

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

(19) World Intellectual Property  
Organization  
International Bureau



(10) International Publication Number

WO 2018/163151 A1

(43) International Publication Date  
13 September 2018 (13.09.2018)

(51) International Patent Classification:

A61K 31/047 (2006.01) A61P 27/02 (2006.01)  
A61K 9/00 (2006.01)

Published:

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

(21) International Application Number:

PCT/IL2018/050184

(22) International Filing Date:

19 February 2018 (19.02.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/467,139 05 March 2017 (05.03.2017) US

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(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, 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).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

(54) Title: EYE DROPS FOR TREATMENT OF IRRITATION NOT DUE TO INFECTION

(57) Abstract: An ophthalmic preparation useful for effective prevention or treatment of irritation to corneal or conjunctival epithelial cells as measured by Lissamine Green staining, in particular irritation due to causes other than infection, is disclosed. The preparation comprises an aqueous solution of glycerol. In other embodiments, the preparation may comprise additional components including high molecular weight polymers for viscosity control and pharmacologically active substances. The use of the preparation in the prevention or treatment of irritation to corneal or conjunctival epithelial cells, and a method for preventing or treating irritation to corneal or conjunctival epithelial cells are also disclosed.

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## EYE DROPS FOR TREATMENT OF IRRITATION NOT DUE TO INFECTION

## FIELD OF THE INVENTION

**[0001]** This invention relates in general to means and methods for treatment and elimination of irritation to corneal or conjunctival epithelial tissue. In particular, it relates to the use of eye drops comprising a solution of glycerol in water for treating devitalized corneal or conjunctival epithelial cells.

## BACKGROUND OF THE INVENTION

**[0002]** Corneal or conjunctival epithelial cell damage occurs in many corneal diseases such as dry eye disease; occupational dry eye caused by insufficient rate of blinking; lack of tear production such as is found, for example, in Sjögren's syndrome; Meibomian oil deficiency; drug or preservative induced cell damage; mechanical cell damage induced by such factors as contact lens wearing; and ocular surface disease.

**[0003]** Treatments for corneal or conjunctival epithelial cell damage known in the art tend to be strictly palliative and are generally aimed at lessening the severity of the symptoms of the condition rather than at treating the condition by healing the damaged cells. For example, Sjögren's syndrome is normally treated by use of moisture replacement therapies such as by application of artificial tears, which have only limited effectiveness, or by drugs that affect the immune system, which during chronic treatment lead to side effects in up to 20% of cases.

**[0004]** U.S. Pat. 5106615, which is hereby incorporated by reference in its entirety, discloses humectant eye drops that are useful for treatment of dry eye syndrome. The eye drops have non-Newtonian rheological properties that mimic the behavior of natural tears, and comprise an aqueous solution of a low molecular weight humectant polyol at approximately isotonic concentration, an anionic polymer having a molecular weight between 500,000 and 4,000,000, and less than 1.5 mM inorganic salt.

**[0005]** U.S. Pat. No. 8912166 (henceforth '**166**'), which is hereby incorporated by reference in its entirety, discloses an ophthalmic preparation and method for treating conjunctivochalasis, a disease of the conjunctival folds. The preparation comprises an aqueous solution of glycerol, a normal component of human blood. In contrast to artificial tear solutions known in the art, the preparation disclosed in '**166**' provides a statistically significant reduction in the severity of the condition as measured by the Lid Parallel Conjunctival Folds (LIPCOF) scale.

**[0006]** Despite these advances, compositions and methods for treating corneal irritation that heal the damaged cells rather than merely ease the symptoms remain a long-felt, but as yet unmet need.

## SUMMARY OF THE INVENTION

**[0007]** The present invention provides a method for treatment of irritated corneal or conjunctival epithelial cells in which the cell irritation is due to a cause other than infection. Irritation of the corneal and epithelial cells will lead to damage of those cells. The method of treatment disclosed herein at a minimum alleviates the severity of, and typically cures entirely, such cell damage. The treatment of the irritation leads to healing of the damaged tissue. The method comprises application of eye drops that contain glycerol as the sole active ingredient to treat the irritation. The present invention also discloses an ophthalmological composition for treatment of such conditions, and the use of the composition in the treatment thereof.

