BIOPHOTONIC COMPOSITIONS, KITS AND METHODS

BACKGROUND

Phototherapy has recently been recognized as having wide range of applications in both the medical, cosmetic and dental fields for use in surgeries, therapies and examinations. For example, phototherapy has been developed to treat cancers and tumors with lessened invasiveness. Phototherapy has also been used to disinfect target sites as an antimicrobial treatment. Phototherapy has also been found to promote wound healing.

Photodynamic therapy is a type of phototherapy which involves a step of systemic administration or uptake of a photosensitive agent into the diseased or injured tissue, which step is followed by site-specific application of activating light (photodynamic therapy). Such regimens, however, are often associated with undesired side-effects, including systemic or localized toxicity due to the direct contact of the photosensitive agents with the tissues. Moreover, such existing regimens often demonstrate low therapeutic efficacy due to, for example, the poor uptake of the photosensitive agents into the target tissues. Therefore, it is an object of the present disclosure to provide new and improved compositions and methods useful in phototherapy.

STATEMENT OF INVENTION

The present disclosure provides biophotonic compositions and methods useful in phototherapy. In particular, the biophotonic compositions of the present disclosure may comprise a chromophore in a medium, such as a gelling agent, that provides a barrier such that the chromophore(s) and other components of the topical biophotonic compositions are not in substantial contact with the target tissues, and/or do not penetrate the target tissues. Put another way, the biophotonic compositions of the present disclosure may contain a chromophore in a medium, such as a gelling agent, which provides a barrier rendering the compositions substantially resistant to leaching in use. The use of such biophotonic compositions in phototherapy would therefore not involve substantial direct contact of the target tissues with a chromophore, which may be potentially toxic the tissues or may cause undesired side effects.

From one aspect, there is provided a biophotonic composition comprising a first chromophore; and a gelling agent present in an amount sufficient to gel the composition and render the biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use.

From one aspect, there is provided a biophotonic composition comprising a first chromophore; and a gelling agent present in an amount sufficient to gel the composition and render the biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use, as measured by (i) placing a 2 mm thick layer of the biophotonic composition onto a top surface of a 2.4-3 cm diameter polycarbonate (PC) membrane with a thickness of 10 microns and a pore size of 3 microns, (ii) contacting a bottom surface of the PC membrane with a phosphate saline buffer solution contained in a receptor compartment, and (iii) after a treatment time at room temperature and pressure, measuring the chromophore content in the receptor compartment.

From another aspect, there is provided a biophotonic composition comprising at least a first chromophore and a gelling agent, wherein the biophotonic composition is a gel or a semi-solid and is substantially resistant to leaching such that less than 15% of the total chromophore amount leaches out of the biophotonic composition into tissue when in contact with tissue in use. In certain embodiments, the biophotonic composition is spreadable so that it can conform to a tissue topography.

From yet another aspect, there is provided a biophotonic composition comprising at least a first chromophore and a gelling agent, wherein the biophotonic composition is substantially translucent and is substantially resistant to leaching such that less than 15% of the total chromophore amount leaches out of the biophotonic composition into tissue when in contact with tissue in use. By substantially translucent is meant having a transmission of more than about 20%.

From a further aspect, there is provided a biophotonic composition comprising at least a first chromophore and a gelling agent, wherein the biophotonic composition and/or the gelling

agent has a viscosity of about 10,000-100,000, 10,000-90,000, 10,000-80,000, 10,000-70,000, 15,000-80,000, 15,000-70,000, 15,000-50,000, or 15,000-45,000 cP when measured using a Wells-Brookfield HB cone/plate viscometer and a CP-51 cone at room temperature at a rotational speed of 2 rpm and a torque >10%, or a Brookfield DV-II+Pro viscometer with a spindle of 7, at 50 rpm, 1 minute.

