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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
16 March 2017 (16.03.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/044743 A1

(51) International Patent Classification:
A61K 38/16 (2006.01) *C07K 14/00* (2006.01)
A61K 38/18 (2006.01)

(21) International Application Number:
PCT/US2016/050945

(22) International Filing Date:
9 September 2016 (09.09.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/217,611 11 September 2015 (11.09.2015) US

(71) Applicant: **THE SCHEPENS EYE RESEARCH INSTITUTE, INC.** [US/US]; 20 Staniford Street, Boston, MA 02114 (US).

(72) Inventors: **CHAUHAN, Sunil**; 173 Pleasant Street, Apt. 106, Cambridge, MA 02139 (US). **DANA, Reza**; 538 Chestnut Street, Newton, MA 02458 (US).

(74) Agents: **BEATTIE, Ingrid, A.** et al.; Mintz Levin Cohn Ferris Glovsky And Popeo, P.C., One Financial Center, Boston, MA 02111 (US).

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

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

Published:

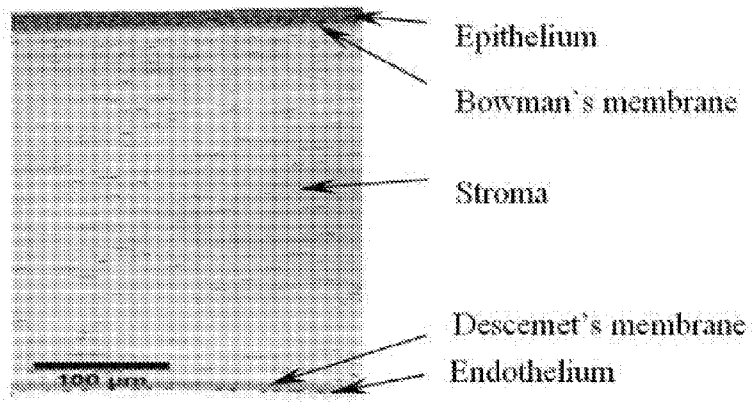
— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF CORNEAL HAZE AND SCARRING

FIG. 1



(57) Abstract: This application discloses ophthalmic formulations and methods for treating and preventing corneal haze and scarring with an hepatocyte growth factor (HGF) agent.

WO 2017/044743 A1

COMPOSITIONS AND METHODS FOR PREVENTION AND TREATMENT OF CORNEAL HAZE AND SCARRING

CROSS-REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Appl. No. 62/217,611, filed September 11, 2015, the content of which is incorporated herein by reference in its entirety and for all purposes.

FIELD OF THE DISCLOSURE

[0002] The present invention relates to compositions and methods for treating and preventing corneal haze and scarring.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING

[0003] The contents of the text file named 36770-548001WO_ST25.TXT, which was created on September 7, 2016, and is 6,610 bytes in size, are hereby incorporated by reference in their entireties.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0004] This invention was made with Government support under Grant No. W81XWH-11-1-0477 awarded by the Department of Defense. The Government has certain rights in this invention.

BACKGROUND OF THE DISCLOSURE

[0005] Corneal diseases and injuries account for the second leading non-refractive - cause of blindness affecting over 10 million people worldwide. A number of pathological conditions can lead to corneal scarring, including: injuries (e.g. chemical burns/industrial accidents); infection (e.g. contact lens-related infection or optical herpes); and laser vision correction (PRK). Ninety percent of blindness is permanent due to scarring and vascularization. Scarring caused via fibrotic cellular responses, heals the tissue, but fails to restore transparency.

[0006] There is a need for prevention or treatment of the significant effects of corneal haze and scarring resulting from disease or injury to prevent visual degradation, including blindness.

SUMMARY OF THE DISCLOSURE

[0007] Prior to the compositions and methods described herein, treatments for corneal haze and scarring were often associated with non-selective actions, harmful immunosuppression and/or secondary infection. The invention described herein provides a solution to these and other problems in the field of corneal haze and scarring. The invention described herein also relates to a pharmaceutical formulation for use in the treatment and prevention of corneal haze and scarring resulting from disease or injury. The invention also provides for methods for the treatment and prevention of corneal haze or scarring in a subject in need of such treatment by administering the formulations of the present invention (e.g., topically or subconjunctivally) directly to the eye, e.g., onto the surface of the cornea, or to a region, e.g., region adjacent to the cornea, of the eye of the subject. The subject is preferably a mammal in need of such treatment, e.g., a subject that has been diagnosed with corneal haze or scarring or a predisposition thereto. For example, the subject has suffered one or more injuries (e.g. chemical burns/industrial accidents), infection (e.g. contact lens-related infection or optical herpes), and/or laser vision correction (PRK) surgery or is expected to undergo a surgery. The mammal can be any mammal, e.g., a human, a primate, a mouse, a rat, a dog, a cat, a horse, as well as livestock or animals grown for food consumption, e.g., cattle, sheep, pigs, chickens, and goats. In a preferred embodiment, the mammal is a human.

[0008] Corneal haze or scarring is a clouding or reduction in transparency of the cornea. It can be a side effect of ocular disease, injury or surgery, e.g., as a result of an agressive wound response. Corneal haze or scarring describes a cloudy or opaque appearance of the cornea. The cornea is normally clear, so corneal haze can greatly impair vision. Although the haze or scarring can occur in any part of the cornea, it is most often found within the thicker, middle layer of the cornea, called the stroma. Corneal haze or scarring is most often caused by inflammatory cells and other debris that is activated during trauma, infection or surgery. Corneal haze or scarring can be graded on a scale of 1 to 4 as shown in Table 1 below. Accordingly, in embodiments, identifying a subject in need of treatment comprises determining or calculating the grade scale (e.g., trace, mild, moderate, or severe) of the subject's cornea transparency.

Table 1. Grading Scale for Corneal Haze/Scarring

None	0	Transparent, clear
Trace	+1	Minimal loss of transparency. Only the epithelium and/or the anterior half of the stroma is involved as observed with an optical section of the slit lamp.
Mild	+2	Dull-glass appearance. The cloudiness extends past the anterior half of the stroma.
Moderate	+3	Involvement of the entire thickness of the stroma. The affected stroma has lost its marble-like appearance and is homogeneously white. With optical section, the endothelium is still visible.
Severe	+4	Involvement of the entire thickness of the stroma. With optical section, cannot clearly visualize the endothelium.

[0009] A method of treating corneal haze or scarring is carried out by identifying a subject who has been identified as having experienced an incident or predilection to corneal haze or scarring, and administering to an ocular or adnexal tissue a composition comprising an effective amount of a purified hepatocyte growth factor (HGF) agent or compound that binds to and/or induces HGF or cMET mediated signal transduction. In the present context an HGF agent may include HGF, or a truncation, modification, mimetic, agonist or analog thereof. An HGF agent is able to induce HGF-mediated signal transduction by HGF-receptor (HGFR or cMET), and may include, without limitation, a natural protein, recombinant protein or peptide and fusion or chimeric protein able to bind HGFR, and biologic or chemical small molecule agonist of HGFR. A small molecule is a compound that is less than 2000 daltons in mass. The molecular mass of the small molecule is preferably less than 1000 daltons, more preferably less than 600 daltons, *e.g.*, the compound is less than 500 daltons, 400 daltons, 300 daltons, 200 daltons, or 100 daltons. HGF agents can be used alone or in conjunction with additional HGF agents or other therapies. However, HGF agents are not inclusive of other growth factors including epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), nerve growth factor (NGF) and synthetic or naturally occurring mixtures thereof including plasma rich in growth factors (PRGF).

[0010] The pharmaceutical formulations of the present invention (e.g., HGF agents) are formulated for ophthalmic delivery, e.g., corneal delivery or administration such that the corneal stroma is permeated. Stromal cells are physiologically and phenotypically different from epithelial cells and it is in the stroma, not any other layer of the cornea, where haze and

scarring is seen. For example, the pharmaceutical compositions are formulated for subconjunctival administration. In embodiments, the administration includes contacting the compositions described herein with corneal stroma or corneal stromal cells in a subject in need thereof. Alternatively, the pharmaceutical compositions are formulated for topical administration to the eye or region of the eye. For example, the formulation may comprise one or more tear substitutes. The formulation alternatively comprises an ophthalmic lubricant.

[0011] The pH of the formulation is between 5.5 and 7.5 (e.g., about 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5). For example, the pH of the formulation is about 7.4. The formulation is in the form of a single dose unit or in the form of a multi-dose system.

[0012] Suitable forms of the composition include a solid, a paste, an ointment, a gel, a liquid, an aerosol, a mist, a polymer, a film, an emulsion, or a suspension. In some cases, the composition is incorporated into or coated onto a contact lens. Preferably, the formulation is an aqueous formulation. The term "aqueous" typically denotes an aqueous composition wherein the carrier is to an extent of >50%, more preferably >75% and in particular >90% by weight water.

[0013] Polynucleotides, polypeptides, or other agents are purified and/or isolated. Specifically, as used herein, an "isolated" or "purified" nucleic acid molecule, polynucleotide, polypeptide, or protein, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized. Similarly, cell populations are substantially free of other cellular material, or culture medium. Purified compounds are at least 60% by weight (dry weight) the compound of interest. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight the compound of interest. For example, a purified compound is one that is at least 90%, 91%, 92%, 93%, 94%, 95%, 98%, 99%, or 100% (w/w) of the desired compound by weight. Purity is measured by any appropriate standard method, for example, by column chromatography, thin layer chromatography, or high-performance liquid chromatography (HPLC) analysis. A purified or isolated polynucleotide (ribonucleic acid (RNA) or deoxyribonucleic acid (DNA)) is free of the genes or sequences that flank it in its naturally-occurring state. A purified or isolated polypeptide is free of the amino acids or sequences that flank it in its naturally-occurring state. Purified also

defines a degree of sterility that is safe for administration to a human subject, e.g., lacking infectious or toxic agents.