**[0008]** It is therefore an object of the present invention to disclose the use of an ophthalmic preparation comprising an aqueous solution of glycerol in a treatment comprising treatment or prevention of irritation of epithelial cells of the eye, wherein said aqueous solution comprises at least 1% (w/v) glycerol and said method comprises applying said ophthalmic preparation to an affected eye.

**[0009]** It is a further object of this invention to disclose such a use in a treatment of irritation of corneal epithelial cells.

**[0010]** It is a further object of the present invention to disclose such a use in a treatment for prevention of irritation of corneal epithelial cells.

**[0011]** It is a further object of the present invention to disclose such a use in a treatment of irritation of conjunctival epithelial cells, wherein said method comprises applying said ophthalmic preparation to an affected eye.

**[0012]** It is a further object of the present invention to disclose such a use in a treatment for prevention irritation to conjunctival epithelial cells, wherein said method comprises applying said ophthalmic preparation to an affected eye.

**[0013]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said irritation is not due to infection.

**[0014]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said treatment is a treatment of cell irritation resulting from a cause selected from the group consisting of Sjögren's syndrome, Meibomian oil deficiency, drug-induced cell irritation, preservative-induced cell irritation, mechanical cell irritation induced by contact lens wearing, and ocular surface disease.

**[0015]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said method comprises determining a level of devitalization of cells by using Rose Bengal staining.

**[0016]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said method comprises determining a level of devitalization of cells by using Lissamine Green staining.

**[0017]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution is characterized by a glycerol concentration of between 1.1% and 4% (w/v).

**[0018]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution is essentially isotonic.

**[0019]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution has a pH between 6.7 and 7.7.

**[0020]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution is characterized by an inorganic salt concentration of less than 0.1% w/v.

**[0021]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution comprises at least one polymer of molecular weight of at least 10,000 Dalton. In some preferred embodiments of the invention, the concentration of said polymer is chosen to bring said solution to a predetermined viscosity. In some particularly preferred embodiments of the invention, said viscosity is between 5 and 125 mPa•s. In some embodiments of the invention, said at least one polymer is anionic. In some preferred embodiments of the invention in which said at least one polymer is anionic, said solution comprises at least one polymer selected from the group consisting of hyaluronates, carbomers, and combinations and mixtures thereof.

**[0022]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution comprises a pharmaceutically effective amount of at least one pharmacologically active agent.

**[0023]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said aqueous solution comprises at least one substance selected from the group consisting of stabilizers, preservatives, antioxidants, and buffers.

**[0024]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said treatment comprises applying said ophthalmic preparation from three to eight times daily.

**[0025]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said treatment comprises applying said ophthalmic preparation until a statistically significant reduction in severity of irritation to corneal epithelial cells, as measured by Lissamine Green staining, is observed. In some embodiments of the invention, said treatment comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding three months. In some embodiments of the invention, said treatment comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding one month.

**[0026]** It is a further object of the present invention to disclose the use as defined in any of the above, wherein said treatment comprises applying prophylactically said ophthalmic preparation at predetermined intervals following completion of a therapeutic course of treatment, thereby maintaining a condition in which said irritation remains significantly reduced relative to its severity prior to said course of therapeutic treatment. In preferred embodiments of the invention, said treatment comprises applying prophylactically applying said ophthalmic preparation daily following a therapeutic course of treatment.

**[0027]** It is a further object of the present invention to disclose a method for treating irritation of corneal epithelial cells, wherein said method comprises applying an ophthalmic preparation comprising an aqueous solution of glycerol to an affected eye.

**[0028]** It is a further object of the present invention to disclose a method for preventing irritation of corneal epithelial cells, wherein said method comprises applying an aqueous solution of glycerol to an affected eye.

**[0029]** It is a further object of the present invention to disclose a method for treating irritation of conjunctival epithelial cells, wherein said method comprises applying an ophthalmic preparation comprising an aqueous solution of glycerol to an affected eye.

**[0030]** It is a further object of the present invention to disclose a method for preventing irritation of conjunctival epithelial cells, wherein said method comprises applying an aqueous solution of glycerol to an affected eye.

**[0031]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said irritation is not due to infection.

**[0032]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said method comprises treating cell irritation resulting from a cause selected from the group consisting of Sjögren's syndrome, Meibomian oil deficiency, drug-induced cell irritation, preservative-induced cell irritation, irritation due to mechanical cell damage induced by contact lens wearing, and ocular surface disease.