From a yet further aspect, there is provided a biophotonic composition, comprising a first chromophore in a carrier medium, wherein the composition is encapsulated in a membrane which membrane limits leaching of the first chromophore such that less than 15% of the total chromophore amount leaches out of the composition in use. In certain embodiments, the membrane is substantially translucent. The membrane can be selected from a lipid, a polymer, gelatin, cellulose, and cyclodextrins. The composition can also comprise a dendrimer, such as including poly(propylene amine). The carrier medium can be a liquid. It can also be a gel or semi-solid.

From another aspect, there is provided a biophotonic composition comprising a first chromophore and a gelling agent, wherein the viscosity of the biophotonic composition is about 10,000 to about 100,000 cP, preferably about 10,000 to about 60,000 cP, more preferably about 10,000 to about 50,000 cP. In certain embodiments, the first chromophore is a fluorophore which can absorb and emit light from within the composition. Preferably, the biophotonic composition has a spreadable consistency.

From a yet further aspect, there is provided a biophotonic composition comprising a first chromophore and a second chromophore in a medium, wherein at least one of the first and second chromophores is a fluorophore. The first chromophore can be Fluorescein, and the second chromophore Eosin Y. The first chromophore can be Eosin Y and the second chromophore one or more of Rose Bengal, Phloxine B and Erythrosine B.

From another aspect, there is provided a biophotonic composition comprising first and second chromophores in a medium, wherein the first chromophore is a fluorophore, and wherein light emitted by the first chromophore after photoactivation can photoactivate the

second chromophore. In some embodiments of the above two aspects, the medium is a gel or is gel-like. The medium can have a spreadable consistency.

By 'in use' is meant during a treatment time which can be up to about 5 minutes, up to about 10 minutes, up to about 15 minutes, up to about 20 minutes, up to about 25 minutes, or up to about 30 minutes. The treatment time may comprise the total length of time that the composition is in contact with tissues.

Substantially resistant to leaching can be understand to mean less than 15% of the total chromophore amount leaching out of the biophotonic composition into a phosphate saline buffer solution contained in a receptor compartment, through a 2.4-3 cm diameter polycarbonate (PC) membrane with a thickness of 10 microns and a pore size of 3 microns, having a top side onto which a 2 mm thick layer of the biophotonic composition is placed for 5 minutes at room temperature and pressure, and a bottom side which is in direct contact with the phosphate saline buffer solution. It will be understood that if the treatment time is longer than 5 minutes, the leaching test needs to be extended to the treatment time.

In certain embodiments of any of the foregoing or following, the biophotonic topical composition allows less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1%, or essentially none of said chromophore content to leach out of the biophotonic composition.

In certain embodiments of any of the foregoing or following, the biophotonic composition is a topical composition. Preferably, the composition is a gel, semi-solid or viscous liquid, which can be spread on to the treatment site. In some embodiments, the composition can remain on the treatment site when the treatment site is inverted or tilted during the treatment time.

In certain embodiments of any of the foregoing or following, the biophotonic composition is substantially translucent and/or transparent. By substantially translucent is meant having a transmission of more than about 20%. In some embodiments, the translucency comprises at least 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95% or 100% transmittance of light through a 2mm thick composition.

In certain embodiments of any of the foregoing or following, the composition and/or the gelling agent has a viscosity of about 10,000-100,000, 10,000-90,000, 10,000-80,000, 10,000-70,000, 15,000-80,000, 15,000-70,000, 10,000-50,000, 10,000-40,000, 15,000-50,000 or 15,000-40,000 cP when measured using a Wells-Brookfield HB cone/plate viscometer and a CP-51 cone at room temperature at a rotational speed of 2 rpm and a torque >10%, or a Brookfield DV-II+Pro viscometer with a spindle of 7, at 50 rpm, 1 minute.

In certain embodiments of any of the foregoing or following, the gelling agent is selected from the group of cross-linked polymers. The polymers can be covalently or physically cross-linked. The gelling agent can be selected from at least one of a hydrophilic material, a hygroscopic material or a hydrated polymer. The gelling agent can be polyanionic in charge character. In some embodiments, the gelling agent comprises carboxylic functional groups, which may contain from 2 to 7 carbon atoms per functional group.

