalloproteinase-9 (MMP-9), which leads to degradation of the corneal stroma. FIG. 5D provides quantification of MMP-9 positivity in the corneal stroma. n= 3 per treatment group.

**[0022]** FIGS. 7A-7D show that treatment with aCT1 peptide reduces corneal neovascularization in NM-exposed corneas. FIG. 7A shows blood vessels in the cornea in each group by H&E staining and FIG. 7B provides a quantification of blood vessel count in each group. Vascular endothelial growth factor (VEGF) is a signaling protein that stimulates neovascularization. FIG. 7C shows staining for VEGF and FIG. 7D provides quantification of the VEGF positivity score. n= 3 per treatment group.

## DETAILED DESCRIPTION

**[0023]** Provided herein are formulations for topical delivery of peptide compositions to the eye, and methods for treating or preventing eye disorders and conditions, such as corneal injuries.

**[0024]** Therapeutics to be used for ophthalmic delivery must meet ICH and USP guidelines governing formulation heterogeneity, stability, viscosity, and pH to ensure safety as well as effective delivery of the active pharmaceutical ingredient to the relevant tissues of the eye. The development of peptide containing formulations for ocular use presents unique challenges including the poor chemical and physical stability of peptides in solution, particularly in the type of solution that provides sufficient stability and viscosity for topical administration to the

eye. Failure to develop peptide containing formulations that exhibit sufficient bioavailability for treatment of ocular disorders is a likely explanation for the lack of peptide based ocular therapeutics that have obtained FDA approval. Few peptide containing ocular formulations have been FDA approved. For those that are approved, the route of administration for these peptide containing formulations is intravitreal injection (Mandal et al. 2018), instead of the safer and less invasive topical route of administration.

**[0025]** A viscoelastic polymer such as hydroxypropyl methylcellulose (HPMC) has not been used in combination with a peptide in a formulation appropriate for eye drop delivery. A formulation with appropriate viscosity, surface tension, and other physical properties is necessary for an eye drop to achieve sufficient contact time with the ocular surface necessary to ensure peptide delivery. Small peptides are expected to exhibit poor solubility in conventional excipients employed to modify eye drop viscosity such as HPMC, carboxymethylcellulose (CMC), hydroxyethyl cellulose (HEC), and PF-127. Peptide aggregation in combination with these ingredients results in precipitate formation that makes the formulation unsuitable for ocular delivery. Thus, conventional teaching in the art is away from a formulation which utilizes a viscoelastic polymer such as HPMC in combination with a small peptide active pharmaceutical ingredient. Instead, current formulations for delivery of small peptide therapeutics involve admixture of non-reducing sugars, amino acids, and surfactants with the peptide or other macromolecules to achieve formulations suitable for ocular delivery (Giannos et al. 2018, Kamerzell et al. 2011).

**[0026]** The present inventors unexpectedly discovered that a combination of a viscoelastic polymer with a small peptide active ingredient resulted in a formulation that maintains peptide solubility and with a kinematic viscosity appropriate for topical delivery of the peptide to the ocular surface. The formulation surprisingly achieves a stable solution state of the peptide in a formulation for eye drop delivery. Peptide stability in solution is an important performance characteristic differentiating the present invention from conventional peptide delivery systems. Due to poor solubility, peptides are known to precipitate out in conventional eye drop delivery systems. It is well known that solutions with high viscosity cannot be filter sterilized; conventional formulations for ocular delivery of macromolecules have used aqueous excipients that do not include viscoelastic polymers to achieve a solution with low viscosity that can be

sterilized by passage through a sterile filter. Thus, there are no examples of HPMC admixed with therapeutic peptides to achieve a formulation suitable for topical delivery to the eye.

**[0027]** However, the present inventors surprisingly achieved a stable formulation comprising aCT1 peptide and HPMC, that was suitable for topical delivery to the eye and effective in treatment of ocular disorders. This formulation was unpredictably superior to formulations comprising CMC, HEC, or pluronic gel (PF-127) instead of HPMC. Unexpectedly, the use of the viscoelastic polymer HPMC with the peptide yielded a formulation having a viscosity sufficient to enable contact time necessary for peptide delivery to the ocular surface, yet that can be sterilized through passage of the solution through a 0.22 μM PVDF or PES membrane filter. Formulation sterility is necessary for delivery of therapeutic peptides to sensitive tissues such as the eye. Passage of formulations through a 0.22 μM PVDF or PES membrane filter produces a formulation with sterility suitable for the delivery of medication to sensitive ocular tissues.

**[0028]** In embodiments, the formulation further comprises sodium chloride (NaCl), potassium chloride (KCl), sodium iodide (NaI), magnesium chloride ($MgCl_2$), potassium fluoride (KF), calcium chloride ($CaCl_2$), sodium tetrafluoroborate ($NaBF_4$), and/or sodium bromide (NaBr). In embodiments, the formulation comprises NaCl. In embodiments, the NaCl surprisingly provides greater stability relative to a formulation that does not comprise NaCl. In embodiments, the NaCl is present at a concentration from about 0.5% to about 2.0%, or about 0.7% to about 1.5%. In embodiments, the NaCl is present at a concentration from about 0.25% to about 0.9%. In embodiments, the NaCl is present at a concentration of about 0.9% (w/w).

**[0029]** In embodiments, the formulations provided herein exhibit stability over time at a range of temperatures. For example, the formulations provide peptide stability for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least two months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 8 months, at least 12 months, at least 18 months, at least 2 years, at least 3 years, or at least 6 years. In embodiments, the formulations provide peptide stability at about -20°, about 5°C, about 25°C, and any temperature therebetween. In certain embodiments, the formulations provide peptide stability at about -20° for at least 6 months, at least 8 months, at least 12 months, at least 18 months, at least 2 years, at least 3 years, at least 4 years, at least 5 years, or at least 6 years. In embodiments, the formulations provided herein comprise a peptide (e.g.,

an alpha connexin peptide), wherein the peptide remains at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, or at least about 95% stable over at least about 1 month. In embodiments, the formulations provided herein comprise a peptide (e.g., an alpha connexin peptide), wherein the peptide remains at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, or at least about 95% stable over at least about 3 months. In embodiments, the formulations provided herein comprise a peptide (e.g., an alpha connexin peptide), wherein the peptide remains at least about 80%, at least about 81%, at least about 82%, at least about 83%, at least about 84%, at least about 85%, at least about 86%, at least about 87%, at least about 88%, at least about 89%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, or at least about 95% stable over at least about 1 year, at least about 2 years, at least about 3 years, at least about 4 years, at least about 5 years, or at least about 6 years. Such stability is achieved at about -20°, about 5°C, about 25°C, and any temperature therebetween when the formulations provided herein are utilized.

**[0030]** In embodiments, the formulations provided herein exhibit no impurities or negligible impurities or an acceptable level of impurities over time at a range of storage temperatures. For example, the formulations exhibit no impurities or negligible impurities or an acceptable level of impurities for at least 1 week, at least 2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least two months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 1 year, at least 2 years, at least 3 years, at least 4 years, at least 5 years, or at least 6 years. In embodiments, the formulations exhibit no impurities or negligible impurities or an acceptable level of impurities at about -20°, about 5°C, about 25°C, and any temperature therebetween. In embodiments, negligible levels of impurities in the formulation may be less than 0.1%. In embodiments, acceptable levels of impurities in the formulation may be less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.5%.

**[0031]** In embodiments, the formulations provided herein are readily filterable (e.g., filterable through a 0.2 μm PES filter). In embodiments, the formulations provided herein are more filterable compared to formulations previously used for ocular administration of peptides. In embodiments, the formulations provided herein comprising HPLC, a peptide (e.g., an alpha connexin peptide), NaCl, and does not require an additional vehicle, buffer, or excipient, to have formulation properties (e.g., viscosity, osmolality, density, pH, filterability) as well as purity and stability profiles suitable for ocular delivery.

**[0032]** In embodiments, the present disclosure provides an eye drop carrier containing a therapeutic peptide (e.g., aCT1 peptide) that is non-irritating, stable, and of appropriate characteristics for topical use in the eye. Thus, in embodiments, the present disclosure provides therapeutic eye drop compositions comprising alpha connexin polypeptides for the treatment of ocular injury or disease. In embodiments, the eye drop formulation further comprises HPMC. In embodiments, the formulation further comprises a buffer and/or excipient which stabilizes the alpha connexin polypeptide during storage. In embodiments, the alpha connexin polypeptide comprises a carboxyl terminal amino acid sequence of alpha connexin. The alpha connexin polypeptides of the present invention may comprise, consist, or include the carboxy-terminal most 4 to 30 contiguous amino acids of an alpha connexin protein or conservative variant thereof. In embodiments, the said at least one alpha connexin polypeptide is linked at its amino terminus to a cellular internalization transporter.

**[0033]** In embodiments, the present disclosure provides a formulation of a stable eye drop carrier that contains aCT1 for therapeutic application in ophthalmic indications, and methods for making the same. In embodiments, the ophthalmic indications include wound healing, inflammatory and immune modulation, tissue regeneration, biomechanical restoration, or treatment of other physiological conditions affecting any part of the cornea or other ocular tissue. The formulations provided herein may be administered to treat acute and chronic injuries and wounds, including military or civilian chemical injuries or corneal lacerations, surgery-related conditions, and acute and chronic manifestations of any primary ocular disorder or other condition causing a secondary ocular condition manifesting or necessitating medical attention. The formulations possess physicochemical, biochemical, and rheological properties that enable its ability to provide a therapeutic and effective amount of aCT1 peptide when applied to injuries, wounds, and conditions affecting proper eye function.

**[0034]** In embodiments, one or more buffering agents in any form added to sterile water may be used to maintain a physiologically relevant pH or to maintain a pH where the addition of pH modulators will result in a physiological-relevant pH. In some embodiments, one or more pH modulators such as sodium borate, citric acid, sodium nitrate, histidine, hydrochloric acid or sodium hydroxide may be added to adjust within the desire therapeutic range of pH 5 to 8. Preferably, buffering agents are non-irritating, non-staining, and non-immunogenic. In embodiments, a preferred buffer is histidine. In embodiments, the histidine is present at a concentration of about 20 mM to about 80 mM. In embodiments, the histidine is present at a concentration of about 40 mM.

**[0035]** In embodiments, the formulation further comprises a tonicity modifier. For example, in embodiments, the formulation further comprises dextrose, glycerin, mannitol, potassium chloride, or magnesium chloride. In embodiments, the formulation further comprises an antioxidant, such as methionine.

**[0036]** In embodiments, the formulations provided herein do not include a buffering agent. In embodiments, additional excipients are excluded from the formulation, such that the formulation does not comprise an excipient. In embodiments, the formulation comprises the active agent peptide, HPMC, and no added excipients. In embodiments, the formulations provided herein do not include any added sugars, amino acids, and/or surfactants. In embodiments, the formulation comprises, consists essentially of, or consists of the active agent (e.g., a connexin peptide), HPMC, NaCl, and water. In embodiments, the HPMC is present in the formulation at a concentration of about 0.2% w/w to about 1.0% w/w. In embodiments, the HPMC is present in the formulation at a concentration of about 0.5% w/w. In embodiments, the HPMC is present in the formulation at a concentration of about 1.0% w/w.

**[0037]** In embodiments, one or more polymers such as HPMC is included in the formulation to stabilize the isolated polypeptide. Preferably, the formulation comprises a stabilizer that is non-irritating, non-staining, and non-immunogenic. The addition of stabilizers enable 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 (e.g., 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 (e.g., at about 0% relative humidity, at about 10% relative humidity, at about 20% relative humidity, at about

30% relative humidity, at about 40% relative humidity, at about 50% relative humidity, at about 60% relative humidity, at about 70% relative humidity, at about 80% relative humidity, at about 90% relative humidity, or at about 100% relative humidity). In embodiments, the present invention may also include a preservative to further maintain the described long-term storage under the stated variety of temperatures and relative humidities.

**[0038]** Exemplary formulations are provided below in Table 1.

| Form No | peptide | pH | His (mM) | NaCl (mM) | MgCl2 (mM) | mannitol (mM) | Met (mM) | HPMC (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 6.5 | 30 | 130 | 0 | 0 | 0 | 0.19 |
| 2 | 20 | 6.0 | 20 | 65 | 50 | 0 | 10 | 0.15 |
| 3 | 20 | 5.5 | 10 | 65 | 0 | 150 | 0 | 0.11 |
| 4 | 20 | 6.0 | 40 | 100 | 25 | 0 | 0 | 0 |
| 5 | 10 | 6.5 | 40 | 65 | 50 | 0 | 5 | 0.19 |
| 6 | 10 | 6.0 | 30 | 130 | 0 | 0 | 0 | 0.15 |
| 7 | 10 | 6.0 | 20 | 100 | 25 | 0 | 0 | 0.11 |
| 8 | 10 | 7.0 | 10 | 65 | 0 | 150 | 20 | 0 |
| 9 | 20 | 7.0 | 30 | 130 | 0 | 0 | 0 | 0.19 |
| 10 | 20 | 6.5 | 20 | 65 | 0 | 150 | 0 | 0.15 |
| 11 | 20 | 7.0 | 20 | 0 | 0 | 270 | 10 | 0.11 |
| 12 | 20 | 6.0 | 20 | 130 | 0 | 0 | 0 | 0 |
| 13 | 5 | 5.5 | 20 | 0 | 0 | 270 | 0 | 0.15 |
| 14 | 5 | 6.0 | 10 | 130 | 0 | 0 | 10 | 0 |
| 15 | 20 | 6.6 | 0 | 150 | 0 | 0 | 0 | 0.5 |
| 16 | 20 | 5.0 | 0 | 150 | 0 | 0 | 0 | 0.5 |

**[0039]** In embodiments, the formulations provided herein may be contained in plastic eye dropper or glass vial containing a single dose or multiple doses for therapeutic administration to a subject in need thereof a topical ophthalmic formulation comprising of at least one aCT polypeptide. In embodiments, the formulation may be contained in a glass container, and may be more stable in glass containers compared to containers made of other materials (e.g., plastic). In embodiments, the formulation may be contained in a plastic container, e.g., a plastic eye dropper. In embodiments, the topical ophthalmic formulation comprises HPMC. In embodiments, the formulations provided herein are in a sterile, ready-to-use eye drop formulation in an administration-appropriate and -designed eye dropper bottle or vial.