**[0033]** It is a further object of the present invention to disclose a method as defined in any of the above, comprising using Rose Bengal staining to determine a level of devitalization of cells due to irritation.

**[0034]** It is a further object of the present invention to disclose a method as defined in any of the above, comprising using Lissamine Green staining to determine a level of devitalization of cells due to irritation.

**[0035]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an aqueous solution of glycerol characterized by a glycerol concentration of between 1% and 4% (w/v).

**[0036]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an essentially isotonic aqueous solution of glycerol.

**[0037]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an aqueous solution of glycerol characterized by a pH between 6.7 and 7.7.

**[0038]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying

an aqueous solution of glycerol characterized by an inorganic salt concentration of less than 0.1% w/v.

**[0039]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an aqueous solution of glycerol comprising at least one polymer of molecular weight of at least 10,000 Dalton. In some preferred embodiments of the method, the concentration of said polymer is chosen to bring said solution to a viscosity of between 5 and 125 mPa•s. In some preferred embodiments of the method, said at least one polymer is anionic. In some preferred embodiments of the method in which said polymer is anionic, said at least one polymer is a hyaluronate. In some preferred embodiments of the method in which said polymer is anionic, said at least one polymer is a carbomer. In some preferred embodiments of the method in which said at least one polymer is anionic, said solution comprises at least one polymer selected from the group consisting of hyaluronates, carbomers, and combinations and mixtures thereof.

**[0040]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an aqueous solution of glycerol comprising a pharmaceutically effective amount of a pharmacologically active agent.

**[0041]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said step of applying an aqueous solution of glycerol comprises applying an aqueous solution of glycerol comprising a substance selected from the group consisting of stabilizers, preservatives, antioxidants, and buffers.

**[0042]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said method comprises applying said ophthalmic preparation from three to eight times daily.

**[0043]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said method comprises applying said ophthalmic preparation until a statistically significant reduction in the severity of irritation to corneal epithelial cells, as measured by Lissamine Green staining, is observed.

**[0044]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said method comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding three months. In some preferred

embodiments of the invention, said method comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding one month.

**[0045]** It is a further object of the present invention to disclose a method as defined in any of the above, wherein said method comprises applying said ophthalmic preparation prophylactically following completion of a therapeutic course of treatment, thereby maintaining a condition in which said irritation remains significantly reduced relative to its severity prior to said course of therapeutic treatment. In preferred embodiments of the method, said step of applying said ophthalmic preparation prophylactically comprises applying said ophthalmic preparation daily.

**[0046]** It is a further object of this invention to disclose an ophthalmic preparation comprising an aqueous solution of glycerol, wherein said ophthalmic preparation comprises at least 1% (w/v) glycerol and is an effective treatment for treatment or prevention of irritation of epithelial cells of the eye.

**[0047]** It is a further object of this invention to disclose an ophthalmic preparation comprising an aqueous solution of glycerol, wherein said ophthalmic preparation is an effective treatment for prevention or treatment of irritation of conjunctival epithelial cells.

**[0048]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, wherein said ophthalmic preparation is an effective treatment for prevention or elimination of irritation to corneal or conjunctival cells as measured by a method selected from the group consisting of Rose Bengal staining and Lissamine Green staining.

**[0049]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, wherein said ophthalmic preparation is characterized by a glycerol concentration of between 1% and 4% (w/v).

**[0050]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, wherein said solution is essentially isotonic.

**[0051]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, wherein said solution has a pH between 6.7 and 7.7.

**[0052]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, wherein said solution is characterized by an inorganic salt concentration of less than 0.1%.

**[0053]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, comprising at least one polymer of molecular weight of at least 10,000 Dalton. In some preferred embodiments of the invention, the concentration of said at least one polymer is chosen to bring said solution to a predetermined viscosity. In some particularly preferred embodiments of the invention, said predetermined viscosity is between 5 and 125 mPa•s. In some preferred embodiments of the invention, said at least one polymer is anionic. In some preferred embodiments of the invention in which said at least one polymer is anionic, said solution comprises at least one polymer selected from the group consisting of hyaluronates, carbomers, and combinations and mixtures thereof.

**[0054]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, comprising a pharmaceutically effective amount of at least one pharmacologically active agent.