The gelling agent can be a synthetic polymer selected from the group consisting of vinyl polymers, polyoxyethylene-polyoxypropylene copolymers, poly(ethylene oxide), acrylamide polymers and derivatives or salts thereof. The gelling agent can be a vinyl polymer selected from the group of polyacrylic acid, polymethacrylic acid, polyvinyl pyrrolidone or polyvinyl alcohol. The gelling agent can be a carboxy vinyl polymer or a carbomer obtained by polymerisation of acrylic acid. The carboxy vinyl polymer or carbomer can be crosslinked.

In certain embodiments, the gelling agent is a high molecular weight, cross-linked polyacrylic acid polymer having a viscosity in the range of about 10,000-100,000; 10,000-80,000; 15,000-80,000; 10,000-70,000; 15,000-70,000; 15,000-40,000, 10,000-60,000; 10,000-50,000; 10,000-40,000; 20,000-100,000; 25,000-90,000; 30,000-80,000; 30,000-70,000; 30,000-60,000; 25,000-40,000 cP. The polymer can be selected from the group consisting of, but not limited to Carbopol® 940, Carbopol® 980, ETD 2020 NF, Carbopol® 1382 Polymer, 71G NF, 971P NF, 974P NF, 980 NF, 981 NF, 5984 EP, ETF 2020 NF, ultrez 10 NF, ultrez 20, ultrez 21, 1342 NF, 934 NF, 934P NF, 940 NF, 941 NF.

In certain embodiments, the gelling agent is a polyacrylic acid polymer cross-linked with alkyl acrylate or allyl pentaerythritol and is present in an amount of about 0.05% to about

5% by weight of the final composition, preferably about 0.5% to about 2% by weight of the final composition.

In certain embodiments of any of the foregoing or following, the gelling agent comprises a protein-based polymer, which can be selected from at least one of sodium hyaluronate, gelatin and collagen. The gelling agent can be gelatin and be present in an amount of equal to or more than about 4 % by weight of the final composition. The gelling agent can be collagen and be present in an amount equal to or more than about 5% by weight of the final composition.

In certain embodiments of any of the foregoing or following, the gelling agent comprises a polysaccharide, which can be selected from at least one of starch, chitosan, chitin, agar, alginates, xanthan, carrageenan, guar gum, gellan gum, pectin, and locust bean gum. The gelling agent can be present in an amount equal to or more than about 0.01% by weight of the final composition.

In certain embodiments of any of the foregoing or following, the gelling agent comprises at least one glycol. The glycol can be selected from ethylene glycol and propylene glycol. The ethylene glycol can be polyethylene glycol.

In certain embodiments, the biophotonic composition can further comprise a humectant, such as glycerine. The biophotonic may further comprise healing factors, preservatives, pH adjusters, chelators or the like.

In certain embodiments of any of the foregoing or following, the biophotonic composition is encapsulated in a membrane, which may be impermeable or breathable to allow permeation of gases but not liquids. The membrane may be translucent. The membrane may comprise a lipid, a polymer or gelatin.

In certain embodiments of any of the foregoing or following, the biophotonic composition further comprises an oxygen-releasing agent which can be a peroxide or a peroxide-releasing agent or water. The oxygen-releasing agent can be selected from hydrogen

peroxide, carbamide peroxide, benzoyl peroxide, peroxy acid, alkali metal peroxides, alkali metal percarbonates, peroxyacetic acid, and alkali metal perborates.

In certain embodiments of any of the foregoing or following, the first chromophore can be in an aqueous or alcohol solution in the composition. The gelling agent and the chromophore solution can form a hydrocolloid.

In certain embodiments of any of the foregoing or following, the first chromophore absorbs light at a wavelength of 200-600 nm, or 400-800nm. In certain embodiments of any of the foregoing or following, the first chromophore absorbs light at a wavelength in the range of the visible spectrum. In some embodiments, the first chromophore is a fluorescent chromophore (fluorophore). The first chromophore can be a xanthene dye. The first chromophore can be selected from Eosin Y, Eosin B, Erythrosin B, Fluorescein, Rose Bengal and Phloxin B. The first chromophore can be present in an amount of about 0.001% to about 40% by weight of the total composition, preferably about 0.005% to about 2% by weight of the total composition, more preferably about 0.01% to about 2% by weight of the total composition.