**[0040]** In embodiments, the present disclosure provides methods for treating and preventing corneal injuries and ocular trauma. In embodiments, the methods include topical administration to the eye of a formulation provided herein comprising an alpha connexin

polypeptide. In embodiments, the injury or trauma is a closed globe ocular injury or wound where damage to the cornea has occurred. The cause of the corneal injury or wound is not limited to and may include blast injuries, chemical and thermal burns, and other insults or conditions causing acute or chronic injury, as either a primary and secondary manifestation of a disorder or disease. In embodiments, the cause of the corneal injury is exposure to a vesicant, or blister agent, such as nitrogen mustard or sulfur mustard (e.g., mustard gas). In embodiments, the disorder or disease is diabetes. In embodiments, the disorder or disease is diabetic keratopathy. In embodiments, the chronic disease is retinal disease. In embodiments, present disclosure provides methods for treating and preventing retinal diseases. For example, in embodiments, the retinal disease is selected from macular degeneration (e.g., age-related macular degeneration (AMD), neovascular age-related macular degeneration (nAMD)), retinitis pigmentosa (RP), retinal detachment, diabetic retinopathy, macular edema, diabetic macular edema (DME), and macular edema occurring after retinal vein occlusion (RVO). In embodiments, the disease or disorder involves corneal defects that occur in a subject when treatment for an ocular disease or disorder (e.g., a retinal disorder) involves vitrectomy and/or one or more intravitreal injections.

**[0041]** In embodiments, the methods provided herein includes treatment and/or prevention of any diseases or disorders leading to corneal scarring or excessive and dysregulated inflammation or an immune response. In embodiments, the subject is a human subject that has a persistent corneal epithelial defect (PED or PCED), which results from the failure of rapid reepithelialization and closure after corneal injury (e.g., within about 2 weeks), even with standard of care supportive treatment. PEDs can result in serious complications including infection and vision loss. In embodiments, the PED is caused by dry eye disease. Accordingly, in embodiments, the formulations and methods provided herein treat a subject suffering from PEDs or otherwise suffering from corneal injury by enhancing the rate of reepithelization following corneal injury. In embodiments, administration of the provided formulations enhances the rate of reepithelialization by about 10%, about 25%, about 50%, about 75%, about 100%, or more. In embodiments, the administration of the provided formulations enhances the rate of reepithelialization compared to the rate of reepithelialization in a control wherein standard of care or no treatment is administered to the eye of the subject. The rate of reepithelialization may be enhanced such that corneal healing occurs within about 2, about 3,

about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, or about 14 days after injury. The formulations and methods provided herein are not limited to exclusive treatment alone and may be used in conjunction with other standard of care treatment(s).

**[0042]** The polypeptides useful in the formulations and methods provided herein may be any polypeptide with properties such as wound healing properties, anti-inflammatory properties, properties relating to protection or regeneration of the corneal stroma, and/or anti-neovascular properties. In embodiments, the polypeptides can be any suitable polypeptide having a molecular weight of about 1.0 kDa to about 10.0 kDa. In embodiments, the polypeptide can be any suitable polypeptide having a molecular weight of about 1.0 kDa, about 2.0 kDa, about 3.0 kDa, about 4.0 kDa, about 5.0 kDa, about 6.0 kDa, about 7.0 kDa, about 8.0 kDa, about 9.0 kDa, or about 10.0 kDa.

**[0043]** In embodiments, the polypeptides can be any polypeptide comprising the carboxy-terminal most amino acids of an alpha Connexin, wherein the polypeptide does not comprise the full-length alpha Connexin protein. Thus, in embodiments, the provided polypeptide does not comprise the cytoplasmic N-terminal domain of the alpha Connexin. In embodiments, the provided polypeptide does not comprise the two extracellular domains of the alpha Connexin. In embodiments, the provided polypeptide does not comprise the four transmembrane domains of the alpha Connexin. In embodiments, the provided polypeptide does not comprise the cytoplasmic loop domain of the alpha Connexin. In embodiments, the provided polypeptide does not comprise that part of the sequence of the cytoplasmic carboxyl terminal domain of the alpha Connexin proximal to the fourth transmembrane domain. There is a conserved proline or glycine residue in alpha Connexins consistently positioned some 17 to 30 amino acids from the carboxyl terminal-most amino acid For example, for human Cx43 a proline residue at amino acid 363 is positioned 19 amino acids back from the carboxyl terminal most isoleucine. In another example, for chick Cx43 a proline residue at amino acid 362 is positioned 18 amino acids back from the carboxyl terminal-most isoleucine. In another example, for human Cx45 a glycine residue at amino acid 377 is positioned 19 amino acids back from the carboxyl terminal most isoleucine. In another example for rat Cx33, a proline residue at amino acid 258 is positioned 28 amino acids back from the carboxyl terminal most methionine. Thus, in embodiments, the provided polypeptide does not comprise amino acids proximal to said

conserved proline or glycine residue of the alpha Connexin. Thus, the provided polypeptide 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, 22, 23, 24, 25, 26, 27, 28, 29, 30 amino acids of the alpha Connexin. Exemplary alpha Connexin polypeptides are disclosed in U.S. Patent Nos. 7,786,074; 7,888,319; 8,357,668; 8,809,257; 8,916,515; 8,859,733; 8,846,605; 9,161,984; 9,394,351; 9,408,381; 9,844,214; 9,855,313; 10,398,140; and 10,398,757, and/or International Patent Application No. PCT/US2018/000035, the entire contents of each of which are hereby incorporated by reference.

**[0044]** Connexins are the sub-unit protein of the gap junction channel, which is responsible for intercellular communication (Goodenough and Paul, 2003). Based on patterns of conservation of nucleotide sequence, the genes encoding Connexin proteins are divided into two families termed the alpha and beta Connexin genes. The carboxy-terminal-most amino acid sequences of alpha Connexins are characterized by multiple distinctive and conserved features. This conservation of organization is consistent with the ability of aCT peptides to form distinctive 3D structures, interact with multiple partnering proteins, mediate interactions with lipids and membranes, interact with nucleic acids including DNA, transit and/or block membrane channels and provide consensus motifs for proteolytic cleavage, protein cross-linking, ADP-ribosylation, glycosylation and phosphorylation. Thus, the provided polypeptide interacts with a domain of a protein that normally mediates the binding of said protein to the carboxy-terminus of an alpha Connexin. For example, nephroblastoma overexpressed protein (NOV) interacts with a Cx43 c-terminal domain (Fu et al., J Biol. Chem. 2004 279(35):36943-50). It is considered that this and other proteins interact with the carboxy-terminus of alpha Connexins and further interact with other proteins forming a macromolecular complex. Thus, the provided polypeptide can inhibit the operation of a molecular machine, such as, for example, one involved in regulating the aggregation of Cx43 gap junction channels.

**[0045]** The polypeptides provided herein comprise a carboxy-terminal amino acid sequence of an alpha Connexin, or a conservative variant thereof. In embodiments, the polypeptide comprises or consists of the amino acid sequence RPRPDDLEI (SEQ ID NO: 2). In embodiments, the polypeptide is aCT1, as described herein. The term "aCT1" is used interchangeably herein with "αCT1," "aCT", "aCT-1", "ACT," and "ACT-1". aCT1 is a 25 aa peptide having a molecular weight of 3597.33 Da that has a compact 2-domain design based

on linkage of an Antennapedia cell internalization domain (1-16aa; RQPKIWFPNRRKPWKK; SEQ ID NO: 7) to the C-terminal PDZ binding domain of the transmembrane gap junction protein Cx43 (17-25aa; RPRPDDLEI; SEQ ID NO:2). Accordingly, the full aCT1 sequence is RQPKIWFPNRRKPWKK RPRPDDLEI (SEQ ID NO: 9). aCT1 and related peptides increase the size and stability of gap junctions by modulating the molecular interaction between Cx43 and its C-terminal binding partners, including the tight junction protein zonula occludens-1 (ZO-1). This leads to phosphorylation of the serine 368 (S368) amino acid on Cx43 and favors a transition of cell-surface Cx43 from hemichannels to gap junction intercellular channels. Phosphorylation of S368 prevents the binding of ZO-1 to the C-terminus of Cx43 long after aCT1 has degraded, permitting therapeutic longevity. Concomitantly, aCT1 stabilizes ZO-1 at the cell membrane, preventing junctional degradation in response to injury and preserving barrier function of epithelial cells. The result is stabilization of gap junctions (intercellular communication) as well as tight junctions (intercellular junctions) leading to a variety of beneficial effects including increased cellular communication, dampened inflammatory responses, and reduction in the infiltration and proliferation of profibrotic cells. Collectively, the molecular and cellular events facilitated by aCT1 preserves tissue integrity, reduces injury spread, dampens pathological inflammation, and accelerates healing and tissue regeneration

**[0046]** In embodiments, the compositions and methods provided herein are related to preventing, treating, and/or mitigating the progression of corneal injuries. In embodiments, the compositions and methods provided herein are related to preventing, treating, and/or mitigating the progression of corneal injuries. In embodiments, the formulations provided herein are for use in preventing, treating, and/or mitigating the progression of corneal injuries. In embodiments, provided herein are uses of aCT1 in the manufacture of a medicament for preventing or treating corneal injuries.

**[0047]** The aCT sequence of the provided polypeptide can be from any alpha Connexin. Thus, the alpha Connexin component of the provided polypeptide can be from a human, murine, bovine, monotrene, marsupial, primate, rodent, cetacean, mammalian, avian, reptilian, amphibian, piscine, chordate, protochordate or other alpha Connexin. Thus, the provided polypeptide can comprise an ACT of a Connexin selected from the group consisting of mouse Connexin 47, human Connexin 47, Human Connexin 46.6, Cow Connexin 46.6, Mouse Connexin 30.2, Rat Connexin 30.2, Human Connexin 31.9, Dog Connexin 31.9, Sheep

Connexin 44, Cow Connexin 44, Rat Connexin 33, Mouse Connexin 33, Human Connexin 36, mouse Connexin 36, rat Connexin 36, dog Connexin 36, chick Connexin 36, zebrafish Connexin 36, morone Connexin 35, morone Connexin 35, Cynops Connexin 35, Tetraodon Connexin 36, human Connexin 37, chimp Connexin 37, dog Connexin 37, Cricetulus Connexin 37, Mouse Connexin 37, Mesocricetus Connexin 37, Rat Connexin 37, mouse Connexin 39, rat Connexin 39, human Connexin 40.1, Xenopus Connexin 38, Zebrafish Connexin 39.9, Human Connexin 40, Chimp Connexin 40, dog Connexin 40, cow Connexin 40, mouse Connexin 40, rat Connexin 40, Cricetulus Connexin 40, Chick Connexin 40, human Connexin 43, Cercopithecus Connexin 43, Oryctolagus Connexin 43, Spermophilus Connexin 43, Cricetulus Connexin 43, Phodopus Connexin 43, Rat Connexin 43, Sus Connexin 43, Mesocricetus Connexin 43, Mouse Connexin 43, Cavia Connexin 43, Cow Connexin 43, Erinaceus Connexin 43, Chick Connexin 43, Xenopus Connexin 43, Oryctolagus Connexin 43, Cyprinus Connexin 43, Zebrafish Connexin 43, Danio aequipinnatus Connexin 43, Zebrafish Connexin 43.4, Zebrafish Connexin 44.2, Zebrafish Connexin 44.1, human Connexin 45, chimp Connexin 45, dog Connexin 45, mouse Connexin 45, cow Connexin 45, rat Connexin 45, chick Connexin 45, Tetraodon Connexin 45, chick Connexin 45, human Connexin 46, chimp Connexin 46, mouse Connexin 46, dog Connexin 46, rat Connexin 46, Mesocricetus Connexin 46, Cricetulus Connexin 46, Chick Connexin 56, Zebrafish Connexin 39.9 cow Connexin 49, human Connexin 50, chimp Connexin 50, rat Connexin 50, mouse Connexin 50, dog Connexin 50, sheep Connexin 49, Mesocricetus Connexin 50, Cricetulus Connexin 50, Chick Connexin 50, human Connexin 59, or other alpha Connexin.

**[0048]** The 20-30 carboxy-terminal-most amino acid sequence of alpha Connexins are characterized by a distinctive and conserved organization. This distinctive and conserved organization includes a type II PDZ binding motif (Φ-x-Φ; wherein x = any amino acid and Φ =a Hydrophobic amino acid) and proximal to this motif, Proline (P) and/or Glycine (G) hinge residues; a high frequency phospho-Serine (S) and/or phospho-Threonine (T) residues; and a high frequency of positively charged Arginine (R), Lysine (K) and negatively charged Aspartic acid (D) or Glutamic acid (E) amino acids. For many alpha Connexins, the P and G residues occur in clustered motifs proximal to the carboxy-terminal type II PDZ binding motif. The S and T phosphor-amino acids of most alpha Connexins also are typically organized in clustered, repeat-like motifs. This organization is particularly the case for Cx43, where 90% of 20

carboxyl terminal-most amino acids are comprised of the latter seven amino acids. In a further example of the high conservation of the sequence, ACT peptide organization of Cx43 is highly conserved from humans to fish.

**[0049]** Thus, in one aspect, the provided polypeptide comprises one, two, three or all of the amino acid motifs selected from the group consisting of 1) a type II PDZ binding motif, 2) Proline (P) and/or Glycine (G) hinge residues; 3) clusters of phospho-Serine (S) and/or phospho-Threonine (T) residues; and 4) a high frequency of positively charged Arginine (R) and Lysine (K) and negatively charged Aspartic acid (D) and/or Glutamic acid (E) amino acids). In another aspect, the provided polypeptide comprises a type II PDZ binding motif at the carboxy-terminus, Proline (P) and/or Glycine (G) hinge residues proximal to the PDZ binding motif, and positively charged residues (K, R, D, E) proximal to the hinge residues.