[0014] In some cases, the method further comprises the administration of a second therapeutic agent. For example, a second therapeutic agent includes a steroid, other biologic or small molecule-based anti-inflammatory treatments (e.g. cytokine targeting).

[0015] The HGF agent is administered at a frequency that affords optimal effectiveness. For example, the HGF agent is administered every 72 hours, every 48 hours, every 24 hours, every 12 hours, every 6 hours, every 3 hours, every 1 hour, or any other appropriate interval. The HGF agent is administered for 1 day, 2 days, 3 days, 4 days, 5 days, 7 days, 14 days, 30 days, 60 days, 90 days, or 120 days. Administration may be following an injury or insult to the corneal, or administered prior to surgery to prevent corneal haze and scarring from occurring. Injury or insult to the cornea may be the result of injuries (e.g. chemical burns/industrial accidents); infection (e.g. contact lens-related infection or optical herpes); and laser vision correction (PRK). Alternatively, the HGF agent is administered for long-term use, i.e., more than 120 days, more than 150 days, more than 180 days, more than 210 days, more than 240 days, more than 270 days, more than 300 days, more than 330 days or more than 360 days.

[0016] Also provided is a method of preventing corneal haze or scarring comprising identifying a subject who is at risk for developing corneal haze or scarring, and administering to an ocular or adnexal tissue a composition comprising an effective amount of an HGF agent. In some cases, the subject is asymptomatic, but at high risk for developing corneal haze or scarring.

[0017] Optionally, the method further comprises the administration of a pharmaceutically acceptable carrier. The phrase "pharmaceutically acceptable" is art-recognized and refers to compositions, polymers and other materials and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The method may further comprise administration of an "ophthalmically acceptable" carrier.

[0018] The phrase "pharmaceutically acceptable carrier" is art-recognized, and refers to, for example, pharmaceutically acceptable materials, compositions or vehicles, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting any supplement or composition, or component thereof, from one organ, or

portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the supplement and not injurious to the patient. Optionally, a pharmaceutically acceptable carrier is non-pyrogenic. Some examples of materials which may serve as pharmaceutically acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, sunflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; (21) liposome-based excipients; (22) cyclodextrin; (23) nanoparticle-based excipients and (24) other non-toxic compatible substances employed in pharmaceutical formulations.

[0019] Excipients for topical administration of the compositions of the present invention preferentially promote drug penetration through the ocular surface and corneal epithelium and into the corneal stroma. Example excipients that are suitable for preferential drug delivery, e.g., at least 10%, 25%, 50%, 75%, 2-fold, 5-fold, 10-fold, or more, to the corneal stroma compared to other ocular anatomical locations include polysaccharides and derivatives thereof (e.g., chitosan, n-carboxymethyl chitosan, chitosan HCL, N-trimethylchitosan, xyloglucan, hyaluronic acid, alginic acid, cellan gum, cyclodextrans, etc.); nanoparticles (e.g., nanoparticles conjugated, e.g., covalently linked, to a drug of the present invention); liposomes, e.g., into which or onto which the compound/therapeutic agent is associated or linked ; surfactants; benzalkonium chloride; and EDTA. A liposome is spherical vessel having at least one lipid bilayer. A nanoparticle is a microscopic particle with at least one dimension less than 100 nm. These excipients may be used as a single excipient or in combinations.

[0020] The phrase "ophthalmically acceptable" refers to compositions comprising excipients, emulsifiers, wetting agents, carriers or fillers that are suitable for application to the tissues of the eye and eye area. Such ophthalmically acceptable compositions may comprise for example, the polyethylene glycols designated 200, 300, 400 and 600, or

Carbowax designated 1000, 1500, 4000, 6000 and 10000, complexing agents, such as disodium-EDTA or EDTA, antioxidants, such as ascorbic acid, acetylcysteine, cysteine, sodium hydrogen sulfite, butyl-hydroxyanisole, butyl-hydroxy- toluene; stabilizers, such as thiourea, thiosorbitol, sodium dioctyl sulfosuccinate or monothioglycerol; or other excipients, such as, for example, lauric acid sorbitol ester, triethanol amine oleate or palmitic acid ester.

[0021] Other carriers and excipients are known in the art, for example those described in: Kreuter, J. "Nanoparticles" *Colloidal Drug Delivery Systems*, edited by Jork Kreuter, Marcel Dekker, New York, N.Y. (USA), chapter 5, page 219 (1994); Gurny, R. "Ocular therapy with nanoparticles" *Polymeric Nanoparticles and Microspheres* edited by P. Guiot and P. Couvreur, Boca Raton, Fla. (USA): CRC Press, page 127 (1986); Gurny, R. "Preliminary study of prolonged acting drug delivery system for the treatment of glaucoma" *Pharm Acta Helv.*, volume 56, page 130 (1981); Zimmer, et al. "J. Microspheres and nanoparticles used in ocular delivery systems" *Advanced Drug Delivery Reviews*, volume 16, number 1, pages 61-73 (1995); Zambito, et al. "Polysaccharides as excipients for Ocular Topical Formulations" *Biomaterials Applications for Nanomedicine*, Prof. Rosario Pignatello (Ed.), ISBN: 978-953-307-661-4, InTech, Available from: http://www.intechopen.com/books/biomaterials-applications-for-nanomedicine/polysaccharidesas-excipients-for-ocular-topical-formulations at pages 253-284.; Kompella, et al. "Recent Advances in Ophthalmic Drug Delivery" *Ther Deliv*, 2010 September 1, 1(3): 435-456.; McCann, J., "Advances and Challenges in Topical Ocular Medications" *Advanced Ocular Care*, March 2011., at pages 23-25; and Calvo, et al. "Comparative in vitro evaluation of several colloidal systems, nanoparticles, nanocapsules, and nanoemulsions, as ocular drug carriers" *J Pharm Sci*, volume 85, number 5. pages 530-536 (May 1996). These references are incorporated in their entirety by reference herein.

[0022] As used herein, the term "tear substitute" refers to molecules or compositions which lubricate, "wet," approximate the consistency of endogenous tears, aid in natural tear build-up, or otherwise provide temporary relief of dry eye symptoms and conditions upon ocular administration.

[0023] The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymers.

[0024] Similarly, by "substantially pure" is meant a nucleotide or polypeptide that has been separated from the components that naturally accompany it. Typically, the nucleotides and polypeptides are substantially pure when they are at least 60%, 70%, 80%, 90%, 95%, or even 99 - 100%, by weight, free from the proteins and naturally-occurring organic molecules with they are naturally associated.

[0025] "Conservatively modified variations" of a particular polynucleotide sequence refers to those polynucleotides that encode identical or essentially identical amino acid sequences, or where the polynucleotide does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance, the codons CGU, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent substitutions" or "silent variations," which are one species of "conservatively modified variations." Every polynucleotide sequence described herein which encodes a polypeptide also describes every possible silent variation, except where otherwise noted. Thus, silent substitutions are an implied feature of every nucleic acid sequence which encodes an amino acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule by standard techniques.

[0026] Similarly, "conservative amino acid substitutions," in one or a few amino acids in an amino acid sequence are substituted with different amino acids with highly similar properties are also readily identified as being highly similar to a particular amino acid sequence, or to a particular nucleic acid sequence which encodes an amino acid. Such conservatively substituted variations of any particular sequence are a feature of the present invention. Individual substitutions, deletions or additions which alter, add or delete a single amino acid or a small percentage of amino acids (typically less than 5%, more typically less than 1%) in an encoded sequence are "conservatively modified variations" where the alterations result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. *See*, e.g., Creighton (1984) Proteins, W.H. Freeman and Company, incorporated herein by reference. Table 2 below provides example amino acid substitutions.

Table 2. Conservative Amino Acid Substitutions.

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr
Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val
Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

[0027] By "isolated nucleic acid" is meant a nucleic acid that is free of the genes which flank it in the naturally-occurring genome of the organism from which the nucleic acid is derived. The term covers, for example: (a) a DNA which is part of a naturally occurring genomic DNA molecule, but is not flanked by both of the nucleic acid sequences that flank that part of the molecule in the genome of the organism in which it naturally occurs; (b) a nucleic acid incorporated into a vector or into the genomic DNA of a prokaryote or eukaryote in a manner, such that the resulting molecule is not identical to any naturally occurring vector or genomic DNA; (c) a separate molecule such as a cDNA, a genomic fragment, a fragment produced by polymerase chain reaction (PCR), or a restriction fragment; and (d) a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a fusion protein. Isolated nucleic acid molecules according to the present invention further include molecules produced synthetically, as well as any nucleic acids that have been altered chemically and/or that have modified backbones. For example, the isolated nucleic acid is a purified cDNA or RNA polynucleotide.

[0028] The term "percent sequence identity" or "percentage sequence identity" refers to the overlap of sequences in an amino acid or nucleic acid sequence. As used herein, the terms

"identity" or "percent identity", refers to the subunit sequence similarity between two polymeric molecules, *e.g.*, two polynucleotides or two polypeptides. When a subunit position in both of the two molecules is occupied by the same monomeric subunit, *e.g.*, if a position in each of two peptides is occupied by serine, then they are identical at that position. The identity between two sequences is a direct function of the number of matching or identical positions, *e.g.*, if half (*e.g.*, 5 positions in a polymer 10 subunits in length), of the positions in two peptide or compound sequences are identical, then the two sequences are 50% identical; if 90% of the positions, *e.g.*, 9 of 10 are matched, the two sequences share 90% sequence identity. The identity between two sequences is a direct function of the number of matching or identical positions. Thus, if a portion of the reference sequence is deleted in a particular peptide, that deleted section is not counted for purposes of calculating sequence identity. Identity is often measured using sequence analysis software *e.g.*, BLASTN or BLASTP (available at the world wide web site ("www") of the National Center for Biotechnology Information (".ncbi") of the National Institutes of Health (".nih") of the U.S. government (".gov"), in the "Blast" directory ("/BLAST/"). The default parameters for comparing two sequences (*e.g.*, "Blast"-ing two sequences against each other), by BLASTN (for nucleotide sequences) are reward for match = 1, penalty for mismatch = -2, open gap = 5, extension gap = 2. When using BLASTP for protein sequences, the default parameters are reward for match = 0, penalty for mismatch = 0, open gap = 11, and extension gap = 1. Additional, computer programs for determining identity are known in the art.