In certain embodiments of any of the foregoing or following, the composition further comprises a second chromophore. The first chromophore can have an emission spectrum that overlaps at least 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70% with an absorption spectrum of the second chromophore. In some embodiments, the first chromophore of the biophotonic topical composition has an emission spectrum that overlaps at least 1-10%, 5-15%, 10-20%, 15-25%, 20-30%, 25-35%, 30-40%, 35-45%, 50-60%, 55-65% or 60-70% with an absorption spectrum of the second chromophore when present.

In certain embodiments of any of the foregoing or following, the first chromophore transfers energy to the second chromophore upon illumination with a light. Illumination of the biophotonic topical composition with light causes a transfer of energy from the first chromophore to the second chromophore. In some embodiments, the second chromophore emits fluorescence and/or generates reactive oxygen species after absorbing energy from the first chromophore. At least one of the chromophores, for example, the first chromophore, can

photobleach during illumination with light. At least one of the chromophores, for example, the first chromophore can emit fluorescence upon illumination with light. In certain embodiments, the biophotonic composition does not generate a substantial amount of heat following illumination with light. In some embodiments, the energy emitted by the biophotonic composition does not cause tissue damage.

In certain embodiments of any of the foregoing or following, the second chromophore can absorb light at a wavelength in the range of the visible spectrum. In some embodiments, the second chromophore has an absorption wavelength that is relatively longer than that of the first chromophore, for example, 10-100 nm, 20-80 nm, 25-70 nm, or 30-60 nm longer.

In certain embodiments of any of the foregoing or following, the first chromophore is Eosin Y, and the second chromophore is one or more selected from Fluorescein, Phloxine B and Erythrosine B. In certain embodiments of any of the foregoing or following, the first chromophore is Fluorescein, and the second chromophore is Eosin Y. Optionally, a third chromophore may be present such as Rose Bengal. In other embodiments, the first chromophore is Rose Bengal. In some embodiments, the biophotonic composition comprises Eosin and Fluorescein. In other embodiments, the biophotonic composition comprises Eosin and Rose Bengal. In other embodiments, the biophotonic composition comprises Fluorescein and Rose Bengal. In other embodiments, the biophotonic composition comprises Fluorescein and Rose Bengal.

The second chromophore can be present in an amount of about 0.0001% to about 40% by weight of the total composition, preferably about 0.0001% to about 2% by weight of the total composition.

In certain embodiments of any of the foregoing or following, the composition comprises a third chromophore. The third chromophore can be a chlorophyll (e.g. chlorophyllin, chlorophyll a, chlorophyll b) or saffron.

In certain embodiments of any of the foregoing or following, the pH of the composition is within the range 4.0 to 7.0, preferably in the range of 4.0 to 6.5, more preferably in the range

of 4.0 to 5.0. The pH of the composition may also be within the range 6.0 to 8.0, preferably in the range 6.5 to 7.5.

In certain embodiments of any of the foregoing or following, the biophotonic composition may be applied to or impregnated into a material such as a pad, a dressing, a woven or non-woven fabric or the like. The impregnated material may be used as a mask (e.g. a face mask) or a dressing.

In certain embodiments of any of the foregoing or following, the biophotonic composition further comprises at least one waveguide within or adjacent to the composition. The waveguide can be a particle, a fibre or a fibrillar network made of a material which can transmit and/or emit light.

In certain embodiments of any of the foregoing or following, the composition does not comprise opaque particles, such as silica.

From a further aspect, there is provided a biophotonic composition, as described above, for use in skin rejuvenation; for use in the treatment of wounds; for use in the treatment or prevention of skin disorders (such as acne, psoriasis); for use in the treatment or prevention of periodontitis; for use in the treatment of acute inflammation; or for use in the treatment of fungal, bacterial or viral infections.