**[0055]** It is a further object of this invention to disclose an ophthalmic preparation as defined in any of the above, comprising a substance selected from the group consisting of stabilizers, preservatives, antioxidants and buffers.

**[0056]** It is a further object of this invention to disclose the use of the ophthalmic preparation as defined in any of the above in a method as defined in any of the above.

**[0057]** It is a further object of this invention to disclose the use of an aqueous solution comprising at least 1% glycerol (w/v) in the preparation of a composition for treatment or prevention of irritation of epithelial cells of the eye. In some preferred embodiments of the invention, said treatment is selected from the group consisting of treatment of irritation of corneal epithelial cells; prevention of irritation of corneal epithelial cells; treatment of irritation of conjunctival epithelial cells; and prevention of irritation of conjunctival epithelial cells. In some preferred embodiments of the invention, said ophthalmic preparation is an effective treatment for prevention or elimination of irritation to corneal or conjunctival cells as measured by a method selected from the group consisting of Rose Bengal staining and Lissamine Green staining.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

**[0058]** In the following description, various aspects of the invention will be described. For the purposes of explanation, specific details are set forth in order to provide a thorough understanding of the invention. It will be apparent to one skilled in the art that there are other

embodiments of the invention that differ in details without affecting the essential nature thereof.

**[0059]** The inventor has discovered that surprisingly, glycerol is an effective substance for treatment of irritation of corneal epithelial cells, irritation of conjunctival epithelial cells, and hence, damage to corneal or conjunctival cells when these conditions are caused by factors other than infection. Topical application of glycerol (e.g. in an aqueous solution) to the affected eye reduces or even eliminates entirely corneal or conjunctival irritation.

**[0060]** The invention herein disclosed is an ophthalmic preparation for treatment or prevention of irritation to corneal or conjunctival epithelial cells, the preparation comprising an aqueous glycerol solution. In typical embodiments of the invention, the solution comprises 1.1% – 4% glycerol (w/v). In some preferred embodiments of the invention, the solution comprises 2.5% glycerol (w/v). In preferred embodiments of the invention, the solution is isotonic.

**[0061]** In preferred embodiments of the invention, the composition comprises an aqueous glycerol solution in which the concentration of inorganic salts is less than 2 mM. In preferred embodiments of the invention, the viscosity of the solution is controlled by addition of a quantity of high molecular weight polymer (MW > 10<sup>4</sup> Dalton) such as hyaluronate, carbomer or a mixture thereof, sufficient to bring the solution to the desired viscosity. All ingredients are of purity sufficient for use in eye drops.

**[0062]** The solutions may then be transferred to a container appropriate for dispensing it as eye drops.

**[0063]** While in some embodiments of the invention, the only active ingredient present in the composition is glycerol, the composition may comprise in addition a pharmaceutically effective concentration at least one pharmacologically active agent. If necessary, any stabilizer, preservative, antioxidant, buffer or combination thereof appropriate for use with the pharmacologically active agent may be added to the solution in any concentration suitable for use in eye drops.

**[0064]** It is within the scope of the invention to disclose the use of the eye drops in the non-surgical treatment of, or prevention of, irritation of and irritation to the corneal or conjunctival epithelial tissue, particularly damage due to causes other than infection, and a method of non-surgical treatment or prevention of irritation of and hence, damage to the corneal or conjunctival epithelial tissue, particularly damage due to causes other than

infection. Non-limiting examples of conditions that can be treated by the eye drop composition disclosed herein include Sjögren's syndrome, Meibomian oil deficiency, drug or preservative induced irritation, irritation due to mechanical cell damage such as cell damage induced by contact lens wearing, and ocular surface disease.

**[0065]** A typical protocol for use of the eye drops disclosed herein to treat or to alleviate corneal and/or conjunctival epithelial cell damage is to place drops in the affected eye three to eight times daily until the severity of condition is reduced to an acceptable level. In particularly severe cases, more frequent applications may be necessary, and in less severe cases, one or two daily treatments may be sufficient. The progress of the treatment can be measured by the use of techniques such as Lissamine Green staining or Rose Bengal staining to track the condition of the epithelial cells. In some preferred embodiments of the treatment, application of the composition disclosed herein is performed for no more than three months, by which time statistically significant improvement of the condition of the corneal or conjunctival epithelial cells is observed. In some preferred embodiments of the treatment, application of the composition disclosed herein is performed for no more than one month, by which time statistically significant improvement of the condition of the corneal or conjunctival epithelial cells is observed.