[0029] "Similarity" or "percent similarity" in the context of two or more polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues, or conservative substitutions thereof, that are the same when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms, or by visual inspection.

[0030] HGF agents of the present invention may include a polypeptide having an amino acid sequence that is at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, or at least 99.9% identical to human HGF (SEQ ID NO:1).

[0031] Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid and the phrase "nucleic acid sequence" refers to the linear list of nucleotides of the nucleic acid molecule, the two phrases can be used interchangeably.

[0032] By the terms “effective amount” and “therapeutically effective amount” of a formulation or formulation component is meant a sufficient amount of the formulation or component, alone or in a combination, to provide the desired effect. For example, by "an effective amount" is meant an amount of a compound, alone or in a combination, required to reduce or prevent corneal haze or scarring disease in a mammal. In some cases, an effective amount is an amount sufficient to inhibit differentiation of corneal fibroblasts upon treatment with a composition described herein for at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the level without treatment of a composition described herein. In some cases, an effective amount is an amount sufficient to inhibit α-smooth muscle actin (αSMA) expression upon treatment with a composition described herein for at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the level without treatment of a composition described herein. In some cases, an effective amount is an amount sufficient to increase stratification of corneal epithelial cells upon treatment with a composition described herein for at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the level without treatment of a composition described herein. In some cases, an effective amount is an amount sufficient to increase c-met expression upon treatment with a composition described herein for at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more compared to the level without treatment of a composition described herein. In some cases, an effective amount is an amount sufficient to restore the thickness of an injured cornea to about 50%, 60%, 70%, 80%, 90%, 95% or higher percentage of the normal (i.e., healthy) cornea thickness. Ultimately, the attending physician or veterinarian decides the appropriate amount and dosage regimen.

[0033] The terms "treating" and "treatment" as used herein refer to the administration of an agent or formulation to a clinically symptomatic individual afflicted with an adverse condition, disorder, or disease, e.g., corneal haze or scarring, so as to effect a reduction in severity and/or frequency of symptoms, eliminate the symptoms and/or their underlying cause, and/or facilitate improvement or remediation of damage.

[0034] The terms "preventing" and "prevention" refer to the administration of an agent or composition to a clinically asymptomatic individual who is susceptible or predisposed to a particular adverse condition, disorder, or disease, and thus relates to the prevention of the occurrence of symptoms and/or their underlying cause, *e.g.* being characterized as a PRK patient, chemical burn victim, or ocular injury victim.

[0035] The transitional term "comprising," which is synonymous with "including," "containing," or "characterized by," is inclusive or open-ended and does not exclude additional, unrecited elements or method steps. By contrast, the transitional phrase "consisting of" excludes any element, step, or ingredient not specified in the claim. The transitional phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention.

[0036] Human hepatocyte growth factor (GenBank Accession No.: P14210.2) has the following amino acid sequence (SEQ ID NO: 1):

1 mwvtkllpal llqhvllhll llpiaipyae gqrkrrntih efkksakttl ikidpalkik
61 tkkvntadqc anrctrnkgl pftckafvfd karkqclwfp fnsmssgvkk efghefdlye
121 nkdyirncii gkgrsykgtv sitksgikcq pwssmipheh sflpssyrgk dlqenycrnp
181 rgeeggpwcf tsnpevryev cdipqcseve cmtcngesyr glmdhtesgk icqrwdhqtp
241 hrhkflpery pdkgfddnyc rnpdgqprpw cytldphtrw eycaiktcad ntmndtdvpl
301 etteciqgqg egyrgtvnti wngipcqrwd sqyphehdmt penfkckdlr enycrnpdgs
361 espwcfttdp nirvgycsqi pncdmshgqd cyrgngknym gnlsqtrsgl tcsmwdknme
421 dlhrhifwep dasklnenyc rnpdddahgp wcytgnplip wdycpisrce gdttptivnl
481 dhpviscakt kqlrvvngip trtnigwmvs lryrnkhicg gslikeswvl tarqcfpsrd
541 lkdyeawlgi hdvhgrgdek ckqvlnvsql vygpegsdlv lmklarpavl ddfvstidlp
601 nygctipekt scsvygwgyt glinydgllr vahlyimgne kcsqhhrgkv tlneseicag
661 aekigsgpce gdyggplvce qhkmrmvlgv ivpgrgcaip nrpgifvrva yyakwihkii
721 ltykvpqs

[0037] HGF agents may comprise full length HGF (SEQ ID NO: 1). HGF agents may also comprise truncated HGF or certain domains of the full length peptide, e.g., fragments of the full length or parent protein, e.g., HGF. The term "fragment," as used herein, means a portion of a polypeptide or polynucleotide that is less than the entire polypeptide or polynucleotide. As used herein, a "functional fragment" of a reference protein, e.g., HGF, is a fragment of the polypeptide that is shorter than the full-length, immature, or mature polypeptide and has at least 25% (e.g., at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or even 100% or more) of the activity of full-length mature reference protein. For example, the activity to reduce corneal haze or scarring. Methods of establishing whether a fragment of HGF is functional/active are known in the art, e.g., as determined by the criteria described in Table 1. For example, fragments of interest can be made by either recombinant, synthetic, or proteolytic digestive methods. Such fragments can then be isolated and tested for their ability to co-stimulate T cells by procedures described herein.

[0038] For example, the mature protein comprises amino acid 32-494 (underlined). Other examples include, amino acids 126-207, 208-289, 302-384, and 388-470 each of which comprise Kringle domains which may each, individually be involved in binding mediators.

[0039] Accordingly, the HGF agents described herein may include a polypeptide having an amino acid sequence of SEQ ID NO: 1. In embodiments, the HGF agents described herein may include a polypeptide having an amino acid sequence of residues 32-494 of SEQ ID NO: 1. In embodiments, the HGF agents described herein may include a polypeptide having an amino acid sequence of residues 126-207 of SEQ ID NO: 1. In embodiments, the HGF agents described herein may include a polypeptide having an amino acid sequence of residues 208-289 of SEQ ID NO: 1. In embodiments, the HGF agents described herein may include a polypeptide having an amino acid sequence of residues 302-384 of SEQ ID NO: 1. In embodiments, the HGF agents described herein may include a polypeptide having an amino acid sequence of residues 388-470 of SEQ ID NO: 1.

[0040] The HGF agents described herein may include a fragment of SEQ ID NO: 1. A fragment can be between 3-10 amino acids, 10-20 amino acids, 20-40 amino acids, 40-56 amino acids in length or even longer. Amino acid sequences having at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% identity, and most preferably 95% identity to the fragments described herein are also included within the scope of the invention described herein.

[0041] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All published foreign patents and patent applications cited herein are incorporated herein by reference. Genbank and NCBI submissions indicated by accession number cited herein are incorporated herein by reference. All other published references, documents, manuscripts and scientific literature cited herein are incorporated herein by reference. In the case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] FIG. 1 is a diagram of corneal anatomy identifying the layers of the cornea.

[0043] FIG. 2A is a photograph showing representative slit-lamp (bright-field and fluorescein) biomicroscopy images of injured cornea. FIG. 2B is a bar graph displaying quantification of corneal opacity (haze/scarring) using Image J software shows a significant reduction in development of corneal opacity in HGF-treated mice compared to albumin-treated control group. FIG. 2C is a bar graph showing quantification of fluorescein-stained area using Image J software shows significant reduction of fluorescein staining (i.e. injured area) in HGF-treated mice compared to control group. (N = 5-6 mice/group). FIG. 2A – FIG. 2C show images and quantification of corneal injury at days 1, 3, 5 and 7 post injury, photographs of injured cornea (with or without fluorescein (green) staining) were captured using slit-lamp biomicroscopy.

[0044] FIG. 3A is a bar graph and FIG. 3B is a series of photographs demonstrating that HGF inhibits expression of α-smooth muscle actin (αSMA: a factor that causes scarring) by corneal keratocytes. *In vitro* analysis of murine corneal keratocytes (MK/T1) shows that HGF significantly inhibits TGFp-induced expression of αSMA in keratocytes as measured by real time PCR (FIG. 3A) and immunohistochemistry (FIG. 3B).

[0045] FIG. 4 is a confocal microscopy image showing immunostaining for pan leukocyte marker CD45 in corneas harvested 3-days post injury. Corneal injury was induced by mechanical removal of the complete corneal epithelium using ALGBERRUSH-II™ in C57BL6 mice. Thereafter, murine recombinant HGF was topically applied (dose: 3µl of 0.01% HGF in PBS per eye) to the injured eye twice daily. A Control group received a similar dosage of mouse serum albumin. Blue coloration indicates DAPI staining of cell nuclei; green coloration indicates the presence of CD45, a pan-leukocyte marker.

[0046] FIG. 5A is an image of representative micrographs showing tissue structure of normal, control injured and HGF-treated corneas. FIG. 5B is a bar graph of cumulative data showing HGF-treated corneas restore their thickness to a thickness similar to that of normal corneas. Injured control corneas show significant increase in the thickness as compared to normal and HGF-treated corneas. (N = 5 mice/group).

[0047] FIG. 6 is a bar graph showing that HGF inhibits differentiation of human corneal fibroblasts into myofibroblasts. Human corneal fibroblasts were stimulated with human recombinant TGFβ1 (100ng/ml, Peprotech) in the presence or absence of rhHGF (10ng/ml, R&D Systems) for 24hrs. αSMA expression (normalized to internal control GAPDH) was

evaluated using real-time PCR. The values shown are the mean ± SD (error bars) from three independent experiments performed in triplicates; * $p<0.02$, ** $p<0.001$.