From a yet further aspect, there is provided use of a biophotonic composition, as described above, for skin rejuvenation, for the treatment of wounds; for the treatment or prevention of skin disorders (such as acne, psoriasis); for the treatment or prevention of periodontitis; for the treatment of acute inflammation; or for the treatment of fungal, bacterial or viral infections.

In another aspect, there is provided a method for providing cosmetic treatment, comprising applying topically to skin a biophotonic composition, as defined above; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore. The cosmetic treatment can promote skin rejuvenation.

In a further aspect, there is provided a method for promoting wound healing, comprising: applying topically to a wound a biophotonic composition as defined above; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore. In certain embodiments of the method, the wound as described herein includes for example chronic or acute wounds, such as diabetic foot ulcers, pressure ulcers, venous ulcers or amputations. In some embodiments of the method for providing biophotonic therapy to a wound, the method promotes reduction of scar tissue formation.

In a yet further aspect, there is provided a method for biophotonic treatment of a skin disorder, comprising applying topically a biophotonic composition as defined above to a target skin tissue afflicted with the skin disorder; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore.

In a further aspect, there is provided a method for biophotonic treatment of acne, comprising: applying topically a biophotonic composition, as defined above, to target tissue, wherein the tissue is an acne lesion or an acne scar; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore.

In another aspect, there is provided a method of biophotonic treatment of periodontal disease, comprising: applying topically a biophotonic composition, as defined above, to a periodontal pocket; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore.

In certain embodiments of any method of the present disclosure, the biophotonic composition is illuminated for any time period per treatment in which the biophotonic composition is activated, for example 1 to 30 minutes, preferably less than 20 minutes, 15 minutes, 10 minutes or about 5 minutes. The treatment time can correspond to, or be longer than a time it takes for the first chromophore to photobleach. In certain embodiments, the method of the present disclosure comprises a step of illuminating the biophotonic composition for a period of at least 30 seconds, 2 minutes, 3 minutes, 5 minutes, 7 minutes, 10 minutes, 15

minutes, 20 minutes, 25 minutes, or 30 minutes. In some embodiments, the biophotonic composition is illuminated for a period of at least 3 minutes. Preferably, the biophotonic composition is illuminated with visible non-coherent light, such as violet and/or blue light. Any other suitable light source can be used.

The distance of the light source from the biophotonic composition can be any distance which can deliver an appropriate light power density to the biophotonic composition and/or the skin tissue, for example 5, 10, 15 or 20 cm. The biophotonic composition is applied topically at any suitable thickness. Typically, the biophotonic composition is applied topically to skin or wounds at a thickness of at least about 2mm, about 2mm to about 10mm.

In certain embodiments of the methods of the present disclosure, the biophotonic composition is removed from the site of a treatment following application of light. Accordingly, the biophotonic composition is removed from the site of treatment within at least 30 seconds, 2 minutes, 3 minutes, 5 minutes, 7 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes or 30 minutes after application. In some embodiments, the biophotonic composition is removed after a period of at least 3 minutes post application of the biophotonic composition to treatment site.

In certain other embodiments, the composition remains in the treatment area and can be re-illuminated as required. The biophotonic composition can be kept in place for up to one, two or three weeks. The composition can be re-illuminated with light, which may include ambient light, at various intervals. In this case, the composition may be covered up in between exposure to light. For example, the biophotonic composition may be soaked in a dressing and placed inside or over a wound and be left in place for an extended period of time (e.g. more than one day).

In certain embodiments of the method for biophotonic treatment acne, the treatment can be applied to the skin tissue, such as on the face, once, twice, three times, four times, five times or six times a week, daily, or at any other frequency. The total treatment time can be one week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, eight weeks, nine weeks, ten weeks, eleven weeks, twelve weeks, or any other length of time deemed

appropriate. In certain embodiments, the face may be split into separate areas (cheeks, forehead), and each area treated separately. For example, the composition may be applied topically to a first portion, and that portion illuminated with light, and the biophotonic composition then removed. Then the composition is applied to a second portion, illuminated and removed. Finally, the composition is applied to a third portion, illuminated and removed.