**[0050]** PDZ domains were originally identified as conserved sequence elements within the postsynaptic density protein PSD95/SAP90, the Drosophila tumor suppressor dlg-A, and the tight junction protein ZO-1. Although originally referred to as GLGF or DHR motifs, they are now known by an acronym representing these first three PDZ-containing proteins (PSD95/DLG/ZO-1). These 80-90 amino acid sequences have now been identified in well over 75 proteins and are characteristically expressed in multiple copies within a single protein. Thus, in one aspect, the provided polypeptide can inhibit the binding of an alpha Connexin to a protein comprising a PDZ domain. The PDZ domain is a specific type of protein-interaction module that has a structurally well-defined interaction ‘pocket’ that can be filled by a PDZ-binding motif, referred to herein as a "PDZ motif". PDZ motifs are consensus sequences that are normally, but not always, located at the extreme intracellular carboxyl terminus. Four types of PDZ motifs have been classified: type I (S/T-x-Φ), type II (Φ-x-Φ), type III (Ψ-x-Φ) and type IV (D-x-V), where x is any amino acid, Φ is a hydrophobic residue (V, I, L, A, G, W, C, M, F) and Ψ is a basic, hydrophilic residue (H, R, K). (Songyang, Z., et al. 1997. Science 275, 73-77). Thus, in one aspect, the provided polypeptide comprises a type II PDZ binding motif.

**[0051]** 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 in 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. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place.

**[0052]** 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. Conservatively substituted variations of each explicitly disclosed sequence are included within the polypeptides provided herein.

**[0053]** 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.

**[0054]** 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.

**[0055]** It is understood that there are numerous amino acid and peptide analogs which can be incorporated into the disclosed compositions. For example, there are numerous D amino acids. The opposite stereoisomers of naturally occurring peptides are disclosed, as well as the stereoisomers of peptide analogs. These amino acids can readily be incorporated into polypeptide chains by charging tRNA molecules with the amino acid of choice and engineering genetic constructs that utilize, for example, amber codons, to insert the analog amino acid into a peptide chain in a site specific way (Thorson et al., Methods in Molec. Biol. 77:43-73 (1991), Zoller, Current Opinion in Biotechnology, 3:348-354 (1992); Ibba, Biotechnology & Genetic Engineering Reviews 13:197-216 (1995), Cahill et al., TIBS, 14(10):400-403 (1989); Benner, TIB Tech, 12:158-163 (1994); Ibba and Hennecke, Bio/technology, 12:678-682 (1994), all of which are herein incorporated by reference at least for material related to amino acid analogs).

**[0056]** D-amino acids can be used to generate more stable peptides, because D amino acids are not recognized by peptidases and such. Systematic substitution of one or more amino acids of a consensus sequence with a D-amino acid of the same type (e.g., D-lysine in place of L-lysine) can be used to generate more stable peptides. Cysteine residues can be used to cyclize or attach two or more peptides together. This can be beneficial to constrain peptides into particular conformations. (Rizo and Gierasch Ann. Rev. Biochem. 61:387 (1992), incorporated herein by reference).

**[0057]** Thus, the provided polypeptide can comprise a conservative variant of the c-terminus of an alpha Connexin (ACT). It is understood that one way to define any variants, modifications, or derivatives of the disclosed genes and proteins herein is through defining the variants, modification, and derivatives in terms of sequence identity (also referred to herein as homology) to specific known sequences. Specifically disclosed are variants of the nucleic acids and polypeptides herein disclosed which have at least 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent sequence identity to the stated or known sequence. Those of skill in the art readily understand how to determine the sequence identity of two proteins or nucleic acids. For example, the sequence identity can be calculated after aligning the two sequences so that the sequence

identity is at its highest level. Another way of calculating sequence identity can be performed by published algorithms.

**[0058]** Thus, the provided polypeptide can comprise an amino acid sequence with at least 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent sequence identity to the c-terminus of an alpha Connexin (ACT). Thus, in one aspect, the provided polypeptide comprises an amino acid sequence with at least 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 percent sequence identity to SEQ ID NO:1, SEQ ID NO: 2, or any sequence provided herein.

**[0059]** In embodiments, the polypeptide comprises a cellular internalization transporter or sequence. The cellular internalization sequence can 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 (Bis-Guanidinium-Tren-Cholesterol). Exemplary cell internalization transporters are provided in Table 2A.

**Table 2A. Exemplary cell internalization sequences**

| Name | Sequence | SEQ ID NO |
|---|---|---|
| Antp | RQPKIWFPNRRKPWKK | (SEQ ID NO: 7) |
| HIV-Tat | GRKKRRQRPPQ | (SEQ ID NO: 14) |
| Penetratin | RQIKIWFQNRRMKWKK | (SEQ ID NO: 15) |
| Antp-3A | RQIAIWFQNRRMKWAA | (SEQ ID NO: 16) |
| Tat | RKKRRQRRR | (SEQ ID NO: 17) |
| Buforin II | TRSSRAGLQFPVGRVHRLLRK | (SEQ ID NO: 18) |
| Transportan | GWTLNSAGYLLGKINKALAALA KKIL | (SEQ ID NO: 19) |
| model amphipathic peptide (MAP) | KLALKLALKALKAALKLA | (SEQ ID NO: 20) |
| K-FGF | AAVALLPAVLLALLAP | (SEQ ID NO: 21) |
| Ku70 | VPMLK-PMLKE | (SEQ ID NO: 22) |

| | | |
|---|---|---|
| Prion | MANLGYWLLALFVTMWTDVGL CKKRPKP | (SEQ ID NO: 23) |
| pVEC | LLIILRRRIRKQAHAHSK | (SEQ ID NO: 24) |
| Pep-1 | KETWWETWWTEWSQPKKKRKV | (SEQ ID NO: 25) |
| SynB1 | RGGRLSYSRRRFSTSTGR | (SEQ ID NO: 26) |
| Pep-7 | SDLWEMMMVSLACQY | (SEQ ID NO: 27) |
| HN-1 | TSPLNIHNGQKL | (SEQ ID NO: 28) |
| BGSC (Bis- Guanidinium-Spermidine- Cholesterol) | (n/a) | |
| BGTC (Bis- Guanidinium-Tren- Cholesterol) | (n/a) | |

**[0060]** Any other internalization sequences now known or later identified can be combined with a peptide of the invention.

**[0061]** The provided polypeptide can comprise any aCT sequence (e.g, any of the aCT peptides disclosed herein) in combination with any of the herein provided cell internalization sequences. Examples of said combinations are provided in Table 2B. Thus, the provided polypeptide can comprise an Antennapedia sequence comprising amino acid sequence SEQ ID NO:7. Thus, the provided polypeptide can comprise the amino acid sequence SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO: 12.

**Table 2B. ACT Polypeptides with Cell Internalization Sequences (CIS) aCT Polypeptides with Cell Internalization Sequences (CIS)**

| CIS/ACT | Sequence | SEQ ID NO |
|---|---|---|
| Antp/ ACT 2 | RQPKIWFPNRRKPWKK PSSRASSRASSRPRPDDLEI | SEQ ID NO:8 |
| Antp/ ACT 1 | RQPKIWFPNRRKPWKK RPRPDDLEI | SEQ ID NO:9 |
| Antp/ ACT 3 | RQPKIWFPNRRKPWKK RPRPDDLEV | SEQ ID NO:10 |
| Antp/ ACT 4 | RQPKIWFPNRRKPWKK RPRPDDVPV | SEQ ID NO:11 |
| Antp/ ACT 5 | RQPKIWFPNRRKPWKK KARSDDLSV | SEQ ID NO:12 |

| | | |
|---|---|---|
| HIV-Tat/ ACT 1 | GRKKRRQRPPQ RPRPDDLEI | SEQ ID NO:56 |
| Penetratin/ ACT 1 | RQIKIWFQNRRMKWKK RPRPDDLEI | SEQ ID NO:57 |
| Antp-3A/ ACT 1 | RQIAIWFQNRRMKWAA RPRPDDLEI | SEQ ID NO:58 |
| Tat/ ACT1 | RKKRRQRRR RPRPDDLEI | SEQ ID NO:59 |
| Buforin II/ ACT 1 | TRSSRAGLQFPVGRVHRLLRK RPRPDDLEI | SEQ ID NO:60 |
| Transportan/ ACT 1 | GWTLNSAGYLLGKINKALAALAKKIL RPRPDDLEI | SEQ ID NO:61 |
| MAP/ ACT 1 | KLALKLALKALKAALKLA RPRPDDLEI | SEQ ID NO:62 |
| K-FGF/ ACT 1 | AAVALLPAVLLALLAP RPRPDDLEI | SEQ ID NO:63 |
| Ku70/ ACT 1 | VPMLKPMLKE RPRPDDLEI | SEQ ID NO:64 |
| Prion/ ACT 1 | MANLGYWLLALFVTMWTDVGLCKKRPKP RPRPDDLEI | SEQ ID NO:65 |
| pVEC/ ACT 1 | LLIILRRRIRKQAHAHSK RPRPDDLEI | SEQ ID NO:66 |
| Pep-1/ ACT 1 | KETWWETWWTEWSQPKKKRKV RPRPDDLEI | SEQ ID NO:67 |
| SynB1/ ACT 1 | RGGRLSYSRRRFSTSTGR RPRPDDLEI | SEQ D NO:68 |
| Pep-7/ ACT 1 | SDLWEMMMVSLACQY RPRPDDLEI | SEQ ID NO:69 |
| HN-1/ ACT 1 | TSPLNIHNGQKL RPRPDDLEI | SEQ ID NO:70 |

**[0062]** Also provided are isolated nucleic acids encoding the polypeptides provided herein. The disclosed nucleic acids are made up of for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell, the expressed mRNA will typically be made up of A, C, G, and U. Thus, provided is an isolated nucleic acid

encoding a polypeptide comprising the amino acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12.

**[0063]** In embodiments, provided herein is a composition comprising one or more of the herein provided polypeptides, nucleic acids, or vectors in a pharmaceutically acceptable carrier. For example, provided is a composition comprising SEQ ID NO:2 or SEQ ID NO:9 in a pharmaceutically acceptable carrier. In embodiments, the composition comprises one or more of the herein provided polypeptides encapsulated in a microcarrier. For example, in embodiments, the composition comprises one or more of the herein provided polypeptides, wherein the polypeptides are in a nanoparticle or exosome.

**[0064]** In embodiments, the compositions provided herein comprise drug loaded microcarrier formulations comprising nanoparticles or exosomes. In embodiments, the size of the nanoparticles is from about 100 nm to about 1000 nm, or about 100 nm to about 500 nm, or about 200 nm to about 250 nm, or about 100 nm to about 200 nm.

**[0065]** In embodiments, formulation comprises about 0.01% w/w to about 3.5% w/w, or about 0.05% w/w to about 3.0% w/w, or about 0.07% w/w to about 2.0% w/w, or about 0.1% w/w to about 1.0% w/w, or about 0.1% w/w to about 0.5% w/w, or about 0.2% w/w to about 0.8% w/w, or about 0.03% w/w to about 0.07% w/w, or about 0.05% w/w, of the polypeptide. In embodiments, the formulation comprises about 0.035% w/w of the polypeptide or about 0.07% w/w of the polypeptide or about 0.1% w/w of the polypeptide, or about 1.0% w/w of the peptide, or about 3.5% w/w of the polypeptide.

**[0066]** In embodiments, the formulation comprises about 0.00035 mg/mL to about 35 mg/mL, or about 0.001 mg/mL to about 20 mg/mL, or about 0.01 mg/mL to about 3.5 mg/mL, or about 0.1 mg/mL to about 1.0 mg/mL, or about 0.2 mg/mL to about 0.8 mg/mL, or about 0.3 mg/mL to about 0.7 mg/mL of the polypeptide. In embodiments, the formulation comprises about 0.1 mg/mL, about 0.2 mg/mL, about 0.3 mg/mL, about 0.35 mg/mL, about 0.4 mg/mL, about 0.5 mg/mL, about 0.6 mg/mL, about 0.7 mg/mL, about 0.8 mg/mL, about 0.9 mg/mL, or about 1.0 mg/mL of the polypeptide. In embodiments, the formulation comprises about 0.35 mg/mL or about 0.7 mg/mL of the polypeptide. In embodiments, the formulation comprises about 1 mg/mL or about 10 mg/mL or about 20 mg/mL of the polypeptide.

**[0067]** In embodiments, the composition is administered to the subject in a formulation comprising about 1 μM to about 100,000 μM, or about 10 μM to about 50,000 μM, or about 100 μM to about 10,000 μM, or about 10 μM to about 9,000 μM, or about 50 μM to about 5,000 μM, or about 100 μM to about 2,000 μM, or about 200 μM to about 2,000 μM, or about 200 μM to about 1,000 μM, or about 50 μM to about 1,500 μM of the polypeptide, or about 100 μM to about 1,000 μM of the polypeptide, or about 500 to about 1,500 μM of the polypeptide. In embodiments, the composition is administered to the subject in a formulation comprising about 1 μM , about 5 μM , about 50 μM, about 100 μM, about 150 μM, about 200 μM, about 300 μM, about 400 μM, about 500 μM, about 600 μM, about 700 μM, about 800 μM, about 900 μM, about 1,000 μM, about 1,500 μM, about 2,000 μM, about 3,000 μM, about 4,000 μM, about 5,000 μM, about 6,000 μM, about 7,000 μM, about 8,000 μM, about 9,000 μM, about 10,000 μM, about 20,000 μM, about 25,000 μM, about 50,000 μM, about 75,000 μM, about 100,000 μM, or more, of the polypeptide.

**[0068]** In embodiments, the formulation is administered to the eye immediately after the event that caused the ocular injury. In embodiments, the formulation is administered to the subject within about 1 hour, within about 2, hours, within about 3 hours, within about 4 hours, within about 5 hours, within about 6 hours, within about 8 hours, within about 12 hours, within about 18 hours, or within about 24 hours of the event the caused the ocular injury. In embodiments, the formulation is administered to the subject at least about 2 hours following the event that caused the ocular injury. In embodiments, the formulation is administered to the eye of the subject daily, e.g., once per day, or twice per day, and/or about every 8 hours or about every 12 hours. In embodiments, the formulation is administered until ocular healing is observed. In embodiments, the formulation is administered for at least about 2 days, at least about 3 days, at least about 4 days, at least about 5 days, at least about 6 days, at least about 7 days, or for longer. In embodiments, the formulation is administered chronically. As used herein, the term "administered chronically" means the formulation is administered for an open-ended dosing regimen, that is, treatment is started and intended to continue for an indefinite period of time and/or until symptoms resolve, etc. In embodiments, for chronic disease conditions, the formulation is administered to the eye upon detection of the chronic disease condition and is administered chronically. In embodiments, the formulation is administered

chronically to subjects with corneal injuries resulting from chronic conditions such as dry eye disease (DED).