[0048] FIG. 7A is a series of images and FIG. 7B is a bar graph showing that HGF augments stratification of epithelial cells after corneal injury. FIG. 7A: At 7 days post injury, corneas were harvested from normal, albumin-treated and HGF-treated mice. Corneal cross sections were stained with the nuclear stain DAPI to visualize corneal epithelial cell layer using confocal microscope (400X). FIG. 7B: Bar chart showing the thickness (μm) of the epithelial cell layer in normal (white bar), control-injured and HGF-treated injured corneas (black bar). The values shown are the mean ± SD (error bars); n=5 mice/group.

[0049] FIG. 8 is bar graph showing that HGF promotes HGF-R (c-met) expression in the cornea after injury. At 3 and 7 days post injury, corneas were harvested from normal (checked bar), mouse albumin-treated control injured (white bar) and HGF-treated (black bar) injured groups. Total RNA was isolated from harvested corneas. HGF-R mRNA expression was quantitated using real-time PCR. GAPDH was used as a internal control. The values shown are the mean ± SD and, each group consists of n=6 mice, *$p<0.03$, **$p<0.01$.

DETAILED DESCRIPTION OF THE INVENTION

[0050] The present invention provides compositions, methods and treatment for corneal haze and scarring. The method for the treatment and prevention of corneal haze and scarring in humans is carried out by therapeutic administration of HGF or a fragment or agonists thereof onto or into the cornea or in combination with either pharmaceutically suitable vehicle or another therapeutic agent. The HGF agent comprises HGF or an agent able to induce HGF-mediated signal transduction by HGF-receptor (HGFR or cMET), and may include, without limitation, a natural protein, recombinant protein or peptide and fusion or chimeric protein able to bind HGFR, and biologic or chemical small molecule agonist of HGFR.

Corneal Anatomy

[0051] The cornea is comprised of multiple layers with different thickness, cellular make-up and function. Layers of the cornea include the epithelium, bowman's membrane or layer, the stroma, Descemet's membrane or layer, and the endothelium. Each of the layers is illustrated in FIG. 1.

[0052] The epithelium is the layer of cells that cover the surface of the cornea. It is only about 5-6 cell layers thick and quickly regenerates when the cornea is injured. If the injury

penetrates more deeply into the cornea, it may leave a scar. Scars leave opaque areas, causing the corneal to lose its clarity and luster.

[0053] Bowman's membrane lies just beneath the epithelium. Because this layer is very tough and difficult to penetrate, it protects the cornea from injury.

[0054] The stroma is the thickest layer and lies just beneath Bowman's membrane. It is composed of tiny collagen fibrils that run parallel to each other. This special formation of the collagen fibrils gives the cornea its clarity. HGF agents act at the stroma of the cornea to prevent and treat corneal haze or scarring. HGF agents inhibit expression of α-smooth muscle actin within the keratocytes of the corneal stroma to suppress keratocyte function and migration of inflammatory cells to corneal stroma that prevent development of corneal haze and scarring. These keratocytes do not exist in other corneal layers.

[0055] Corneal haze is not an epithelial or endothelial cell event. Corneal haze and scarring is a stromal condition, which primarily occurs due to the dysfunction of corneal stroma components, including excessive expression of actin and collagen fibers by keratocytes, and infiltration of inflammatory cells and differentiation of keratocytes into myofibroblasts. These cellular processes are distinct from corneal epithelial cell proliferation.

[0056] Descemet's membrane lies between the stroma and the endothelium.

[0057] The endothelium is just underneath Descemet's and is only one cell layer thick. This layer pumps water from the cornea, keeping it clear. If damaged or disease, these cells will not regenerate.

[0058] Previous methodologies utilized steroid therapy for the treatment of corneal haze and scarring. Table 3 below details the improvements of HGF therapy described herein compared with steroid therapy.

Table 3.		**STEROIDS**	**HGF**
Therapeutic Effects			
	Anti-inflammatory	+	+
	Anti-fibrotic	+	+
	Cell Death	Decreases	Decreases
	Corneal Epithelial cell proliferation and migration	Decreases	Increases
Adverse Effects			
	Action Spectrum	Non-selective	Targeted
	Intraocular Pressure	++	
	Secondary Infection	+	-

[0059] The mechanism of action of HGF is cell-specific in that it targets HGF-R expressing cells. Steroids are broad spectrum and non-selective in their action, which commonly lead to non-specific immunosuppression and secondary infection. As shown in Table 3, the risk of secondary infection is reduced using the compositions and methods described herein and cell proliferation is increased relative to treatment with steroids leading to reduced side effects and increased rates and further degree of healing and prevention.

Methods of Use

[0060] Provided here are methods of treating or preventing corneal haze or scarring by identifying a subject in need thereof; administering to the subject a HGFR-binding composition that includes at least one purified HGF agent. In embodiments, the administering comprises contacting the composition with corneal stroma of the subject. In embodiments, the identifying comprises calculating a grade scale of the subject's cornea transparency as described in Table 1.

[0061] The topical ophthalmic formulations are useful to treat corneal haze or scarring. Thus, the invention also provides methods for the treatment of corneal haze or scarring in a subject in need of such treatment by administering a composition described herein (e.g., an ophthalmic formulation of the present invention) directly to the eye or region of the eye of the subject. For example, administering step may comprise contacting the ophthalmic formulations described herein with corneal stroma or corneal stromal cells.

[0062] Pharmaceutical formulations comprising HGF agents fragments, or agonists thereof of the invention may be used for the treatment of corneal haze or scarring. For example, the pharmaceutical compositions are formulated for topical administration to the eye (e.g., subconjunctival administration; eye drops). Optionally, the pharmaceutical compositions may further comprise a tear substitute. Suitable tear substitutes may comprise glycerin, propylene glycol, HPMC (hydroxypropyl methylcellulose, hypromellose), Dextran 70, mineral oil, petrolatum, Carbopol 980, povidone, CMC (carboxyl methylcellulose sodium), PVA (polyvinyl alcohol) or other ingredients both active an inactive.

[0063] Also provided are methods for treating or preventing corneal haze or scarring in a subject in need thereof comprising administering to the eye surface of the subject a pharmaceutical composition comprising an effective amount of at least one (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, *etc.*) HGF agent(s). Optionally, the administration of HGF agent(s) to the eye of a subject in need of treatment or preventing corneal haze or scarring is also effective to mitigate or reduce one or more symptoms associated with a disease or condition on the

corneal surface. An effective amount is an amount that reduces the haze/scarring score by at least one unit, e.g., the units shown in Table 1. For example, an effective amount reduces the score from a "+3" to a "+2". The subject is preferably a human, but may be another mammal, for example a dog, a cat, a rabbit, a mouse, a rat, or a non-human primate.

[0064] The formulations may contain an effective amount of HGF agent and optionally one or more additional active ingredients that are effective for the intended use. Particular dosages are also selected based on a number of factors including the age, sex, species and condition of the subject. Effective amounts can also be extrapolated from dose-response curves derived from *in vitro* test systems or from animal models. The term "effective amount" means an amount of HGF agent(s) that is sufficient to prevent, eliminate, or reduce corneal haze or scarring.

[0065] The effective amount is the amount sufficient for the treatment or prevention of corneal haze or scarring. "Treatment" in this context refers to reducing or ameliorating at least one symptom as a result of corneal haze or scarring. "Prevention" in this context refers to a reduction in the frequency of, or a delay in the onset of, symptoms associated with a disease or condition, relative to a subject who does not receive the composition. In some cases, the methods described herein inhibit differentiation of corneal fibroblasts for at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more upon administration of a composition described herein compared to the level without administration of a composition described herein. In some cases, the methods described herein inhibit α-smooth muscle actin (αSMA) expression for at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more upon administration of a composition described herein compared to the level without administration of a composition described herein. In some cases, the methods described herein increase stratification of corneal epithelial cells for at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more upon administration of a composition described herein compared to the level without administration of a composition described herein. In some cases, the methods described herein increase c-met expression for at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more upon administration of a composition described herein compared to the level without administration of a composition described herein. In some cases, the methods described herein restore the thickness of an injured cornea to about 50%, 60%, 70%, 80%, 90%, 95% or higher percentage of that of a normal (i.e., healthy) cornea thickness upon administration of a composition described herein. In some cases, the methods described herein inhibit trafficking of inflammatory leukocytes to an injured cornea

for at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more upon administration of a composition described herein compared to the level without administration of a composition described herein.

[0066] The invention features methods of treating corneal haze or scarring in a subject comprising use of the formulations described above. For example, a method of treating corneal haze or scarring may comprise administering to the eye surface of the subject a pharmaceutical composition comprising an effective amount of at least one HGF agent and a tear substitute in a pharmaceutically acceptable carrier.

Ophthalmic Formulations

[0067] HGF agents may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the HGF agent, and a pharmaceutically acceptable carrier (excipient). Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art.

[0068] For example, the pharmaceutical compositions of the invention may comprise combinations of at least one (*e.g.*, 1, 2, 3, 4, 5, 6, *etc.*) HGF agent. In embodiments, the pharmaceutical compositions are formulated for subconjunctival administration. For example, the pharmaceutical compositions are formulated for topical administration to the eye (e.g., subconjunctival administration; eye drops). The pharmaceutical compositions may further comprise a tear substitute.

[0069] The concentration of HGF agents are from about 0.001% to about 10.0% (w/v), e.g., about 0.001% to about 5%, about 0.001% to about 2.5%, about 0.001% to about 1%, about 0.001% to about 0.5%, about 0.005 to about 0.5%, about 0.005% to about 0.05%, about 0.01%. By way of example, the concentration of HGF agent is effective to restore corneal thickness to that of a normal corneal and/or to inhibit trafficking of inflammatory leukocytes to an injured cornea.

[0070] Preferably, the pharmaceutical compositions according to the present invention are formulated as solutions, suspensions and other dosage forms for topical administration. Aqueous solutions are generally preferred, based on ease of formulation, as well as a patient's ability to easily administer such compositions by means of instilling one to two drops of the solutions in the affected eyes. However, the compositions may also be suspensions, viscous or semi-viscous gels, or other types of solid or semi-solid compositions.