**[0066]** It is within the scope of the invention to include within the method prophylactic application of the composition disclosed herein in order to prevent recurrence of the condition. After the course of therapeutic treatment, which typically lasts no more than three months, a maintenance regimen comprising prophylactic application of the eye drops is begun. Application of the eye drops one to three times daily is usually sufficient to prevent recurrence of the irritation.

**[0067]** In contrast to methods known in the art, in particular, those that use drugs that affect the immune system, no side effects were observed in any of the treatment protocols in which the invention disclosed herein was tested. In particular, no side effects were observed with long-term use of eye drops containing as much as 2.5% (w/v) glycerol.

**[0068]** The following examples of the preparation and use of the ophthalmological composition herein disclosed are intended to assist a person having ordinary skill in the art to make and use the invention, and are not to be construed as being in any way limiting.

## EXAMPLE 1: Anti-irritation eye drops

[0069] A solution was prepared containing:

[0070] Glycerol	2.5 g
[0071] Carbomer 981	0.05 g
[0072] Water	to 100 ml
[0073] The solution was buffered to pH of 7.2.	

## EXAMPLE 2: Anti-irritation eye drops

[0074] A solution was prepared containing:

[0075] Glycerol	2.5 g
[0076] Sodium hyaluronate	0.015 g
[0077] Carbomer 981	0.015 g
[0078] Water	to 100 ml
[0079] The solution was adjusted to a pH of approximately 7.	

## EXAMPLE 3 Anti-glaucoma eye drops

[0080] A solution was prepared containing:

[0081] Glycerol	2.5 g
[0082] Latanoprost	5 mg
[0083] Carbomer 981	0.03 g
[0084] Water	to 100 ml
[0085] The solution was adjusted to pH of between 6.8 and 7.6.	

A suitable concentration of preservative may optionally be added.

## EXAMPLE 4

[0086] A composition was prepared as described in Example 2 above and was tested on 21 patients suffering from Sjögren's syndrome. Results of the study are summarized in Table 1; the value in each column is the mean score with the standard error of the mean given in parentheses. Lissamine Green staining evaluated by Oxford Grade is a measure of the severity of dry eye syndrome, while OSDI (Ocular Surface Disease Index) is a measure of patient satisfaction. At the conclusion of the treatment, the patients' eyes were free of measurable damage.

TABLE 1

Time	Oxford Grade	OSDI Index
Initial	1.86 (0.1)	55.8 (3.2)
1 month	0.85 (0.21)	37.7 (4.3)
3 months	0.25 (0.13)	32.5 (4.2)

**[0087]** These results are surprising and unexpected, since the Schirmer's test, which measures the level of tear formation was very low at the start of the study ( $1.6 \pm 0.3$  mm) and did not change significantly after 3 months of treatment ( $1.7 \pm 0.3$  mm). That is, in the patients treated according to the method herein disclosed, using the composition herein disclosed, objective measures of the level of eye irritation symptomatic of Sjögren's Syndrome due to the severely reduced tear production characteristic of the condition showed a significant decrease, even though tear production did not increase. This observation cannot be explained by the known physicochemical moisturizing effect of glycerol.

#### EXAMPLE 5

**[0088]** An experiment was performed to investigate the effect *in vitro* of a solution containing glycerol on human corneal epithelial cells, in particular, on the expression of barrier genes Involucrin, Occludin, Filaggrin, and Cadherin-1.

**[0089]** Immortalized human corneal epithelial cells (HCEC cell line) cultured in DMEM/F12 with 5% FBS and 10 ng/ml human epidermal growth factor (Invitrogen – Gibco). The cells were treated for three hours with one of the following three compositions: (a) an aqueous solution of glycerol (0.27% w/v); (b) 20  $\mu$ g/ml Polyinosinic:polycytidylic acid (p(I:C), an activator of TLR3 to induce inflammation; and (c) a combination of the two previous compositions.

**[0090]** The expression of the barrier genes was determined at the mRNA level by use of quantitative "real-time" PCR (Q-PCR). Q-PCR was performed on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA) using the 5' nuclease assay. Total RNA was isolated using TRIzol (Invitrogen) and 3  $\mu$ g of total RNA were reverse-transcribed into cDNA by using 15 U of AMV reverse transcriptase (Promega, Madison, WI, USA) and 0.025  $\mu$ g/ $\mu$ l random primers (Promega). PCR amplification was performed by using the TaqMan primers and probes. As internal controls, transcripts of cyclophilin A (PPIA) were determined.