**[0069]** As used herein, "subject" include vertebrates, more specifically a mammal (e.g., a human, horse, pig, rabbit, dog, sheep, goat, non-human primate, cow, cat, guinea pig or rodent), a fish, a bird or a reptile or an amphibian. In embodiments, the subject is a human subject. The term does not denote a particular age or sex. Thus, adult and newborn subjects, as well as fetuses, whether male or female, are intended to be covered. In embodiments, a patient refers to a subject afflicted with a disease or disorder. In embodiments, a patient population refers to a particular, defined set of subjects having a disease or disorder or at risk of developing a particular disease or disorder.

**[0070]** As used herein, "inhibit," "inhibiting," and "inhibition" mean to decrease an activity, response, condition, disease, or other biological parameter. This can include, but is not limited to, the complete loss of activity, response, condition, or disease. Thus, the reduction can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels.

**[0071]** Ranges and values may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, also specifically contemplated and considered disclosed is the range from the one particular value and/or to the other particular value unless the context specifically indicates otherwise. All of the individual values and sub-ranges of values contained within an explicitly disclosed range are also specifically contemplated and should be considered disclosed unless the context specifically indicates otherwise. The foregoing applies regardless of whether in particular cases some or all of these embodiments are explicitly disclosed. As used herein, the term “about” and the like, when used in the context of a value, generally means plus or minus 10% of the value stated. For example, about 0.5 would include 0.45 and 0.55, about 10 would include 9 to 11, about 1000 would include 900 to 1100. It

**[0072]** By "treat" or "treatment" is meant a method of reducing the effects of a disease or condition. Treatment can also refer to a method of reducing the underlying cause of the disease or condition itself rather than just the symptoms. The treatment can be any reduction from native levels and/or any improvement of clinical signs of the disease and/or any increase in survival or function; and can be but is not limited to the complete ablation of the disease,

condition, or the symptoms of the disease or condition. For example, a disclosed method for treating corneal injury is considered to be a treatment if there is a reduction in one or more symptoms of the injury or if there is an improvement in the condition of the subject when compared to native levels in the same subject or control subjects. Thus, the reduction or improvement can be a 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%, or any amount of reduction in between as compared to native or control levels. By "prevent" or "prevention" and the like is meant a method of preventing the onset or reducing the incidence or severity of corneal injury.

**[0073]** Publications, patents and patent applications cited herein are specifically incorporated by reference in their entireties. While the described invention has been described with reference to the specific embodiments thereof it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adopt a particular situation, material, composition of matter, process, process step or steps, to the objective spirit and scope of the described invention. All such modifications are intended to be within the scope of the claims appended hereto

**[0074]** The present disclosure is further illustrated by reference to the following Examples. However, it should be noted that these Examples, like the embodiments described above, are illustrative and are not to be construed as restricting the scope of the disclosure in any way.

## EXAMPLES

### Example 1. Comparative studies testing buffered formulations

**[0075]** Formulations comprising citrate buffer and either hydroxyethylcellulose (HEC) or hydroxypropyl methylcellulose (HPMC) were subjected to filtration feasibility, peptide stability, and mechanical viscosity studies. Stability and impurity analyses were performed using high performance liquid chromatography (HPLC) of final aCT1 eye drop formulations with a validated analytical method. Studies were also conducted to compare peptide filtration feasibility and peptide stability of formulations comprising different buffers and combinations of excipients.

**[0076]** First, HEC and HPMC solutions containing citrate buffer were prepared and tested for filtration feasibility. The HEC solutions tested contained 0.2% w/w, 0.15% w/w, or 0.125% w/w HEC, in 10 mM citrate buffer at pH 6.0. the HPMC solution tested contained 0.5% w/w

HPMC in 10 mM citrate buffer at pH 6.0. Both solutions contained 0.07% ACT peptide, a 3.6 kDa peptide.

**[0077]** The solutions were tested for filterability through a 0.2 μm PES filter. The results of the study showed that the HPMC solution was easy to filter at the 0.5% (w/w) concentration through a 0.2 μm PES membrane 25 mm syringe filter. In contrast, the highest HEC solution (0.2%(w/w)) was difficult to filter through the PES syringe filter, as well as difficult to filter through 0.2 μm PES filter using a unit with a vacuum pump, when prepared with a low shear mixer (magnetic stir bar). When prepared with a higher shear homogenizer, the higher concentration HEC solution was easier to filter. Both of the lower concentration HEC solutions could be filtered through the 0.2μm PES filter.

**[0078]** A mechanical viscosity study was conducted using a TA AR1000 Rheometer. Viscosity at shear stress of 0.01 to 1,000 Pa was determined with sample gap of 500 μM, at 25°C, and equilibration time 2 minutes. Viscosity of the 0.5% w/w HPMC solution was 57.3 mPaS; viscosity of the 0.15% w/v HEC solution was 3.7 mPaS.

**[0079]** Similar formulations comprising 0.07% aCT1 peptide, 10 mM citric acid, 0.9% NaCl, and either 0.2% HEC or 0.2% HPMC (pH 6.0) were assessed for peptide stability. Impurities were detected at 0 and 3 month timepoints.

**[0080]** In summary, while the formulations could be sterile filtered, viscosity was not considered to be within a range appropriate for retention on the ocular surface. A minimum shear viscosity of 10.2 mPaS is necessary for ocular retention in humans (Zaki et al., 1986). United States Pharmacopeia (USP) guidelines state 18-28 mPaS is the optimal viscosity range for eye drop formulations. Further, impurities were detected at 0 and 3 month stability timepoints. Thus, these formulations were not appropriate for ocular delivery. Taken together, the studies indicated that the inclusion of citrate buffer surprisingly produced a formulation with unfavorable characteristics.

**[0081]** Formulations comprising citrate buffer were compared to formulations comprising phosphate buffer. The solutions shown below in Table 3 were prepared. 100 mM citrate buffers of pH 4, 5, or 6 and 100 mM phosphate buffers of 6, 7, or 8 were prepared. HPMC solutions at 0.5% w/V HPMC were prepared using each of the individual buffers. The HPMC solutions were filtered using a 0.2 μM PES filter. 3.5 mg/mL aCT1 peptide was added to the filtered

HPMC solution and mixed on a stir plate until a uniform, clear solution was obtained. The solution was then filtered into 10 mL clear vials with crimp seal.

**Table 3. Citrate and Phosphate buffer formulations**

| Formulation ID | Description |
|---|---|
| **5776** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM citrate buffer pH 4.0 |
| **5777** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM citrate buffer pH 5.0 |
| **5778** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM citrate buffer pH 6.0 |
| **5779** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM phosphate buffer pH 6.0 |
| **5780** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM phosphate buffer pH 7.0 |
| **5781** | 3.5mg/mL aCT1, 0.5% w/w HPMC in 100mM phosphate buffer pH 8.0 |

**[0082]** It was very difficult to filter the solutions at lower pH (4 and 5) in citrate buffer. Filtration of 0.5% w/v HPMC solution in 100 mM citrate buffer pH 6.0 and 100 mM phosphate buffer pH 6.0 were a bit difficult to filter when compared to 0.5% HPMC solution in 10 mM citrate buffer pH 6.0 (which was used in filtration feasibility studies). Of all the solutions, FID5780 (0.5% HPMC in 100 mM phosphate buffer pH 7.0) was easy to filter. Ease of filtration of each formulation (Formulation ID or FID as shown above in Table 3): FID 5780>5781>5779>5778. aCT1 peptide dissolved quickly in all the solutions.

**[0083]** However, impurities were detected in each of the formulations at 2 week and 4 week stability time points (Table 4). Accordingly, the inclusion of phosphate buffer, like citrate buffer, did not yield a formulation with favorable peptide stability.

**Table 4. Impurities at 2 and 4 week stability time points**

| Sample Description | Temp. | T0 Total impurities / Largest Impurity | T2W Total impurities / Largest Impurity | T4W Total impurities / Largest Impurity |
|---|---|---|---|---|
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 4.0 | 25°C | 0.75% / 0.37% | 11.14% / 6.39% | 17.03% / 8.48% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 4.0 | 40°C | | 30.38% / 11.46% | 84.56% / 40.97% |

| | | | | |
|---|---|---|---|---|
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 5.0 | 25°C | 0.49% / 0.22% | 8.74% / 1.72% | 11.47% / 3.10% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 5.0 | 40°C | | 14.90% / 2.69% | 19.13% / 4.67% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 6.0 | 25°C | 1.55% / 0.41% | 28.79% / 11.57% | 38.92% / 15.92% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM citrate buffer pH 6.0 | 40°C | | 27.72% / 8.97% | 31.03% / 8.68% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 6.0 | 25°C | 1.05% / 0.24% | 24.37% / 9.34% | 34.80% / 13.59% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 6.0 | 40°C | | 27.8% / 5.92% | 37.31% / 15.77% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 7.0 | 25°C | 0.49% / 0.20% | 13.44% / 7.62% | 21.91% / 13.55% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 7.0 | 40°C | | 53.82% / 35.64% | 71.95% / 49.63% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 8.0 | 25°C | 1.06% / 0.51% | 37.92% / 24.69% | 56.67% / 37.74% |
| 0.35% aCT1, 0.5% HPMC, in 10 mM phosphate buffer pH 8.0 | 40°C | | 96.29% / 61.37% | 72.82% / 63.04% |

**[0084]** To investigate low assay T0 testing in pH stability study and to evaluate filter suitability of formulations with and without HPMC, the following formulations shown in Table 5 were made.

**Table 5. Further Citrate Buffer and HPMC formulations**

| Sample ID | Description |
|---|---|
| LBR1421-001-13A | aCT10.35mg/mL, Sodium chloride 0.8% w/v, HPMC 0.5%w/v in 10mM citrate buffer pH 5.0 |
| LBR1421-001-13B | aCT10.35mg/mL, Sodium chloride 0.8% w/v in 10mM citrate buffer pH 5.0 |
| LBR1421-001-13C | aCT13.5mg/mL, Sodium chloride 0.8% w/v, HPMC 0.5%w/v in 10mM citrate buffer pH 5.0 |

| | |
|---|---|
| LBR1421-001-13D | aCT1 3.5mg/mL, Sodium chloride 0.8% w/v in 10mM citrate buffer pH 5.0 |

**[0085]** Each solution was filtered using 0.2 μM PES filter or 0.2 μM PVDF filter. Results are provided below in Table 6.

**Table 6. Evaluation of filter suitability and assay results with or without HPMC in the formulation**

| **Sample Description** | **Lot** | **Filter** | **Peptide 328967 Assay (% Label Claim)** |
|---|---|---|---|
| 0.35 mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13A1 | Pre-Filter | 118.5% |
| 0.35mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13A2 | PES | 116.4% |
| 0.35mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13A3 | PVDF | 113.9% |
| 0.35mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13B1 | Pre-Filter | 107.3% |
| 0.35mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13B2 | PES | 107.8% |
| 0.35mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13B3 | PVDF | 105.8% |
| 3.5mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13C1 | Pre-Filter | 113.2% |
| 3.5mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13C4 | Pre-Filter (Plastic Container) | 106.7% |
| 3.5mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13C2 | PES | 109.4% |
| 3.5mg/mL aCT1, 0.5% HPMC, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13C3 | PVDF | 109.1% |
| 3.5mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13D1 | Pre-Filter | 103.9% |
| 3.5mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13D4 | Pre-Filter (Plastic Container) | 103.0% |
| 3.5mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13D2 | PES | 103.2% |

| | | | |
|---|---|---|---|
| 3.5mg/mL aCT1, 0.8% NaCl pH 5.0 (Citrate) | LBR1421-001-13D3 | PVDF | 103.1% |

**[0086]** The formulations containing citrate buffer were very difficult to filter at pH 4 or 5. Formulations containing citrate and phosphate buffers at pH 6 were very difficult to filter. Thus, while the amount of peptide determined to be in the formulation after filtering relative to the expected amount (% Label Claim; Table 6) was generally within a suitable range (generally a range of 97%-115% is considered suitable), the formulations were not suitably filterable using PES or PVDF filters.

**[0087]** Data from 3 month stability studies of citrate buffer vs. phosphate buffer formulations, with or without glycerin as the viscosity enhancer, is provided below in Tables 7-10. While aCT1 peptide stability was favorable in several of these formulations, impurity profiles (Tables 9-10) tested for formulations stored at 5°C and 25°C were outside acceptable ranges.