[0071] Any of a variety of carriers may be used in the formulations of the present invention including water, mixtures of water and water-miscible solvents, such as C1- to C7-alkanols, vegetable oils or mineral oils comprising from 0.5 to 5% non-toxic water-soluble polymers, natural products, such as gelatin, alginates, pectins, tragacanth, karaya gum, xanthan gum, carrageenin, agar and acacia, starch derivatives, such as starch acetate and hydroxypropyl starch, and also other synthetic products, such as polyvinyl alcohol, polyvinylpyrrolidone, polyvinyl methyl ether, polyethylene oxide, preferably cross-linked polyacrylic acid, such as neutral Carbopol, or mixtures of those polymers. The concentration of the carrier is, typically, from 1 to 100000 times the concentration of the active ingredient. Additional ingredients that may be included in the formulation include tonicity enhancers, preservatives, solubilizers, non-toxic excipients, demulcents, sequestering agents, pH adjusting agents, co-solvents and viscosity building agents.

[0072] For the adjustment of the pH, preferably to a physiological pH, buffers may especially be useful. The pH of the present solutions should be maintained within the range of about 4.0 to 8.0 (e.g., about 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8), more preferably about 4.0 to 6.0 (e.g., about 4, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6) , more preferably about 6.5 to 7.8 (e.g., about 6.5, 6.6, 6.7, 6.8, 6.9, 7, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8) . Suitable buffers may be added, such as boric acid, sodium borate, potassium citrate, citric acid, sodium bicarbonate, TRIS, and various mixed phosphate buffers (including combinations of Na_2HPO_4, NaH_2PO_4 and KH_2PO_4) and mixtures thereof. Borate buffers are preferred. Generally, buffers will be used in amounts ranging from about 0.05 to 10 percent by weight.

[0073] Tonicity is adjusted if needed typically by tonicity enhancing agents. Such agents may, for example be of ionic and/or non-ionic type. Examples of ionic tonicity enhancers are alkali metal or earth metal halides, such as, for example, $CaCl_2$, KBr, KCl, LiCl, Nal, NaBr or NaCl, Na_2SO_4 or boric acid. Non-ionic tonicity enhancing agents are, for example, urea, glycerol, sorbitol, mannitol, propylene glycol, or dextrose. The aqueous solutions of the present invention are typically adjusted with tonicity agents to approximate the osmotic pressure of normal lachrymal fluids which is equivalent to a 0.9% ± 0.1% solution of sodium chloride or a 2.5% ± 0.3% solution of glycerol. An osmolality of about 225 to 400 mOsm/kg is preferred, more preferably 280 to 320 mOsm.

[0074] The at least one HGF agent(s) may be administered by the use of or in the form of hydrogels, drug-eluting contact lenses, and nanosystems (liposomal systems, dendrimers, solid biodegradable nanoparticles, nanogels), and/or irrigating solutions.

[0075] Ophthalmic formulations, eye ointments, creams, salves, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Eye Drops

[0076] The eye drop may be formulated with or without one or more tear substitutes. Also provided are pharmaceutical compositions comprising an effective amount of one or more (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, *etc.*) HGF agent(s), and a tear substitute in a pharmaceutically acceptable carrier for the treatment of corneal haze or scarring. The HGF agents and tear substitute may act synergistically to provide a longer dwell time of the HGF agent on the cornea, thus increasing duration and efficacy of action.

[0077] A variety of tear substitutes are known in the art and include, but are not limited to: monomeric polyols, such as, glycerol, propylene glycol, and ethylene glycol; polymeric polyols such as polyethylene glycol; cellulose esters such hydroxypropylmethyl cellulose, carboxy methylcellulose sodium and hydroxy propylcellulose; dextrans such as dextran 70; water soluble proteins such as gelatin; vinyl polymers, such as polyvinyl alcohol, polyvinylpyrrolidone, and povidone; and carbomers, such as carbomer 934P, carbomer 941, carbomer 940 and carbomer 974P. Many such tear substitutes are commercially available, which include, but are not limited to cellulose esters such as Bion Tears®, Celluvisc®, Genteal®, OccuCoat®, Refresh®, Teargen II®, Tears Naturale®, Tears Natural II®, Tears Naturale Free®, and TheraTears®; and polyvinyl alcohols such as Akwa Tears®, HypoTears®, Moisture Eyes®, Murine Lubricating®, and Visine Tears®. Tear substitutes may also be comprised of paraffins, such as the commercially available Lacri-Lube® ointments. Other commercially available ointments that are used as tear substitutes include Lubrifresh PM®, Moisture Eyes PM® and Refresh PM®.

[0078] In one aspect, the tear substitute contains hydroxypropylmethylcellulose. The tear substitute is Genteal® lubricating eye drops. GenTeal® (CibaVision--Novartis) is a sterile lubricant eye drop containing hydroxypropyl methylcellulose 3 mg/g and preserved with sodium perborate.

[0079] The pharmaceutical compositions of the invention may comprise combinations of one or more HGF agent(s) and one or more tear substitutes.

Therapeutic Administration

[0080] The effective amount of the active agents in the formulation will depend on absorption, inactivation, and excretion rates of the drug as well as the delivery rate of the compound from the formulation. It is to be noted that dosage values may also vary with the severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions. Typically, dosing will be determined using techniques known to one skilled in the art.

[0081] The dosage of any compound of the present invention will vary depending on the symptoms, age and other physical characteristics of the patient, the nature and severity of the disorder to be treated or prevented, the degree of comfort desired, the route of administration, and the form of the supplement. Any of the subject formulations may be administered in a single dose or in divided doses. Dosages for the formulations of the present invention may be readily determined by techniques known to those of skill in the art or as taught herein.

[0082] An effective dose or amount, and any possible effects on the timing of administration of the formulation, may need to be identified for any particular formulation of the present invention. This may be accomplished by routine experiment as described herein. The effectiveness of any formulation and method of treatment or prevention may be assessed by administering the formulation and assessing the effect of the administration by measuring one or more indices associated with the efficacy of the active agent and with the degree of comfort to the patient, as described herein, and comparing the post-treatment values of these indices to the values of the same indices prior to treatment or by comparing the post-treatment values of these indices to the values of the same indices using a different formulation.

[0083] The precise time of administration and amount of any particular formulation that will yield the most effective treatment in a given patient will depend upon the activity, pharmacokinetics, and bioavailability of a particular compound, physiological condition of the patient (including age, sex, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), route of administration, and the like. The guidelines presented herein may be used to optimize the treatment, *e.g.*, determining the optimum time and/or amount of administration, which will require no more

than routine experimentation consisting of monitoring the subject and adjusting the dosage and/or timing.

[0084] The combined use of several active agents formulated into the compositions of the present invention may reduce the required dosage for any individual component because the onset and duration of effect of the different components may be complimentary. In such combined therapy, the different active agents may be delivered together or separately, and simultaneously or at different times within the day.

Packaging

[0085] The formulations of the present invention may be packaged as either a single dose product or a multi-dose product. The single dose product is sterile prior to opening of the package and all of the composition in the package is intended to be consumed in a single application to one or both eyes of a patient. The use of an antimicrobial preservative to maintain the sterility of the composition after the package is opened is generally unnecessary.

[0086] Multi-dose products are also sterile prior to opening of the package. However, because the container for the composition may be opened many times before all of the composition in the container is consumed, the multi-dose products must have sufficient antimicrobial activity to ensure that the compositions will not become contaminated by microbes as a result of the repeated opening and handling of the container. The level of antimicrobial activity required for this purpose is well known to those skilled in the art, and is specified in official publications, such as the United States Pharmacopoeia ("USP") and corresponding publications in other countries. Detailed descriptions of the specifications for preservation of ophthalmic pharmaceutical products against microbial contamination and the procedures for evaluating the preservative efficacy of specific formulations are provided in those publications. In the United States, preservative efficacy standards are generally referred to as the "USP PET" requirements. (The acronym "PET" stands for "preservative efficacy testing.")

Kits

[0087] This invention provides kits for the packaging and/or storage and/or use of the formulations described herein, as well as kits for the practice of the methods described herein. Thus, for example, kits may comprise one or more containers containing one or more ophthalmic solutions, tablets, or capsules of this invention. The kits can be designed to facilitate one or more aspects of shipping, use, and storage.

[0088] The kits may optionally include instructional materials containing directions (i.e., protocols) disclosing means of use of the formulations provided therein. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g. CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

Example 1: Topical HGF inhibits development of corneal haze and scarring, and promotes wound healing

[0089] Corneal injury was induced by mechanical removal of the complete corneal epithelium using Algerbrush-II in C57131_6 mice. Complete removal of the corneal epithelium results in injury to the layers below including the corneal stroma. Under a dissecting microscope, the central area of the cornea was demarcated with a 3-mm trephine, and rotated gently to cut into the stroma. The circular area was traced with a sharp pair of surgical forceps, and then corneal epithelium and basement membrane, including the anterior portion of the stroma, were removed using a hand-held ALGERBRUSH II™ (Alger Equipment Co., Tx). This type of wound leaves bare stroma with the epithelium and basement membrane removed, leading to a significant inflammatory response. Following injury, corneas will be flushed with sterile saline and subsequently HGF or control treatments were applied. Thereafter, murine recombinant HGF was topically applied (dose: 3µl of 0.01% HGF in PBS per eye) to the injured eye twice daily up to 7 days post injury. A control group received a similar dosage of mouse serum albumin. At days 1, 3, 5 and 7 post injury, photographs of injured cornea (with or without fluorescein (green) staining) were captured using slit-lamp biomicroscopy. A smaller area of fluorescein (green) staining represents faster repair of corneal injury. FIG. 2A indicates reduced opacity and increased wound healing in the days following injury in treated animals relative to control. The decrease in opacity in treated animals is statistically significant at 5 days post injury (FIG. 2B). The decreased in wounded area indicated by the green staining is statistically decreased at 1, 3 and 5 days post injury (FIG. 2C).