**[0091]** The pro-inflammatory challenge p(I:C) (20 µg/ml) markedly decreased the expressions of Involucrin, Occludin, Filaggrin, and Cadherin-1. However, of greatest importance, co-incubation of the human corneal epithelial cells with glycerol (0.27%) during the p(I:C)challenge significantly prevented the barrier-impairing actions of the TLR3 agonist.

**[0092]** These results indicate the pro-differentiating, barrier-repairing, anti-inflammatory and protective effects of glycerol on human corneal epithelial cells. Without wishing to be bound by theory, the results of this experiment may help explain the surprising results observed in the treatment protocol described in the previous example.

## CLAIMS

We claim:

1. The use of an ophthalmic preparation comprising an aqueous solution of glycerol in a treatment comprising treatment or prevention of irritation of epithelial cells of the eye, wherein said aqueous solution comprises at least 1% (w/v) glycerol and said method comprises applying said ophthalmic preparation to an affected eye.
2. The use according to claim 1, wherein said treatment comprises treatment of irritation of corneal epithelial cells.
3. The use according to claim 1, wherein said treatment comprises prevention of irritation of corneal epithelial cells.
4. The use according to claim 1, wherein said treatment comprises treatment of irritation of conjunctival epithelial cells.
5. The use according to claim 1, wherein said treatment comprises prevention of irritation of conjunctival epithelial cells.
6. The use according to claim 1, wherein said irritation is not due to infection.
7. The use according to claim 1, wherein said treatment is a treatment of cell irritation resulting from a cause selected from the group consisting of Sjögren's syndrome, Meibomian oil deficiency, drug-induced cell irritation, preservative-induced cell irritation, irritation due to mechanical cell damage induced by contact lens wearing, and ocular surface disease.
8. The use according to claim 1, wherein said method comprises determining a level of devitalization of cells by using Rose Bengal staining.
9. The use according to claim 1, wherein said method comprises determining a level of devitalization of cells by using Lissamine Green staining.
10. The use according to claim 1, wherein said aqueous solution is characterized by a glycerol concentration of between 1% and 4% (w/v).
11. The use according to claim 1, wherein said aqueous solution is essentially isotonic.
12. The use according to claim 1, wherein said aqueous solution has a pH between 6.7 and 7.7.
13. The use according to claim 1, wherein said aqueous solution is characterized by an inorganic salt concentration of less than 0.1% w/v.

14. The use according claim 1, wherein said aqueous solution comprises at least one polymer of molecular weight of at least 10,000 Dalton.
15. The use according to claim 14, wherein the concentration of said at least one polymer is chosen to bring said solution to a predetermined viscosity.
16. The use according to claim 15, wherein said viscosity is between 5 and 125 mPa•s.
17. The use according to claim 14, wherein said polymer is anionic.
18. The use according to claim 17, wherein said aqueous solution comprises at least one polymer selected from the group consisting of hyaluronates, carbomers, and mixtures and combinations thereof.
19. The use according claim 1, wherein said aqueous solution comprises a pharmaceutically effective amount of at least one pharmacologically active agent.
20. The use according claim 1, wherein said aqueous solution comprises at least one substance selected from the group consisting of stabilizers, preservatives, antioxidants, and buffers.
21. The use according claim 1, wherein said treatment comprises applying said ophthalmic preparation from three to eight times daily.
22. The use according claim 1, wherein said treatment comprises applying said ophthalmic preparation until a statistically significant reduction in irritation of corneal epithelial cells, as measured by Lissamine Green staining, is observed.
23. The use according to claim 22, wherein said treatment comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding three months.
24. The use according to claim 22, wherein said treatment comprises applying said ophthalmic preparation three to eight times daily for a period of time not exceeding one month.
25. The use according claim 1, wherein said treatment comprises applying prophylactically said ophthalmic preparation at predetermined intervals following completion of a therapeutic course of treatment, thereby maintaining a condition in which symptoms of said irritation remain significantly reduced relative to their severity prior to said course of therapeutic treatment.
26. The use according to claim 25, wherein said treatment comprises applying prophylactically applying said ophthalmic preparation daily following a therapeutic course of treatment.