**Table 7. 5°C Assay Results**

| Formulation ID (FID) | Sample Description | T0[2] Assay (% LC[1]) | T3M[3] Assay (% LC) | T6M[4] Assay (% LC) |
|---|---|---|---|---|
| 5903 | 0.35% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 96.5% | 102.3% (Plastic)<br>102.1% (Glass) | 100.7% (Plastic)<br>102.2% (Glass) |
| 5905 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 95.6% | 101.8% (Plastic)<br>101.9% (Glass) | 100.7% (Plastic)<br>101.7% (Glass) |
| 5904 | 0.035% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 97.9% | 93.5% (Plastic)<br>92.0% (Glass) | 88.0% (Plastic)<br>88.1% (Glass) |
| 5906 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 99.7% | 91.0% (Plastic)<br>95.6% (Glass) | 91.1% (Plastic)<br>94.2% (Glass) |

[1] % LC = Percentage of Label Claim
[2] T0 = Time zero
[3] T3M = Time 3 months of storage

[4] T6M = Time 6 months of storage

**Table 8. 25°C / 40% RH Assay Results**

| Table 6 - aCT1 Stability in Citrate or Phosphate Buffer Formulations Stored in Accelerated Conditions (25°C/40% RH) | | | | |
|---|---|---|---|---|
| **Formulation ID (FID)** | **Sample Description** | **T0[2] Assay (% LC[1])** | **T3M[3] Assay (% LC)** | **T6M[4] Assay (% LC)** |
| 5903 | 0.35% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 96.5% | 94.7% (Plastic)<br>95.0% (Glass) | 88.4% (Plastic)<br>95.0% (Glass) |
| 5905 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 95.6% | 84.2% (Plastic)<br>90.8% (Glass) | NT[5] (Plastic)<br>80.9% (Glass) |
| 5908 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM phosphate buffer pH 5 | 96.6% | 84.0% (Plastic)<br>91.4% (Glass) | NT[5] (Plastic)<br>NT[5] (Glass) |
| 5904 | 0.035% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 97.9% | 73.5% (Plastic)<br>78.6% (Glass) | 47.2% (Plastic)<br>65.4% (Glass) |
| 5906 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 99.7% | 35.8% (Plastic)<br>81.6% (Glass) | NT[6] (Plastic)<br>NT[6] (Glass) |
| 5909 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM phosphate buffer pH 5 | 101.0% | 42.8% (Plastic)<br>80.8% (Glass) | 9.7% (Plastic)<br>59.2% (Glass) |

[1] % LC = Percentage of Label Claim
[2] T0 = Time zero
[3] T3M = Time 3 months of storage
[4] T6M = Time 6 months of storage

[5] Not Tested

**Table 9. 5°C Impurity Results**

| Formulation ID (FID) | Sample Description | T0[1] Total Impurities/ Largest Impurity | T3M[2] Total Impurities/ Largest Impurity | T6M[3] Total Impurities/ Largest Impurity |
|---|---|---|---|---|
| 5903 | 0.35% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 2.11%/0.69% | 3.80%/1.59% (Plastic) 3.90%/1.62% (Glass) | 4.81%/1.80% (Plastic) 4.41%/1.80% (Glass) |
| 5905 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 2.15%/0.73% | 4.20%/1.52% (Plastic) 3.80%/1.46% (Glass) | 5.52%/2.20% (Plastic) 4.73%/1.91% (Glass) |
| 5904 | 0.035% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 3.35%/2.02% | 6.01%/3.93% (Plastic) 7.03%/4.13% (Glass) | 11.70%/5.61% (Plastic) 11.61%/5.53% (Glass) |
| 5906 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 3.94%/2.46% | 8.63%/6.91% (Plastic) 5.75%/3.02% (Glass) | 12.24%/6.82% (Plastic) 9.61%/3.60% (Glass) |

**Table 10. 25°C / 40% RH Impurity Results**

| Formulation ID (FID) | Sample Description | T0[1] Total Impurities/ Largest Impurity | T3M[2] Total Impurities/ Largest Impurity | T6M[3] Total Impurities/ Largest Impurity |
|---|---|---|---|---|
| 5903 | 0.35% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 2.11%/0.69% | 13.64%/3.36% (Plastic) 9.50%/3.04% (Glass) | 22.69%/9.11% (Plastic) 13.28%/3.67% (Glass) |
| 5905 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 2.15%/0.73% | 18.24%/5.90% (Plastic) 11.80%/3.08% (Glass) | NT[4] (Plastic) 25.44%/4.71% (Glass) |

| | | | | |
|---|---|---|---|---|
| 5908 | 0.35% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM phosphate buffer pH 5 | 2.07%/0.69% | 22.72%/8.06% (Plastic) 11.10%/2.82% (Glass) | $NT^4$ (Plastic) $NT^4$ (Glass) |
| 5904 | 0.035% aCT1, 0.5% HPMC, 0.8% sodium chloride, in 10 mM citrate buffer pH 5 | 3.35%/2.02% | 18.70%/13.34% (Plastic) 8.36%/3.90% (Glass) | 57.81%/25.91% (Plastic) 31.14%/10.60% (Glass) |
| 5906 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM citrate buffer pH 5 | 3.94%/2.46% | 41.44%/36.45% (Plastic) 17.61%/4.15% (Glass) | $NT^4$ (Plastic) $NT^4$ (Glass) |
| 5909 | 0.035% aCT1, 0.5% HPMC, 2.25% glycerin, in 10 mM phosphate buffer pH 5 | 3.25%/1.86% | 45.53%/32.92% (Plastic) 18.53%/6.72% (Glass) | 86.10%/44.39% (Plastic) 40.49%/10.47% (Glass) |

**[0088]** Taken together, the studies suggested that surprisingly, only formulations comprising HPMC (and not HEC, CMC, or glycerin) and excluding any buffer exhibit favorable properties for topical ocular administration.

**Example 2. Comparative studies testing HPMC formulations with or without additives**

**[0089]** The additives mannitol, edetate disodium, sodium metabisulfite, and vitamin E TPGS were individually tested in the formulation provided in Table 11. These are FDA approved excipients for ocular use.

**Table 11. Formulation used to test additives**

| Component | Concentration |
|---|---|
| aCT1 Peptide | 0.035% (w/w) |
| NaCl | 0.9% (w/w) |
| HPMC | 0.5% (w/w) |
| Additive (1.0% mannitol, 0.2% edetate disodium, 0.2% sodium metabisulfite, *or* 0.25% vitamin E TPGS) | (w/w as indicated at left) |

| | |
|---|---|
| Purified water | q.s. |
| pH | 4.0 - 8.0 |

**Table 12. 5°C Assay Results – Plastic container**

| Sample Description | T0 Assay (%LC) | T1M Assay (%LC) | T3M Assay (%LC) | T6M Assay (%LC) |
|---|---|---|---|---|
| 0.7 mg/mL aCT1 peptide (Vit. E, Mannitol, EDTA) | 100.4 | 102.2 | 99.3 | 103.0 |
| 0.7 mg/mL aCT1 peptide (Vit E, TPGS, EDTA) | 101.0 | 103.0 | 99.5 | 102.8 |
| 0.7 mg/mL aCT1 peptide (Vit E, TPGS) | 100.3 | 102.3 | 100.0 | 103.9 |
| 0.7 mg/mL aCT1 peptide (EDTA) | 101.5 | 102.8 | 99.2 | 100.2 |
| 0.7 mg/mL aCT1 peptide (Control – no additive) | 100.7 | 102.3 | 99.1 | 100.7 |

**Table 13. 5°C Assay Results – Glass container**

| Sample Description | T0 Assay (%LC) | T1M Assay (%LC) | T3M Assay (%LC) | T6M Assay (%LC) Prep1 / Prep 2 |
|---|---|---|---|---|
| 0.7 mg/mL aCT1 peptide (Vit. E, Mannitol, EDTA) | 101.4 | 103.3 | 100.2 | 123.8/104.1 |
| 0.7 mg/mL aCT1 peptide (Vit E, TPGS, EDTA) | 101.2 | 103.2 | 102.0 | 107.4/112.1 |
| 0.7 mg/mL aCT1 peptide (Vit E, TPGS) | 100.5 | 102.6 | 100.8 | 110.9/105.3 |
| 0.7 mg/mL aCT1 peptide (EDTA) | 100.7 | 103.4 | 100.2 | 117.4/105.3 |
| 0.7 mg/mL aCT1 peptide (Control – no additive) | 100.6 | 102.7 | 99.5 | 103.8/103.5 |

**[0090]** Impurity results showed inconsistencies prep-to-prep in glass container samples and presence of impurities with these tested formulations. None of the additives tested improved peptide stability in the formulation. Accordingly, formulations excluding all of these additives was selected for further testing, including in vivo testing described below in Examples 4-6.

**Example 3. Comparative in vivo studies of formulations comprising a peptide for eye drop administration**

**[0091]** The excipients shown below in Table 14 were used to prepare formulations containing aCT1 peptide at concentrations ranging from 100 µM to 10,000 µM, and the solubility and potential for effective delivery to the eye were tested. Specifically, formulations

containing NaCl, polymer hydroxypropyl methylcellulose (HPMC), Pluronic® F-127 (PF-127), hydroxyethylcellulose (HEC), and carboxymethylcellulose (CMC) were compared. The formulations tested did not include any additives or buffers.

**Table 14. Vehicles for comparative analysis**

| Vehicle | Concentration (w/v) |
|---|---|
| Hydroxypropyl methylcellulose (HPMC) | 0.5-1% |
| PF-127 | 20-25% |
| Hydroxyethylcellulose (HEC) | 0.5-1% |
| Carboxymethylcellulose (CMC) | 0.5% |
| NaCl | 0.9% |

**[0092]** The formulations were administered topically to the eye of Dutch Belted rabbits. aCT1 peptide was soluble in saline at all concentrations, however the formulation did not remain in contact with the ocular surface for a period of time sufficient to obtain delivery of the medication to the cornea. The addition of the viscosity modifier carboxymethylcellulose (CMC) to the dose formulation caused aggregation. In contrast, the addition of the viscoelastic polymer hydroxypropyl methylcellulose (HPMC) to the formulation did not cause aCT1 peptide aggregation or precipitation.

**Example 4 Tolerability and distribution study of twice daily administration**

**[0093]** A study was conducted to determine the safety, tolerability, and biodistribution of twice daily ocular administration of aCT1 peptide in an HPMC formulation, for 7 consecutive days.

**[0094]** Dose formulations were prepared by mixing the appropriate amount of test article in the vehicle (1% HPMC) to achieve the target concentration (see dose concentrations in Table 15, below). The formulation comprised 1% w/w HPMC and 0.9% w/w NaCl, and excluded buffers, preservatives, other vehicles, and other excipients. The formulation pH was recorded after filtration and was between 5.9 to 7.6, depending on the concentration of the formulation. Each does of the day was given 8 hours apart in a dosing volume of 0.1 mL to each eye.

**Table 15. Experimental design**

| Group | Treatment | Dose level (μmol/eye/dose) | Dose concentration (μM) | pH |
|---|---|---|---|---|
| 1 | Vehicle control | 0 | 0 | 7.2-7.6 |
| 2 | aCT1 | 0.01 | 100 | 5.9 |
| 3 | aCT1 | 0.1 | 1,000 | 6.3 |
| 4 | aCT1 | 1.0 | 10,000 | 5.9-6.0 |

**[0095]** Topical ocular administration of aCT1 twice daily for seven consecutive days was well-tolerated and did not result in any adverse findings in cage-side or clinical observations, body weight, clinical pathology, organ weight, or necropsy. Examination of the eyes showed irritation of eyelids and conjunctiva as well as stippling of cornea, and flaking of the eyelids for some animals in all groups including the control group; indicating that these findings were attributed to the viscosity of the vehicle and not to treatment with aCT1. There were also no microscopic findings in the eye tissues that were exposed to aCT1.

**[0096]** Tissue distribution of aCT1 was determined in rabbits administered 0.1 μmol/eye/dose. Whole blood concentrations of aCT1 were low but measurable in all three male rabbits at 0.25 hr on Day 1 at 0.25 hr were 3.26 ± 5.28 ng/ml. All other blood samples from Day 1 and all from Day 7 were below the lower limit of quantitation (LLOQ). The highest concentrations of aCT1 in ocular tissues were observed in the palpebral conjunctiva in both males and females. All animals sampled had quantifiable concentrations of aCT1 in this tissue after dosing on Days 1 and 7. The highest mean concentration was on Day 1 at 0.25 hr, 939 ± 228 ng/g (males) and 2730 ± 697 ng/g (females). The cornea and aqueous humor had the next highest mean levels of aCT1, 181 ± 50.0 ng/g (cornea, Day 1, 0.25 hr in females) and 115 ± 76.3 ng/g (aqueous humor, Day 1, 0.25 hr in males). The vitreous humor contained quantifiable levels of aCT1 in at least one animal at each timepoint after dose administration on Days 1 and 7. aCT1 was measurable in retinal tissues of all rabbits on Day 1 at 0.25 hr, with concentrations ranging from 1.91 ng/g (female 040) to 24.2 ng/g (female 24.2 ng/g).

**[0097]** Concentrations of aCT1 in all tissues tended to decrease with time. There were no consistent differences in aCT1 distribution observed in male and female rabbits. All blood and

tissue samples collected prior to dose administration were below the lower limit of quantitation for aCT1. Systemic exposure to aCT1 was minor as shown by low levels of the test article in blood at 0.25 hr after ocular administration to the eye on Day 1. One male also had quantifiable levels of aCT1 at 4 hr on Day 7. aCT1 was rapidly absorbed into ocular tissues as the Tmax was 0.25 hr on Day 1, for all eye tissues.

**[0098]** In conclusion, the highest concentrations of aCT1 were observed in the palpebral conjunctiva, a tissue that is in direct contact with the dose formulation. The cornea and aqueous humor also had high exposure to aCT1. The solution also achieved delivery of ACT to the innermost tissues of the eye including the retina, a surprising result given the challenges associated with delivery of peptides to the eye as described herein.

**[0099]** Despite exposure of eye tissues and blood to aCT1, there were no adverse effect that could be attributed to the treatment with aCT1 and histopathology of the eyes exposed to aCT1 did not show any abnormal findings. Therefore, no maximum tolerated dose (MTD) was identified in the study. The no observed adverse effect level (NOAEL) is estimated to be at 1.0 μmol/eye when administered twice daily for 7 days.

**[0100]** Taken together, the results of the study indicated that HPMC can be used in an ophthalmic delivery system for aCT1 peptide and safely achieve therapeutic levels of aCT1 in tissues of interest including the retina.

**Example 5. Corneal regeneration and reepithelialization following corneal injury and treatment with aCT1 peptide in HPMC**

**[0101]** There are no FDA approved therapeutics that effectively accelerate corneal reepithelialization. Studies were conducted to determine if aCT1 peptide can induce and/or accelerate corneal healing following ocular injury.

**[0102]** In one study, 200 μM and 5 mM aCT1 formulations in 1% HPMC were tested for their ability to promote corneal regeneration following heptanol-induced corneal erosion (chemical burn injury) in rabbit eyes. The formulation comprised 1% w/w HPMC and 0.9% w/w NaCl, and excluded buffers, preservatives, other vehicles, and other excipients. aCT1 eye drops were administered immediately post-injury and then twice daily for 2 days. aCT1 accelerated corneal healing following chemical burn injury as measured by fluorescein staining (FIGS. 1 and 2A) and as measured by central corneal thickness (FIG. 2B).