Example 2: HGF inhibits expression of α-smooth muscle actin (αSMA: a factor that causes scarring) by corneal keratocytes

[0090] *In vitro* analysis of murine keratocytes (MK/T1) shows that HGF significantly inhibits TGFβ-induced expression of αSMA in keratocytes as measured by real time PCR (FIG. 3A) and immunohistochemistry (FIG. 3B). These keratocytes are present in the corneal stroma. HGF action in these cell types is indicative of HGF's functioning in the specialized cells of the corneal stroma, the corneal layer crucial for visual clarity.

[0091] Corneal haze and scarring in the stromal layer of the cornea can lead to visual degradation and blindness. Prevention, inhibition or reduction of scarring via inhibition of α-SMA in these tissues is effective to treat corneal haze and scarring and therefor aid improvement of vision.

Example 3: Topical HGF treatment inhibits trafficking and homing of inflammatory leukocytes to the injured corneas

[0092] Corneal injury was induced by mechanical removal of the complete corneal epithelium using ALGERBRUSH-II™ in C57BL6 mice. Thereafter, murine recombinant HGF was topically applied (dose: 3µl of 0.01% HGF in PBS per eye) to the injured eye twice daily. A control group received a similar dosage of mouse serum albumin. At day 3 post injury, corneas were harvested and immunostained for pan leukocyte marker CD45, and examined using confocal microscopy. (N = 5 mice/group). Results are shown in FIG. 4: Blue coloration indicates DAPI staining of cell nuclei and green indicates staining for CD45, a pan-leukocyte marker.

Example 4: Topical HGF treatment restores corneal tissue structure and thickness in injury

[0093] Corneal injury was induced by mechanical removal of the complete corneal epithelium using ALGERBRUSH-II™ in C57BL6 mice. Thereafter, murine recombinant HGF was topically applied (dose: 3µl of 0.01% HGF in PBS per eye) to the injured eye twice daily. A control group received a similar dosage of mouse serum albumin. At day 7 post injury, corneas were harvested and cross sections were stained with hematoxylin and eosin (H&E). FIG. 5A shows representative micrographs showing tissue structure of normal, control injured and HGF-treated corneas. FIG. 5B presents cumulative data showing HGF-treated corneas restore their thickness similar to normal corneas. Injured control corneas show significant increase in the thickness as compared to normal and HGF-treated corneas. (N = 5 mice/group).

Example 5: HGF treatment augments stratification of epithelial cells after corneal injury

[0094] As shown in FIG. 7A and 7B, HGF augments stratification of epithelial cells after corneal injury. At 7 days post injury, corneas were harvested from normal, albumin-treated

and HGF-treated mice. Corneal cross sections were stained with the nuclear stain DAPI to visualize corneal epithelial cell layer using confocal microscope (400X) (FIG. 7A). Bar chart in FIG. 7B shows the thickness (μm) of the epithelial cell layer in normal (white bar), control-injured and HGF-treated injured corneas (black bar). The values shown are the mean ± SD (error bars); n=5 mice/group.

Example 6: HGF promotes HGF-R (c-met) expression

[0095] As shown in FIG. 8, HGF promotes HGF-R (c-met) expression in the cornea after injury. At 3 and 7 days post injury, corneas were harvested from normal (checked bar), mouse albumin-treated control injured (white bar) and HGF-treated (black bar) injured groups. Total RNA was isolated from harvested corneas. HGF-R mRNA expression was quantitated using real-time PCR. GAPDH was used as a internal control. The values shown are the mean ± SD and, each group consists of n=6 mice, *$p<0.03$, **$p<0.01$.

OTHER EMBODIMENTS

[0096] While the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

[0097] The patent and scientific literature referred to herein establishes the knowledge that is available to those with skill in the art. All United States patents and published or unpublished United States patent applications cited herein are incorporated by reference. All published foreign patents and patent applications cited herein are hereby incorporated by reference. Genbank and NCBI submissions indicated by accession number cited herein are hereby incorporated by reference. All other published references, documents, manuscripts and scientific literature cited herein are hereby incorporated by reference.

[0098] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

WHAT IS CLAIMED IS:

1. A method of treating or preventing corneal haze or scarring, the method comprising: identifying a subject in need of treating or preventing corneal haze or scarring; administering to the subject a hepatocyte growth factor receptor (HGFR)-binding composition comprising at least one purified hepatocyte growth factor (HGF) agent.

2. The method of claim 1, wherein the HGF agent comprises a polypeptide comprising an amino acid sequence of SEQ ID NO: 1 or a fragment thereof.

3. The method of claim 2, wherein the HGF agent comprises a polypeptide comprising an amino acid sequence having 70% sequence identity to SEQ ID NO: 1.

4. The method of claim 1, wherein the HGF agent comprises an agonist of HGF.

5. The method of claim 1, wherein the composition comprising at least one purified HGF agent further comprises a corneal stroma permeating excipient.

6. The method of claim 1, wherein the composition acts to inhibit α-smooth muscle actin (α-SMA) in the cornea.

7. The method of claim 1, wherein the purified HGF agent is present in a concentration of 0.001% to 1% w/v.

8. The method of claim 7, wherein the purified HGF agent is present in a concentration of 0.005% to 0.05% w/v.

9. The method of claim 8, wherein the purified HGF agent is present in a concentration of about 0.01% w/v.

10. The method of claim 1, wherein the compositions is administered daily for 5 days after an injury to the cornea.

11. The method of claim 1, wherein the composition is administered daily for 3 days after an injury to the cornea.

12. The method of claim 1, wherein the compositions is administered daily for 1 day after an injury to the cornea.

13. A composition for the treatment of corneal haze or scarring comprising at least one HGF agent.

14. The composition of claim 13 wherein the at least one HGF agent comprises a polypeptide comprising an amino acid sequence of SEQ ID NO: 1 or a fragment thereof.

15. The composition of claim 13, wherein the at least one HGF agent comprises a polypeptide comprising an amino acid sequence having 70% sequence identity to SEQ ID NO: 1.

16. The composition of claim 13, wherein the at least one HGF agent comprises an agonist of HGF.

17. The composition of claim 13, wherein the composition further comprises a corneal stroma permeating excipient.

18. The composition of claim 13, wherein the at least one HGF agent is present in a concentration of 0.001% to 1% w/v.

19. The composition of claim 18, wherein the at least one HGF agent is present in a concentration of 0.005% to 0.05% w/v.

20. The composition of claim 19, wherein the at least one HGF agent is present in a concentration of about 0.01% w/v.

21. The composition of claim 13, wherein the at least one HGF agent is present in a concentration effective to restore corneal thickness to that of a normal corneal.

22. The composition of claim 13, wherein the at least one HGF agent is present in a concentration effective to inhibit trafficking of inflammatory leukocytes to an injured cornea.

23. The composition of claim 13, wherein the at least one HGF agent is present in a concentration effective to inhibit α-SMA expression in cornea.

24. The method of claim 1, wherein the identifying comprises calculating a grade scale of the subject's cornea transparency.

25. The method of claim 1, wherein the administering comprises topical administration or subconjunctival administration.

26. The method of claim 1, wherein the administering comprises contacting the composition with corneal stroma of the subject.

27. The method of claim 1, wherein the composition is formulated as an eye drop.

28. The method of claim 1, wherein the purified HGF agent comprises a polypeptide comprising an amino acid sequence of residues 32-494 of SEQ ID NO:1.

29. The method of claim 1, wherein the purified HGF agent comprises a polypeptide comprising an amino acid sequence of residues 126-207 of SEQ ID NO:1.

30. The method of claim 1, wherein the purified HGF agent comprises a polypeptide comprising an amino acid sequence of residues 208-289 of SEQ ID NO:1.

31. The method of claim 1, wherein the purified HGF agent comprises a polypeptide comprising an amino acid sequence of residues 302-384 of SEQ ID NO:1.

32. The method of claim 1, wherein the purified HGF agent comprises a polypeptide comprising an amino acid sequence of residues 388-470 of SEQ ID NO:1.