27. An ophthalmic preparation comprising an aqueous solution of glycerol, wherein said ophthalmic preparation comprises at least 1% (w/v) glycerol and is an effective treatment for treatment or prevention of irritation of epithelial cells of the eye.
28. The ophthalmic preparation according to claim 27, wherein said ophthalmic preparation is an effective treatment for at least one condition selected from the group consisting of treatment of irritation of corneal epithelial cells; prevention of irritation of corneal epithelial cells; treatment of irritation of conjunctival epithelial cells; and prevention of irritation of conjunctival epithelial cells.
29. The ophthalmic preparation according to claim 27, wherein said ophthalmic preparation is an effective treatment for prevention or elimination of irritation to corneal or conjunctival cells as measured by a method selected from the group consisting of Rose Bengal staining and Lissamine Green staining.
30. The ophthalmic preparation according to claim 27, wherein said ophthalmic preparation is characterized by a glycerol concentration of between 1% and 4% (w/v).
31. The ophthalmic preparation according to claim 27, wherein said solution is essentially isotonic.
32. The ophthalmic preparation according to claim 27, wherein said solution has a pH between 6.7 and 7.7.
33. The ophthalmic preparation according to claim 27, wherein said solution is characterized by an inorganic salt concentration of less than 0.1%.
34. The ophthalmic preparation according to claim 27, comprising at least one polymer of molecular weight of at least 10,000 Dalton.
35. The ophthalmic preparation according to claim 34, wherein the concentration of said at least one polymer is chosen to bring said solution to a predetermined viscosity.
36. The ophthalmic preparation according to claim 35, wherein said predetermined viscosity is between 5 and 125 mPa•s.
37. The ophthalmic preparation according to claim 34, wherein said at least one polymer is anionic.

38. The ophthalmic preparation according to claim 37, wherein said solution comprises at least one polymer selected from the group consisting of hyaluronates, carbomers, and combinations and mixtures thereof.
39. The ophthalmic preparation according to claim 27, comprising a pharmaceutically effective amount of at least one pharmacologically active agent.
40. The ophthalmic preparation according to claim 27, comprising a substance selected from the group consisting of stabilizers, preservatives, antioxidants and buffers.
41. The use of an aqueous solution comprising at least 1% glycerol (w/v) in the preparation of a composition for treatment or prevention of irritation of epithelial cells of the eye.
42. The use according to claim 41, wherein said treatment is selected from the group consisting of treatment of irritation of corneal epithelial cells; prevention of irritation of corneal epithelial cells; treatment of irritation of conjunctival epithelial cells; and prevention of irritation of conjunctival epithelial cells.
43. The use according to claim 42, wherein said ophthalmic preparation is an effective treatment for prevention or elimination of irritation to corneal or conjunctival cells as measured by a method selected from the group consisting of Rose Bengal staining and Lissamine Green staining.

# INTERNATIONAL SEARCH REPORT

International application No  
PCT/IL2018/050184

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. A61K31/047 A61K9/00 A61P27/02  
ADD.

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)  
A61K A61P

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

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

EPO-Internal, CHEM ABS Data, BIOSIS, EMBASE, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SCRIVANTI M ET AL: "Dry-eye syndrome: Hyaluronic acid eyedrop (L02A)", OPHTHALMOLOGIE 1996 FR, vol. 10, no. 1, 1996, pages 24-27, XP009505133, ISSN: 0989-3105</p> <p>the whole document</p> <p>-----</p> <p style="text-align: center;">-/-</p>	1-6, 8, 10, 11, 13-18, 20, 21, 23, 24, 27-30, 32-38, 40-43

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  
"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
7 May 2018	18/05/2018
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer  Pacreu Largo, Marta

## INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2018/050184

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>SOLOMON ABRAHAM ET AL: "The effect of a new tear substitute containing glycerol and hyaluronate on keratoconjunctivitis sicca", JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS, MARY ANN LIEBERT, INC., NEW YORK, NY, US, vol. 14, no. 6, 1 December 1998 (1998-12-01), pages 497-504, XP002682606, ISSN: 1080-7683, DOI: 10.1089/jop.1998.14.497 page 498 - page 499 page 502</p> <p>-----</p>	1-6, 8, 10, 11, 13-18, 20, 21, 23, 24, 27-31, 33-38, 40-43
X	<p>HUBA J. KISS ET AL: "Isotonic Glycerol and Sodium Hyaluronate Containing Artificial Tear Decreases Conjunctivochalasis after One and Three Months: A Self-Controlled, Unmasked Study", PLOS ONE, vol. 10, no. 7, 14 July 2015 (2015-07-14), page e0132656, XP055471810, DOI: 10.1371/journal.pone.0132656 See Results and Discussion</p> <p>-----</p>	1-6, 9-11, 13-18, 20-24, 27-31, 33-38, 40-43
X	<p>WILLIAM G. GENSHIMER ET AL: "Novel Formulation of Glycerin 1% Artificial Tears Extends Tear Film Break-Up Time Compared with Systane Lubricant Eye Drops", JOURNAL OF OCULAR PHARMACOLOGY AND THERAPEUTICS., vol. 28, no. 5, 1 October 2012 (2012-10-01), pages 473-478, XP055471806, US ISSN: 1080-7683, DOI: 10.1089/jop.2011.0053 page 474 page 477</p> <p>-----</p>	1-3, 6, 10, 14, 20, 27, 28, 30, 34, 40-42
X	<p>US 5 106 615 A (DIKSTEIN SHABTAY [IL]) 21 April 1992 (1992-04-21) cited in the application</p> <p>columns 4,6; examples 1-7</p> <p>-----</p> <p>-/-</p>	1-6, 10, 11, 13-21, 23, 24, 27, 30, 31, 33-39, 41

## INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2018/050184

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 895 645 A (DABROWSKI HENRY P [US] ET AL) 20 April 1999 (1999-04-20)  claim 9 -----	1,4, 10-12, 14,15, 20, 27-32, 34,35, 40,41
X	WO 2006/123324 A1 (RESDEVCO RES AND DEV CO LTD [IL]; DIKSTEIN SHABTAY [IL]) 23 November 2006 (2006-11-23)  page 9 - page 11; claims 3-7,18-20; examples 1-5 -----	1-8, 10-14, 17,18, 20,21, 23,24, 27, 29-34, 37,38, 40-43
X	WO 98/32421 A1 (BAUSCH & LOMB [US]) 30 July 1998 (1998-07-30)  page 1; claims 7,9 -----	1,10,12, 14,20, 27,30, 32,34, 40,41
A	SUVARNA P. PHADATARE ET AL: "A Comprehensive Review on Dry Eye Disease: Diagnosis, Medical Management, Recent Developments, and Future Challenges", ADVANCES IN PHARMACEUTICS, vol. 150, no. 1, 1 January 2015 (2015-01-01), pages 50-12, XP55272049, ISSN: 2356-6841, DOI: 10.1155/2015/704946 page 3 -----	1-43

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No PCT/IL2018/050184
---

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 5106615	A 21-04-1992	CA 1311418 C DE 3734835 A1 FR 2604906 A1 GB 2196255 A IL 80298 A IT 1211852 B US 5106615 A		15-12-1992 01-06-1988 15-04-1988 27-04-1988 31-01-1993 03-11-1989 21-04-1992
US 5895645	A 20-04-1999	NONE		
WO 2006123324	A1 23-11-2006	DK 1888024 T3 DK 2465493 T3 EP 1888024 A1 EP 2465493 A1 ES 2393927 T3 ES 2433271 T3 IL 168603 A PT 1888024 E PT 2465493 E US 2008207771 A1 US 2017354735 A1 WO 2006123324 A1		26-11-2012 28-10-2013 20-02-2008 20-06-2012 02-01-2013 10-12-2013 31-05-2011 04-12-2012 11-11-2013 28-08-2008 14-12-2017 23-11-2006
WO 9832421	A1 30-07-1998	AU 723024 B2 BR 9807117 A CA 2278802 A1 DE 69817788 D1 DE 69817788 T2 EP 0969812 A1 ES 2205449 T3 HK 1026363 A1 JP 2001511135 A KR 20000070492 A US 5800807 A WO 9832421 A1		17-08-2000 25-04-2000 30-07-1998 09-10-2003 05-08-2004 12-01-2000 01-05-2004 09-07-2004 07-08-2001 25-11-2000 01-09-1998 30-07-1998