**[0103]** In another study, rabbits were anesthetized and the corneas (bilateral) received a central 6.0 mm diameter x 150 μm deep injury (transepithelial PTK injury). Immediately after the injury, the cornea was stained with fluorescein and imaged to being monitoring the size of the injury. Eyes were treated with 150 μM aCT1 0.5% HPMC or a vehicle control. The results are shown in FIG. 3. Eyes treated with aCT1 peptide showed accelerated corneal healing. Given that timely epithelial resurfacing is critical to prevent loss of function, ocular morbidity, and vision loss, the early (within 3 days) difference between the aCT1 treated and control eyes is highly clinically significant.

**Example 6. aCT1 peptide treatment of sulfur mustard-induced ocular injury**

**[0104]** Sulfur mustard (SM) and nitrogen mustard (NM) are potent vesicating chemical warfare agents affecting the eyes, skin, and respiratory system. Among vesicating agents, SM has been most widely used in warfare resulting in injuries and battlefield causalities. The eye is the most sensitive tissue to vesicant exposure, with symptoms appearing 2-6 hrs after exposure, and healing occurring a few weeks later. Ocular exposure is associated with delayed injury symptoms: dryness, conjunctival scarring, decreased visual acuity, persistent corneal defects, inflammation, and neovascularization leading to progressive visual deterioration. Currently, there is no approved therapy for ocular exposure to ocular vesicants, including SM and NM.

**[0105]** Accordingly, a study was conducted to assess the therapeutic potential of aCT1 eye drops in the treatment of such a vesicating warfare agent. New Zealand white rabbits (n = 3 per treatment group) were exposed to 25 μL 1% nitrogen mustard in saline (NM). Corneas were treated with vehicle control (0.5% HPMC) or with 200 μM aCT1 in 0.5% HPMC, or with 5 mM aCT1 in 0.5% HPMC. The formulations comprised 0.5% w/w HPMC and 0.9% w/w NaCl, and excluded buffers, preservatives, other vehicles, and other excipients. Treatments were applied at 2 hours post-exposure, and then every 12 hours for 7 days. Healthy (uninjured) corneas were also untreated or treated with vehicle only. Animals were euthanized at 7 days post-exposure. Corneas were collected and processed for histology (H&E staining) or immunohistochemistry.

**[0106]** The results of the study are provided in FIGS. 4-7. ACT treatment limited corneal edema following NM exposure. FIGS. 4A and 4B show that aCT1 peptide limited corneal

edema damage compared to untreated and vehicle-treated NM-exposed corneas. aCT1 treatment also decreased pro-inflammatory response in the cornea following NM exposure (FIGS. 5A-5D). A pro-inflammatory response and the recruitment of inflammatory cells can exacerbate tissue damage leading to corneal opacity and scarring. H&E staining showed that aCT1 significantly decreases inflammatory cell infiltration in the corneal stroma in NM-exposed cornea compared to untreated and vehicle-treated NM-exposed corneas (FIGS. 5A and 5B). Expression of the pro-inflammatory enzyme COX-2 was also reduced in aCT1 treated groups (FIGS. 5C and 5D), which may in part mediate the reduction in inflammatory cell infiltration.

**[0107]** Collagen synthesis by corneal fibroblasts (also referred to as corneal keratocytes) is essential to stromal maintenance and regeneration, and increased expression and activity of matrix metallopeptidase-9 (MMP-9) in the corneal stroma leads to its degradation. aCT1 treatment prevented degradation and promoted regeneration of the corneal stroma following NM exposure, as evidenced by the protection of corneal fibroblasts/keratocytes in the aCT1 treated cornea (FIGS. 6A and 6B) as well as the reduction in MMP-9 expression in the stroma in aCT1 treated corneas (FIGS. 6C and 6D).

**[0108]** Ocular exposure to vesicating agents also induces corneal neovascularization, which results in corneal opacity and dysfunction. The study showed that aCT1 prevented corneal neovascularization. FIGS. 7A and 7B show that aCT1 treatment limited the formation of new blood vessels in the corneal stroma. The lack of new blood vessels in the treatment group corresponded to a pattern of decreased vascular endothelial growth factor (VEGF, a signaling factor that stimulates neovascularization) expression (FIGS. 7C and 7D).

**[0109]** Taken together, the results of the study showed that the administration of the aCT1 formulation was surprisingly potent in protecting against sulfur mustard-induced ocular injury and speeding the regeneration of the corneal stroma following such injury. The aCT1 peptide has positive effects on several cell types and activities necessary to corneal healing and thus is uniquely capable of effective treatment and prevention of corneal injuries.

**Example 7. Evaluation of an optimal aCT1 formulation for therapeutic use.**

**[0110]** Studies were conducted to evaluate characteristics of a novel aCT1 eye drop formulation composed of aCT1 peptide, sodium chloride, and HPMC (4000 mPaS; Table 16).

Surprisingly, this formulation, free of preservatives, excipients, or buffer solutions, provided a formulation that possessed a viscosity within recommended range for topical delivery to the eye and demonstrated peptide stability during storage. Furthermore, recovery testing of this formulation demonstrated feasibility of sterile filtration as well as compatibility of the formulation with validated HPLC test methods to confirm eye drop specifications (Tables 17 and 18). Assays were conducted to evaluate the compatibility of this formulation with sterile filtration and analytical method for determining peptide concentration. Compatibility with the analytical method is necessary for ensuring the product remains within specification. Complete recovery of aCT1 peptide was demonstrated following sterile filtration of this optimal formulation following storage in glass or plastic (Table 17). Peptide stability at 0, 1 and 3 months storage at -20°C, 5°C, and 25°C for formulations comprising 0.7% w/w or 1.8% w/w aCT1 peptide are shown in Table 19.

**[0111]** Accordingly, provided herein is a stable eye drop formulation having superior formulation properties for delivery of a peptide to an eye, including superior properties relative to various other vehicles tested and compared to formulations that include preservatives, excipients, or buffer solutions (see Examples 1-3). The formulations provided herein may be used to optimally deliver a peptide therapeutic agent such as aCT1 peptide to an eye for therapeutic use.

**Table 16. Optimal Eye Drop Formulation**

| **Components** | **Concentration (%w/w)** |
|---|---|
| aCT1 peptide | 0.08; 0.4; and 2.0 |
| Sodium Chloride (NaCl) | 0.9 |
| Hydroxypropyl Methylcellulose (HPMC), 4000 mPa.s | 0.5 |
| Purified Water | Q.S. |

**Table 17. Formulation Assay Results**

| **Sample** | **Storage** | **Peptide Conc (mg/mL)** | **% Label claim** |
|---|---|---|---|
| 0.8mg/mL (Tubing/filter study) | Stored in plastic falcon tube overnight at 2-8°C | 0.81 | 101.29 |

| | | | |
|---|---|---|---|
| 0.8 mg/mL (Tubing/filter study) | Stored in glass bottle overnight at 2-8°C | 0.82 | 101.97 |
| 0.8 mg/mL solution in amber vials with pump | Frozen (-20°C) | 0.82 | 102.51 |

**Table 18. Optimal Formulation Properties**

| **Property** | **Eye Drop Formulation** |
|---|---|
| Density | 0.99 g/mL |
| pH | 5.3 |
| Viscosity | 20.4 mPa.s |
| Osmolality | 288 mOsm/kg |

**Table 19. Peptide Stability testing of optimal eye drop formulation.**

| | **0.07% w/w (0.7 mg/ml) aCT1 peptide** | | | **1.8% w/w (18 mg/ml) aCT1 peptide** | | |
|---|---|---|---|---|---|---|
| **Temperature** | **0 months** | **1 months** | **3 months** | **0 months** | **1 months** | **3 months** |
| **-20° C** | 88.2 | 84.8 | 84.2 | 90.5 | 87.4 | 87.5 |
| **5° C** | 88.2 | 85.2 | 83.2 | 90.5 | 87.7 | 86.0 |
| **25° C** | 88.2 | 84.5 | 81.2 | 90.5 | 87.2 | 85.3 |

**Example 8. Excipient Effects**

**[0112]** Effects of various formulation components were assessed. Results are provided in Tables 20 and 21. NaCl provided better stabilization compared to sorbitol, particularly at concentrations above 50 mM. In addition, greater stability was observed at higher peptide concentrations.

**Table 20. Effects of NaCl vs. sorbitol (20 mg/mL peptide at pH 6.5)**

| Loss of Purity (%) | [NaCl] (mM) | Sorbitol (mM) |
|---|---|---|
| 0.67 | 0.67 | 0.67 |
| 0.24 | 0.24 | 0.24 |
| 0.03 | 0.03 | 0.03 |
| 0.76 | 0.76 | 0.76 |
| 1.05 | 1.05 | 1.05 |

**Table 21. Effects of [peptide]/pH**

| Loss of Purity (%) | [peptide] (mg/mL) | pH |
|---|---|---|
| 0.44 | 0.8 | 6.5 |

| | | |
|---|---|---|
| 0.39 | 5 | 6.5 |
| 0.30 | 10 | 6.5 |
| 0.19 | 15 | 6.5 |
| 0.06 | 20 | 6.5 |
| 0.33 | 20 | 7.0 |
| 0.50 | 20 | 7.5 |

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CLAIMS

1. A formulation comprising an active peptide having a molecular weight of about 1.0 kDa to about 10.0 kDa and hydroxypropyl methylcellulose (HPMC), wherein the formulation is suitable for topical ocular delivery.

2. The formulation of claim 1, wherein the HPMC is present in the formulation at a concentration of about 0.01% (w/w) to about 2.0% (w/w).

3. The formulation of claim 2, wherein the HPMC is present in the formulation at a concentration of about 0.05% (w/w) to about 0.5% (w/w).

4. The formulation of any one of claims 1-3, wherein the formulation further comprises sodium chloride (NaCl).

5. The formulation of claim 4, wherein the NaCl is present at a concentration of about 0.7% to about 1.5% (w/w).

6. The formulation of any one of claims 1-5, wherein the active peptide is present in the composition at a concentration of about 0.05% (w/w) to about 0.1% (w/w).

7. The formulation of any one of claims 1-6, wherein the formulation has a viscosity of about 18 to about 28 mPaS.

8. The formulation of any one of claims 1-7, wherein the active peptide is an alpha connexin peptide.

9. The formulation of claim 8, wherein the alpha connexin peptide comprises the carboxy terminal-most 4 to 30 contiguous amino acids of an alpha connexin.

10. The formulation of claim 9, wherein the alpha connexin peptide consists of the carboxy terminal-most 4 to 30 contiguous amino acids of an alpha connexin.

11. The formulation of claim 9, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5.

12. The formulation of claim 11, wherein the peptide comprises the amino sequence of SEQ ID NO: 2.

13. The formulation of any one of claims 1-12, wherein the peptide comprises a cellular internalization sequence.

14. The formulation of claim 13, wherein the cellular internalization sequence comprises an amino acid sequence of a protein selected from a group consisting of Antennapedia, TAT, HIV-Tat, Penetratin, Antp-3A (Antp mutant), Buforin II, Transportan, MAP (model amphipathic peptide), K-FGF, Ku70, Prion, pVEC, Pep-1, SynB 1, Pep-7, HN-1, BGSC (Bis-Guanidinium-Spermidine-Cholesterol) and BGTC (Bis-Guanidinium-Tren-Cholesterol).

15. The formulation of claim 14, wherein the cellular internalization sequence is Antennapedia, and wherein the sequence comprises the amino acid sequence of SEQ ID NO:7.

16. The formulation of claim 15, wherein the peptide comprises the amino acid sequence selected from the group consisting of SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12.

17. The formulation of claim 16 wherein the peptide comprises the amino acid sequence of SEQ ID NO:9.

18. The formulation of any one of claims 1-17, wherein the formulation is suitable for administration via eye drop.

19. A method of treating or preventing an ocular injury in a subject in need thereof, comprising topically administering a formulation of any one of claims 8-18 to the eye of the subject.

20. The method of claim 19, wherein the formulation is administered to the immediately after the event that caused the ocular injury.

21. The method of claim 19, wherein the polypeptide is administered to the subject at least about 2 hours following the event that caused the ocular injury.

22. The method of any one of claims 19-21, wherein the ocular injury is a corneal injury.

23. The method of any one of claims 19-21, wherein the ocular injury is caused by a burn or explosion.

24. The method of any one of claim 19-21, wherein the ocular injury is a chemical or thermal burn injury.

25. The method of any one of claims 19-21, wherein the ocular injury is caused by a chronic disease.

26. The method of claim 25, wherein the chronic disease is diabetes.

27. The method of claim 26, wherein the chronic disease is diabetic keratopathy.

28. The method of claim 25, wherein the chronic disease is retinal disease.

29. The method of any one of claims 19-21, wherein the injury is secondary to an ocular surgery.

30. The method of any one of claims 18-20, wherein the subject suffers from a persistent corneal epithelial defect.

31. The method of claim 30, wherein the persistent corneal epithelial defect arises from dry eye disease.

32. The method of any one of claims 19-21, wherein the ocular injury is a retinal injury.

33. A method for accelerating corneal reepithelialization following an ocular injury in a subject, the method comprising topically administering a formulation of any one of claims 7-17 to the eye of the subject.

34. The method of claim 33, wherein the ocular injury results from a surgery, a chemical injury, a corneal laceration injury, or chronic disease.

35. The method of claim 33, wherein the subject suffers from a persistent corneal epithelial defect.

36. A formulation according to any one of claims 8-18 for use in treating or preventing an ocular injury in a subject in need thereof.

37. A formulation according to any one of claims 8-18 for use in accelerating corneal reepithelialization following an ocular injury in a subject.