FIG. 1

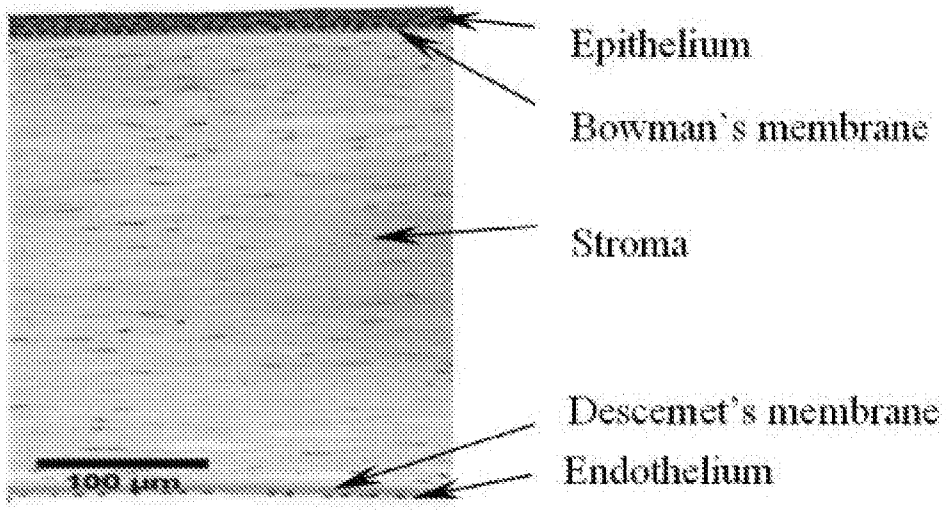

Epithelium
Bowman`s membrane
Stroma
Descemet's membrane
Endothelium
100 µm

FIG. 2A

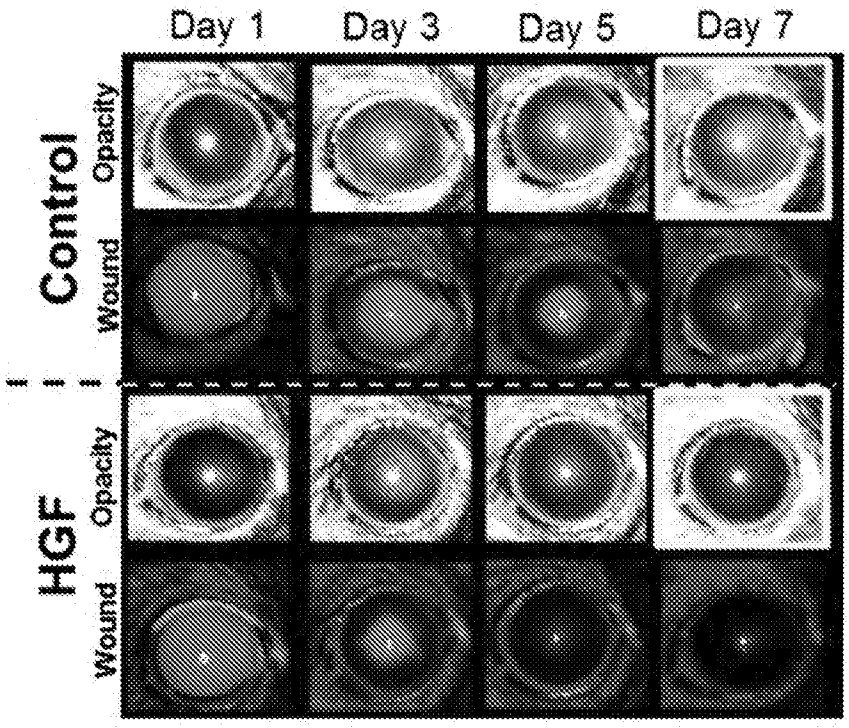

Day 1
Day 3
Day 5
Day 7
Control
Opacity
Wound
HGF
Opacity
Wound

FIG. 2B

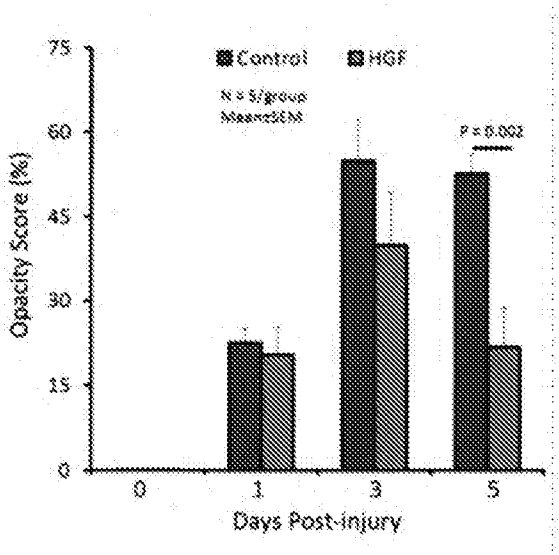

Control
HGF
N = 5/group
Mean±SEM
P = 0.002
Opacity Score (%)
75
60
45
30
15
0
0
1
3
5
Days Post-injury

FIG. 2C

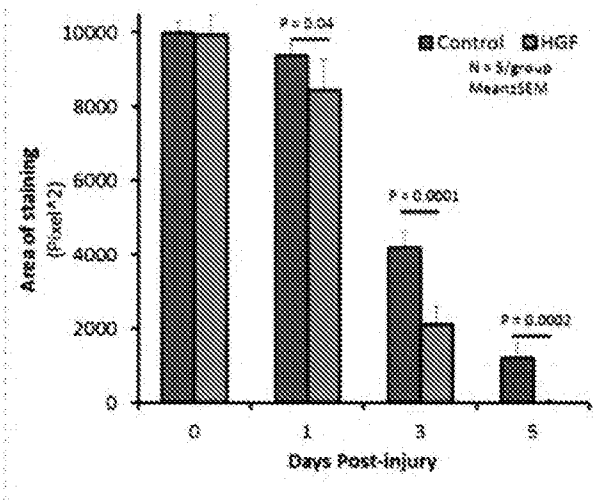

Control
HGF
N = 5/group
Mean±SEM
P = 0.04
P = 0.0001
P = 0.0003
Area of staining
(Pixel^2)
10000
8000
6000
4000
2000
0
0
1
3
5
Days Post-injury

FIG. 3A

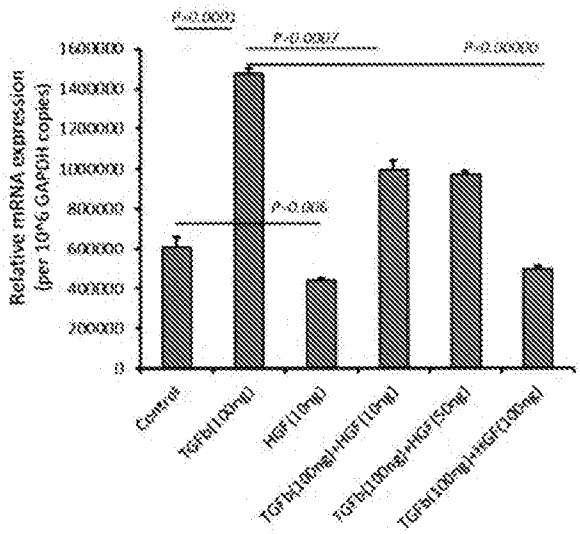

P=0.0001
P=0.0007
P=0.00000
P=0.006
Relative mRNA expression
(per 10^6 GAPDH copies)
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Control
TGFb(100ng)
HGF (10ng)
TGFb(100ng)+HGF (10ng)
TGFb(100ng)+HGF (50ng)
TGFb(100ng)+HGF(100ng)

FIG. 3B

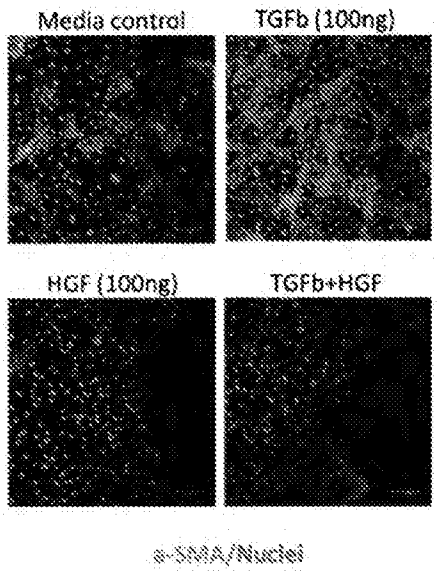
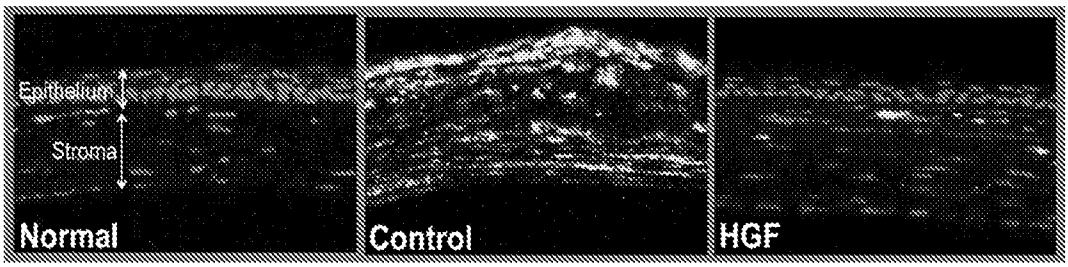
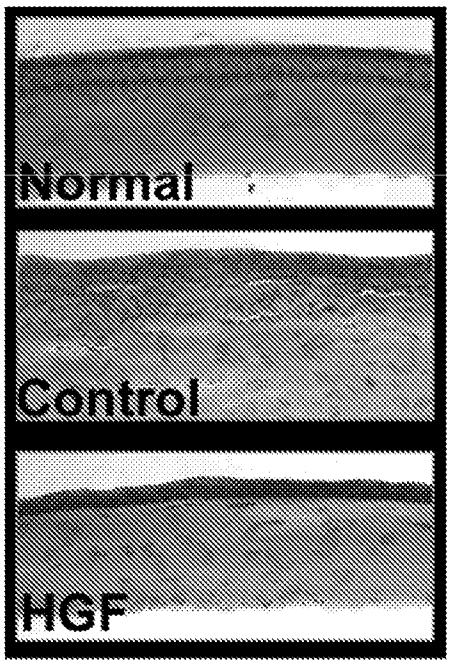

Media control
TGFb (100ng)
HGF (100ng)
TGFb+HGF
a-SMA/Nuclei

FIG. 4



(Blue: DAPI, nuclear stain; Green: CD45, pan-leukocyte marker)

FIG. 5A



(H&E staining of corneal sections)

FIG. 5B

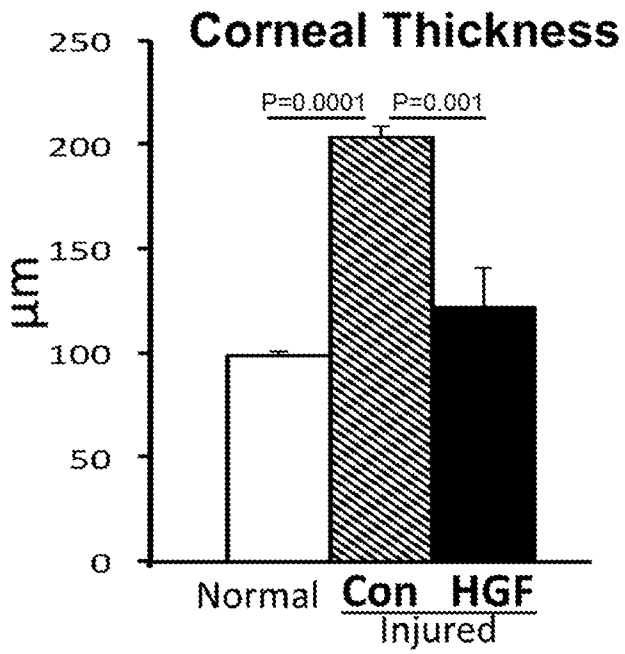

Corneal Thickness
P=0.0001
P=0.001
250
200
150
100
50
0
µm
Normal
Con
HGF
Injured