38. The formulation of claim 36 or 37, wherein the subject suffers from a persistent corneal epithelial defect.

FIG. 1

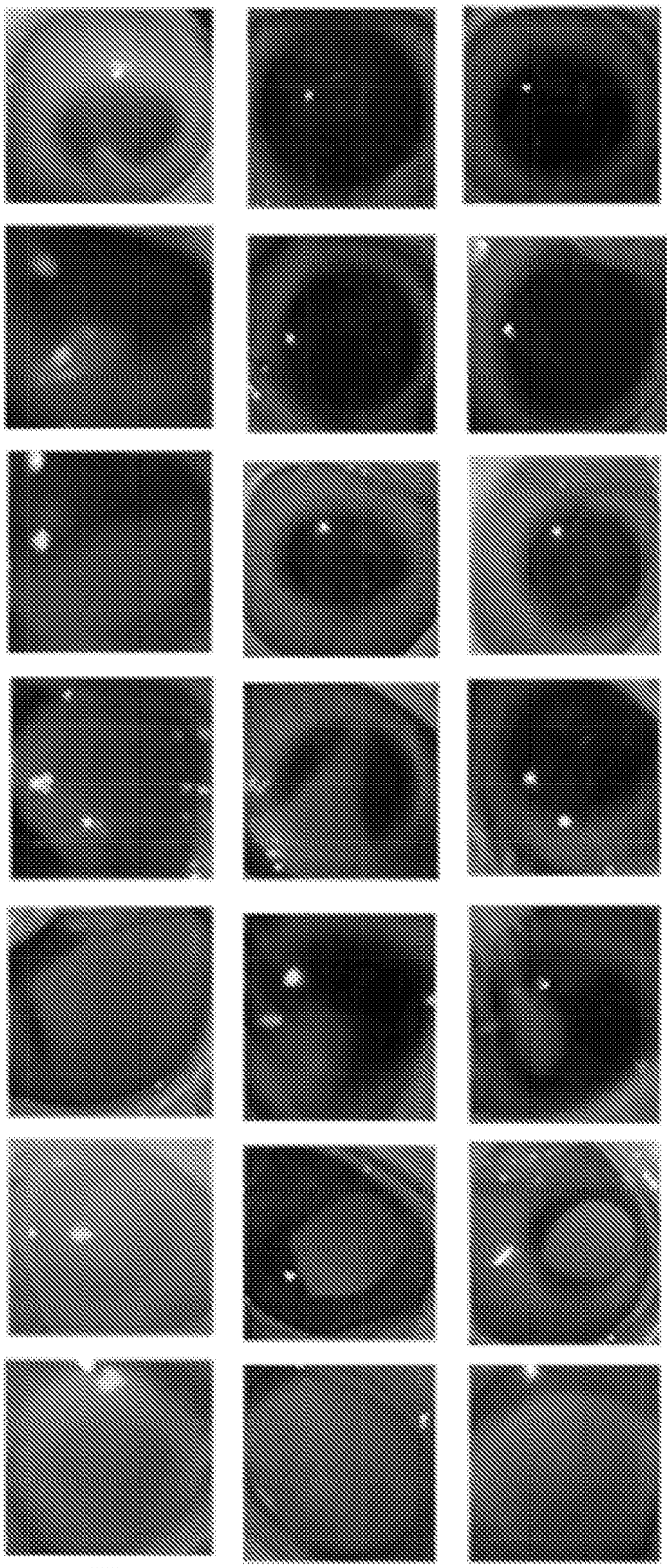

10 hrs
24 hrs
36 hrs
48 hrs
72 hrs
96 hrs
120 hrs
Vehicle Control
aCT1_200uM
aCT1_5mM

FIG. 2A

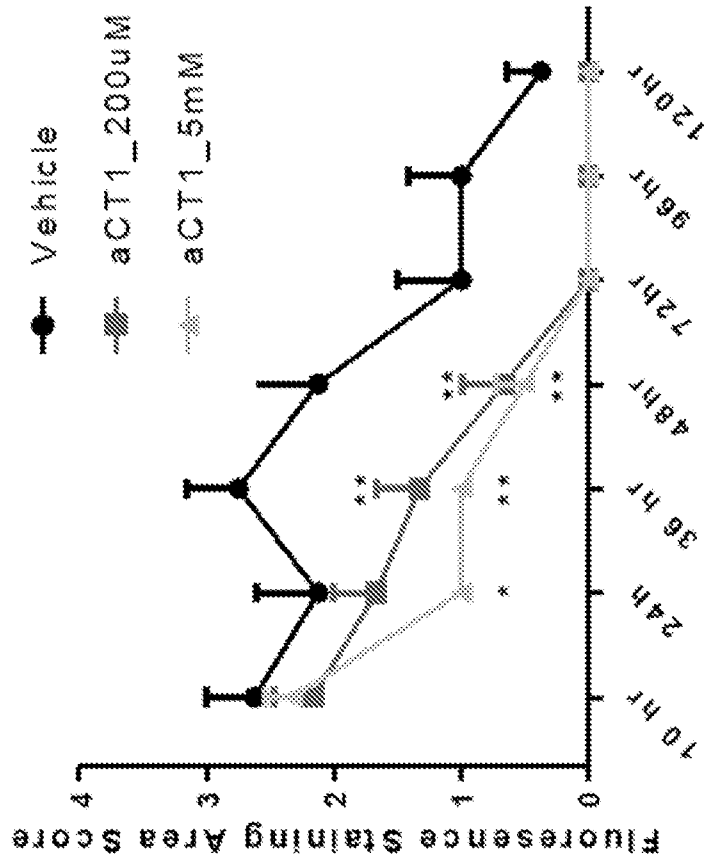

Vehicle
aCT1_200uM
aCT1_5mM
Fluorescence Staining Area Score
0
1
2
3
4
10 hr
24h
36 hr
48hr
72hr
96hr
120hr
*
**
**
**
**
**

FIG. 2B

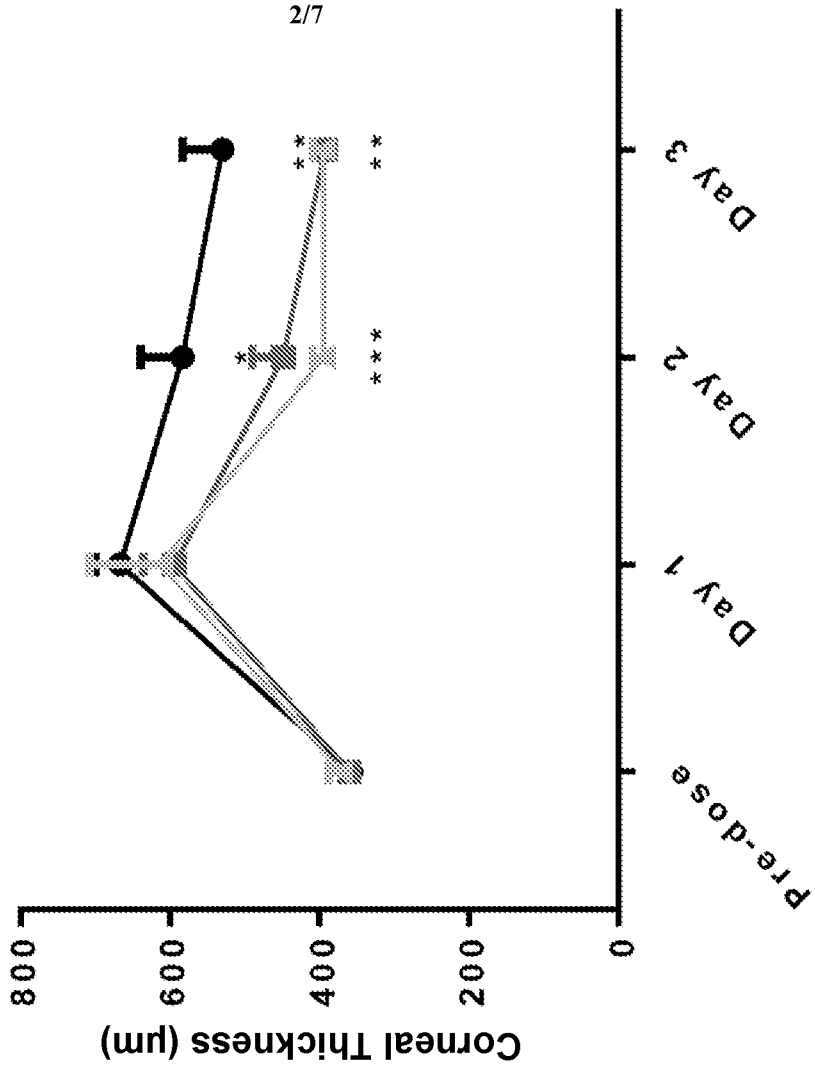

Corneal Thickness (μm)
0
200
400
600
800
Pre-dose
Day 1
Day 2
Day 3
*
**
***
**
**

FIG. 3

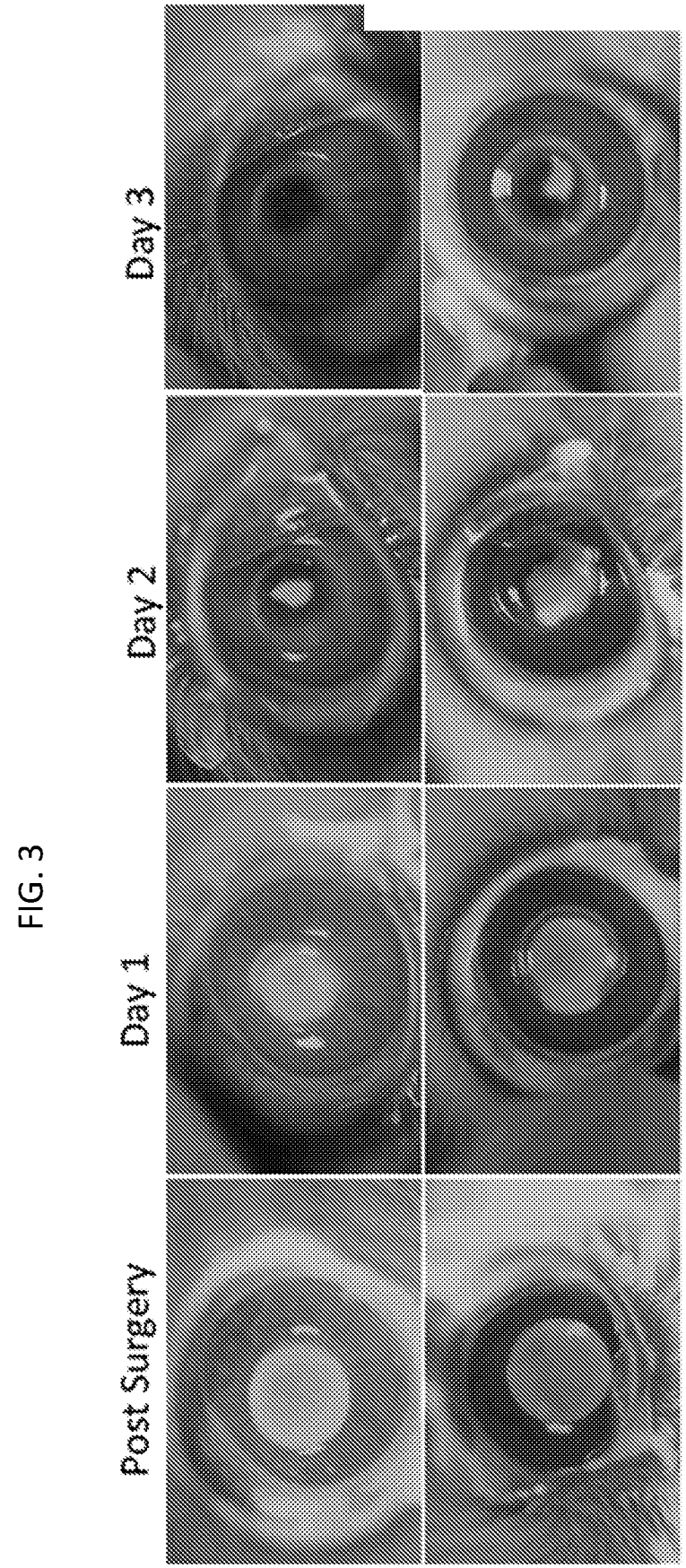


FIG. 4A-4B

FIG. 4A

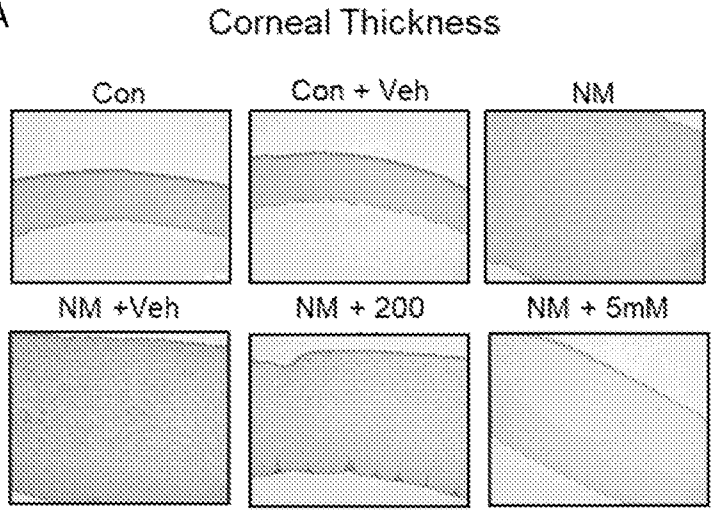



FIG. 4B

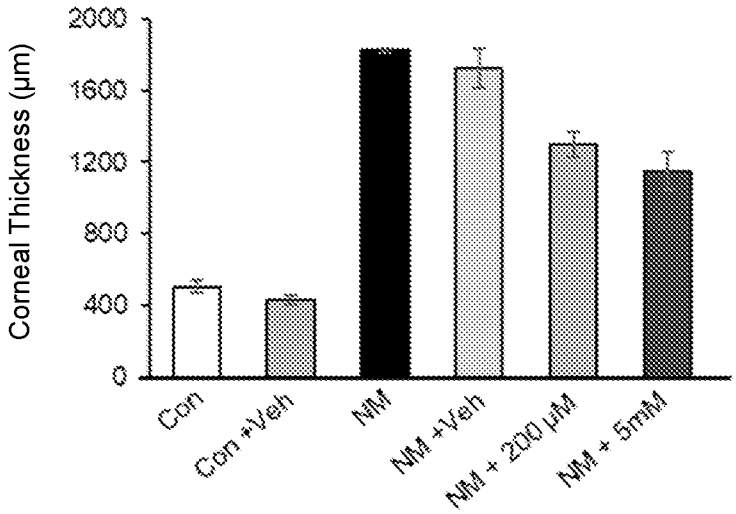


FIG. 5A-5D

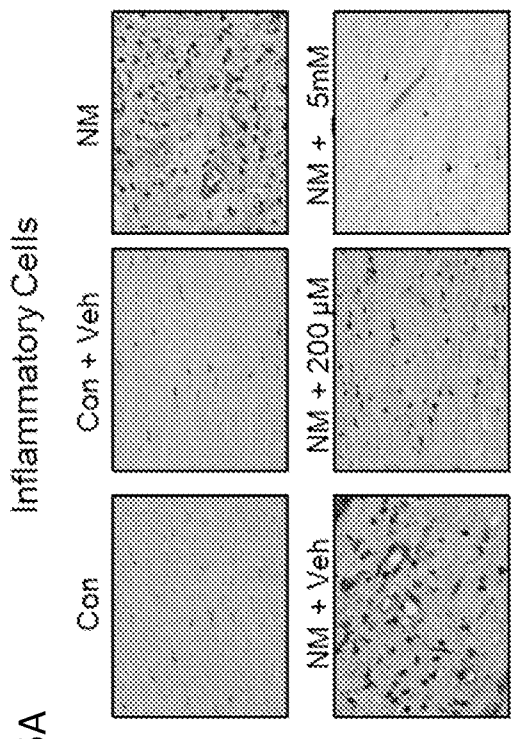



FIG. 5A

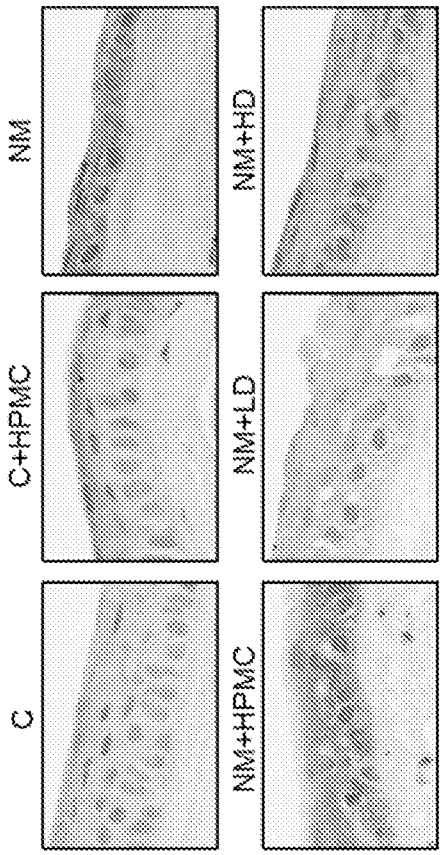



FIG. 5C

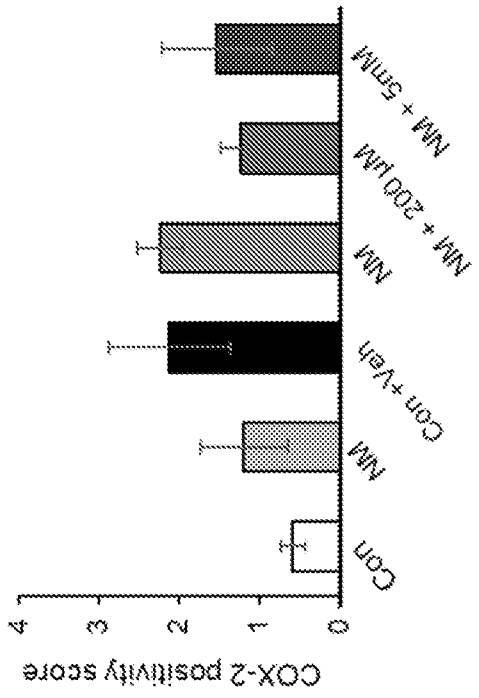



FIG. 5D

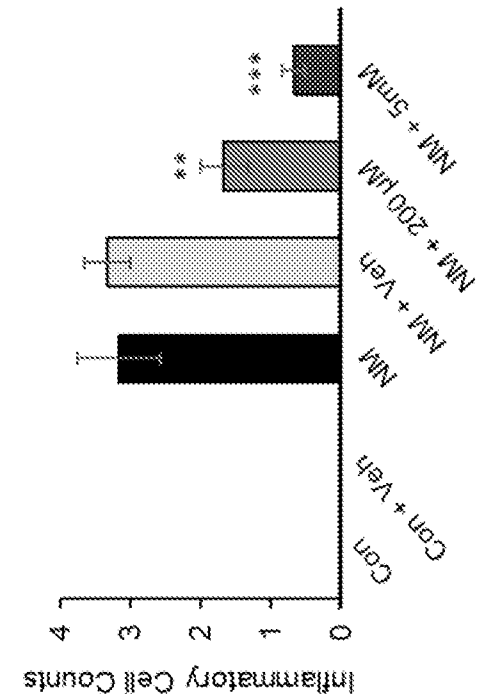



FIG. 5B

FIG. 6A-6D

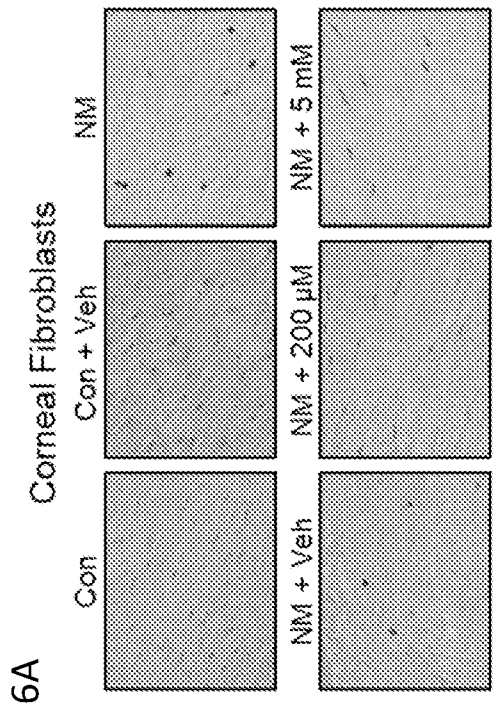



FIG. 6A

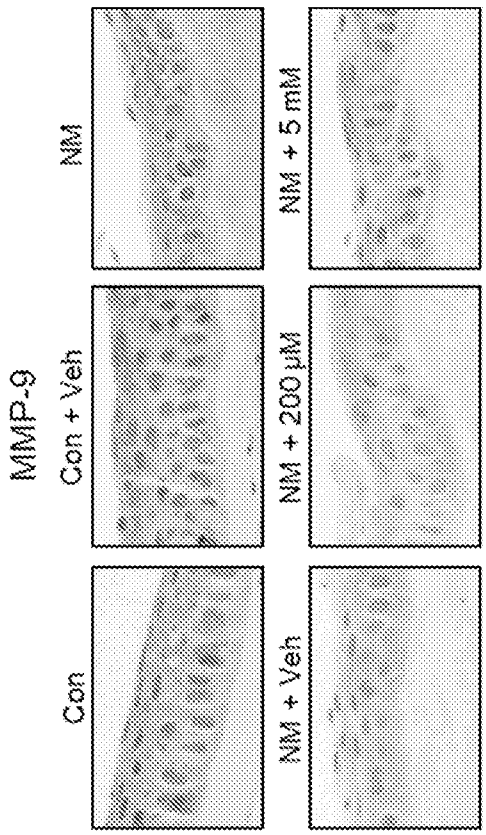



FIG. 6C

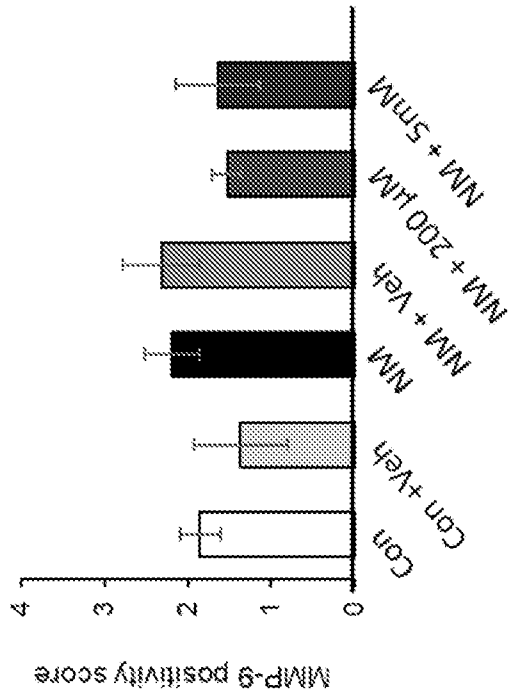



FIG. 6D

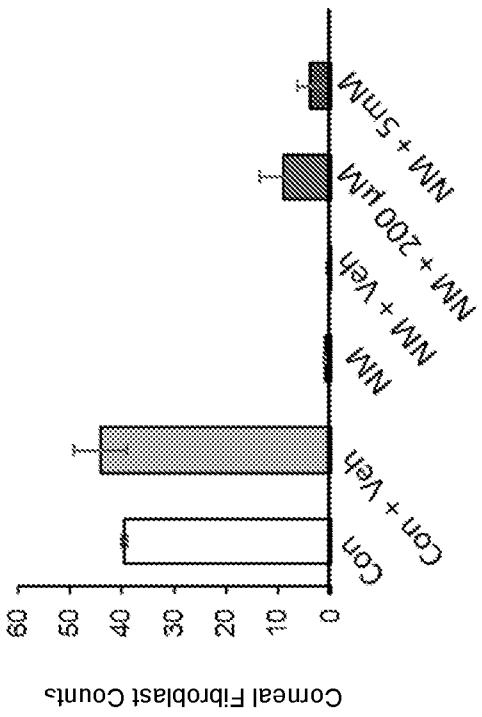



FIG. 6B

FIG. 7A-7D

FIG. 7A

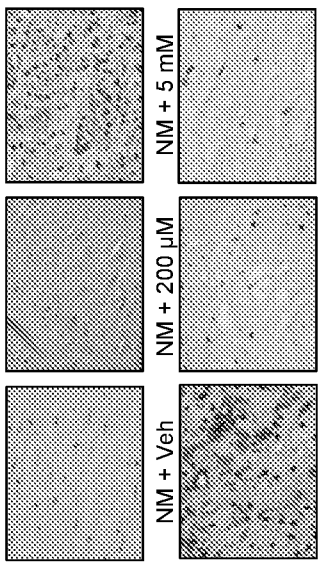



FIG. 7C

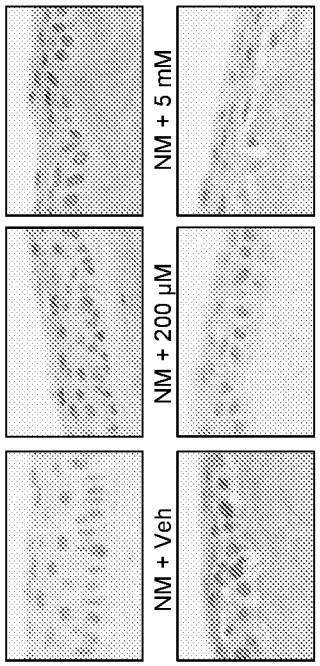



FIG. 7B

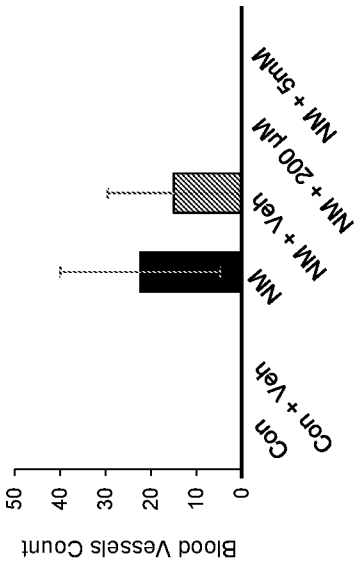
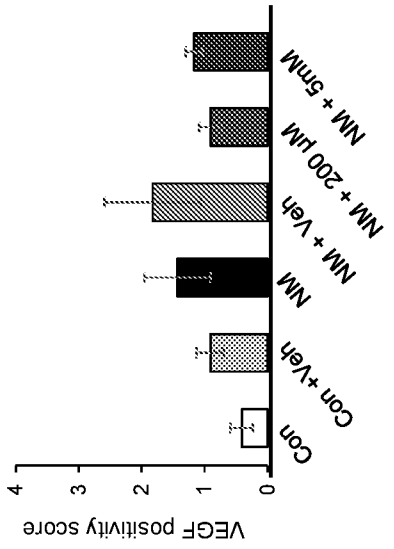



FIG. 7D



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/56231

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 27/02; C07K 14/705; A61K 38/08; A61K 38/14; A61K 38/45; A61K 38/50; A61K 45/06 (2021.01)

CPC - A61P 27/02; C07K 14/705; A61K 38/1709; A61K 38/08; A61K 38/14; A61K 38/164; A61K 38/168; A61K 38/45; A61K 38/50; A61K 45/06; A61K 9/1635; A61K 9/1694; A61K 9/19; A61K 9/5138; C07K 17/08; A61K 2300/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 2011/0130345 A1 (ROHRER BAERBEL) 02 June 2011; paragraphs [0013], [0029], [0039], [0203]; table 2 | 1-5 |
| Y | US 2002/0115589 A1 (NIXON JON C) 22 August 2002; paragraphs [0029], [0031], [0033], [0036] | 1-5 |
| A | US 2010/0317725 A1 (GARNER WILLIAM) 16 December 2010; see entire document for additional information | 1-5 |
| A | WO 2020/056144 A1 (FIRSTSTRING RESEARCH, INC.) 19 March 2020; see entire document for additional information | 1-5 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 26 January 2022 (26.01.2022) | FEB 14 2022 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Shane Thomas<br>Telephone No. PCT Helpdesk: 571-272-4300 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/56231

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US21/56231

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

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

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

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

3. ☒ Claims Nos.: 6-38
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

**Remark on Protest**

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

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

☐ No protest accompanied the payment of additional search fees.