FIG. 6

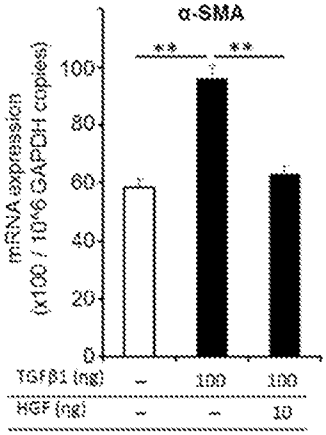

α-SMA
**
**
mRNA expression
(x100 / 10^6 GAPDH copies)
100
80
60
40
20
0
TGFβ1 (ng) - 100 100
HGF (ng) - - 10

FIG. 7A

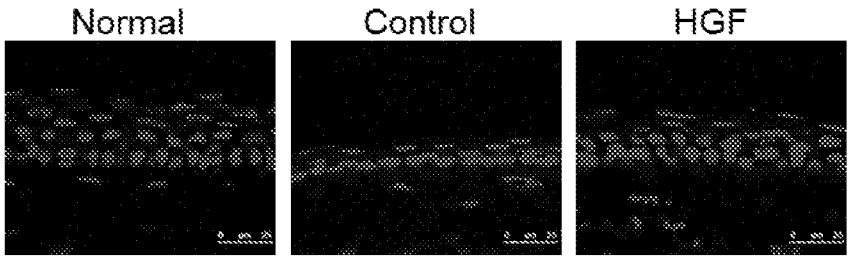

Normal
Control
HGF

FIG. 7B

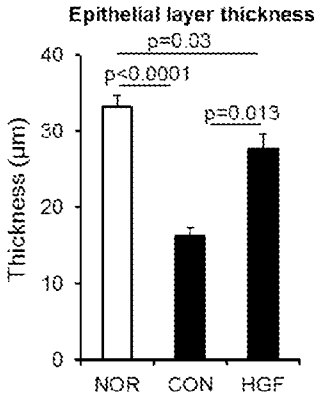
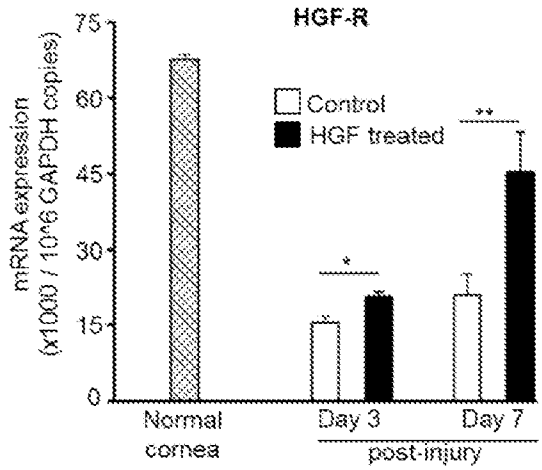

Epithelial layer thickness
p=0.03
p<0.0001
p=0.013
Thickness (μm)
40
30
20
10
0
NOR
CON
HGF

FIG. 8


HGF-R
Control
HGF treated
mRNA expression
(x1000 / 10^6 GAPDH copies)
75
60
45
30
15
0
*
**
Normal cornea
Day 3
Day 7
post-injury

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/50945

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/16, A61K 38/18, C07K 14/00 (2017.01)
CPC - A61K 38/16, A61K 38/18, C07K 14/00
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)
IPC(8) - A61K 38/16, A61K 38/18, C07K 14/00 (2017.01)
CPC - A61K 38/16, A61K 38/18, C07K 14/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 530/324, 424/9.1
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar, Google Web
Search terms: HGF, hepatocyte growth factor, scatter factor, cornea, haze, opacity, transparency, scar, treat, prevent, injury, smooth muscle actin, myofibroblasts, HGFR, c met, cMET, met receptor, FGF, keratocyte, kringle, neovascularization, agonist, eye, permeating

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUN et al. H-RN, a peptide derived from hepatocyte growth factor, inhibits corneal neovascularization by inducing endothelial apoptosis and arresting the cell cycle. BMC Cell Biol. (24 February 2013) Vol 14, No 8, pp 1-10, Especially abstract, pg 1 col 1 para 1 - pg 1 col 2 para 1, pg 4 col 2 para 1, pg 6 col 2 para 3, pg 8 col 1 para 2	1-3,7-9
Y	ANITUA et al. Plasma rich in growth factors (PRGF-Endoret) stimulates corneal wound healing and reduces haze formation after PRK surgery. Exp Eye Res. October 2013, Vol 115, pp 153-61, Especially abstract, pg 155 col 2 para 4, pg 155 col 2 para 5, pg 156 Table 1, pg 160 col 1 para 1, pg 160 col 1 para 4 - col 2 para 1	1,4-6,10-12,24-29
Y	JIANG et al. HGF suppresses the production of collagen type III and alpha-SMA induced by TGF-beta1 in healing fibroblasts. Eur J Appl Physiol. July 2008, Vol 103, No 5, pp 489-93, Especially title, abstract, pg 490 col 2 para 1	1,4-6,10-12,24-29
Y	US 2014/0378391 A1 (GNT, LLC) 25 December 2014 (25.12.2014) Especially title, abstract, para [0139], [0113]-[0114], [0117]	5,26

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
25 January 2017	21 FEB 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/50945

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.:
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:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

--please see continuation on extra sheet--

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.:
1-12, 24-29 limited to amino acid residues 126-207 of SEQ ID NO: 1

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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 16/50945

Continuation of Box No. III Observations where unity of invention is lacking

Group I+, claims 1-12, 24-32, directed to a method of treating corneal haze or scarring, the method comprising: administering to the subject at least one purified hepatocyte growth factor (HGF) agent. The method of treating corneal haze or scarring will be searched to the extent that the HGF agent encompasses an amino acid sequence of residues 126-207 of SEQ ID NO: 1 (see claim 29). It is believed that claims 1-12, 24-29 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass an amino acid sequence of residues 126-207 of SEQ ID NO: 1. Additional HGF agents will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected HGF agents. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be an amino acid sequence of residues 208-289 of SEQ ID NO: 1 (Claims 1-12, 24-28, 30).

Group II, claims 13-23, directed to a composition for the treatment of corneal haze or scarring comprising at least one HGF agent.

The inventions listed as Groups I+ and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

Group I+ includes the special technical feature of a method of treating or preventing corneal haze or scarring, not required by Group II.

Group II includes the special technical feature of a composition comprising a HGF agent, not required by Group I+.

Another special technical feature of the inventions listed as Group I+ is the specific HGF peptide fragments, recited therein am. Each of the inventions of Group I+ requires a unique HGF peptide fragment, not required by the other inventions.

Common Technical Features

Groups I+ and II share the technical feature of treating corneal haze or scarring with a HGF agent.

However, this shared technical feature does not represent a contribution over prior art, because the shared technical feature is made obvious by the article entitled "H-RN, a peptide derived from hepatocyte growth factor, inhibits corneal neovascularization by inducing endothelial apoptosis and arresting the cell cycle" by SUN et al. (hereinafter "SUN") [BMC Cell Biology 14:8, page 1-10; published 24 February 2013].

SUN teaches a method of treating or preventing corneal neovascularization (abstract, "a novel peptide H-RN, derived from the hepatocyte growth factor kringle 1 domain (HGF K1) ... H-RN has anti-angiogenic activity ... in a mouse model of VEGF-induced corneal neovascularization"; pg 4 col 2 para 1, "We infer that H-RN has the potential for treating pathological corneal neovascularization, and sustained release delivery may be an effective drug delivery option"), the method comprising: identifying a subject in need of treating or preventing corneal neovascularization (pg 1 col 2 para 1, "The prevalence of corneal neovascularization ranges from 125,000-470,000 individuals in the population wearing soft contact lenses"); administering to the subject a hepatocyte growth factor receptor (HGFR)-binding composition comprising at least one purified hepatocyte growth factor (HGF) agent (abstract, pg 6, col 2, para 3, H-RN, RNPRGEEGGPW (100% match to amino acid residues 178-188 of SEQ ID NO: 1); pg 4 col 2 para 1, "In the mouse cornea micropocket assay ... Neovascularization ... was significantly inhibited following administration of H-RN"). SUN does not specifically teach identifying a subject in need of treating or preventing corneal haze or scarring. However, SUN teaches that "The transparency and refraction properties of a normal, healthy cornea are predominantly mediated by its avascularity" (pg 1 col 1 para 1), and "many pathological processes affecting the cornea... may lead to corneal neovascularization... While this process may be beneficial in wound healing... neovascularization may reduce corneal transparency by growing into the normally vascular-free cornea tissue, bleeding into the cornea and causing lipid deposition, tissue swelling and scarring" (pg 1 col 1 para 1 - pg 1 col 2 para 1). Given the relationship between corneal transparency and avascularity, and the relationship between neovascularization, as taught by SUN, one of ordinary skill in the art would have recognized that abnormal vascularization would result in changes to transparency and refraction properties, i.e., would cause corneal haze. Accordingly, one of ordinary skill in the art would have been recongized that treating corneal haze or scarring could have been accomplished by targeting corneal neovascularization with a HGF agent, such as H-RN, RNPRGEEGGPW [Note, see instant claim 29, the HGFR-binding agent comprises amino acid residues 126-207 of SEQ ID NO: 1. Since H-RN is a peptide fragment comprising amino acid residues 178-188 of SEQ ID NO: 1, thus, it is inherently a HGFR-binding agent].

As the technical feature was known in the art at the time of the invention, it cannot be considered a special technical feature that would otherwise unify the groups.

Groups I+ and II therefore lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.