In certain embodiments of the method for biophotonic treatment of wounds, the treatment can be applied in or on the wound once, twice, three times, four times, five times or six times a week, daily, or at any other frequency. The total treatment time can be one week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, eight weeks, nine weeks, ten weeks, eleven weeks, twelve weeks, or any other length of time deemed appropriate.

The disclosed methods for treating acne, wounds or other skin conditions may further include, for example, administering a systemic or topical drug before, during or after the biophotonic treatment. The drug may be an antibiotic, a hormone treatment, or any other pharmaceutical preparation which may help to treat acne or wounds. The combination of a systemic treatment together with a topical biophotonic treatment can reduce the duration of systemic treatment time.

From another aspect there is provided a kit comprising a composition as described above, and one or more of a light source for activating the chromophore, instructions for use of the composition and/or the light source, a dressing, and a device for applying and/or removing the composition from a treatment area.

From another aspect, there is provided a kit comprising a first component comprising a first chromophore; and a second component comprising a gelling agent present in an amount sufficient to gel or thicken the composition and render the biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use.

From a yet further aspect, there is provided a kit comprising a first component comprising a first chromophore; and a second component comprising a gelling agent, wherein, in combination, the first component and the second component form a biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use. The first component and/or the second component may individually also be resistant to leaching.

From another aspect, there is provided a kit comprising: a first component comprising a composition as described above, and a second component comprising an oxygen-releasing agent. Specifically, the first component may comprise a first chromophore and a gelling agent, wherein the composition of the first component, as well as the combined first and second component composition, are substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out in use.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts absorption of light in the various layers of the skin (Samson et al. *Evidence Report/Technology Assessment* 2004, *111*, pages 1-97).

Figure 2 illustrates the Stokes' shift.

Figure 3 illustrates the absorption and emission spectra of donor and acceptor chromophores. The spectral overlap between the absorption spectrum of the acceptor chromophore and the emission spectrum of the donor chromophore is also shown.

Figure 4 is a schematic of a Jablonski diagram that illustrates the coupled transitions involved between a donor emission and acceptor absorbance.

Figure 5 depicts the experimental setup of an *in vitro* release test for evaluating leaching of the chromophore(s) of the biophotonic compositions (Example 6).

Figures 6a and 6b are absorbance and emission spectra, respectively, of a composition according to certain embodiments of the present disclosure which includes Eosin and Fluorescein in a gel (Example 1).

Figures 7a and 7b are absorbance and emission spectra, respectively, of a composition according to certain embodiments of the present disclosure which includes Eosin and Fluorescein in an aqueous solution (Example 2).

Figures 8a and 8b are absorbance and emission spectra, respectively, of a composition according to certain embodiments of the present disclosure which includes Eosin, Fluorescein and Rose Bengal in a gel (Example 3).

Figures 9a and 9b are absorbance and emission spectra, respectively, of a composition according to certain embodiments of the present disclosure which includes Eosin, Fluorescein and Rose Bengal in an aqueous solution (Example 4).

Figure 10 is an emission spectrum showing the intensity over time of the light being emitted from a biophotonic composition of the disclosure tested in Example 5.

Figure 11 is an emission spectrum showing the intensity over time of the light being emitted from a biophotonic composition of the disclosure tested in Example 7.

Figure 12 shows the effect of a biophotonic composition of the disclosure on Ki67 expression (Example 10).

Figure 13 shows that emitted fluorescence from chromophore in a composition increases rapidly with increasing composition but slows down to a plateau with further concentration increase for Eosin Y (top) and Fluorescein (bottom) (Example 13).

Figure 14 shows that Eosin and Rose Bengal act in a synergistic manner (Example 14).

DETAILED DESCRIPTION

(1) Overview

Photodynamic therapy regimens have been developed to promote wound healing, rejuvenate facial skins and treat various skin disorders. However, these methods require direct application of a photosensitive agent to the target skin and/or uptake of the photosensitive

agent into the skin cells. As mentioned above, the direct contact of the photosensitive agent with the tissue can lead to undesired side-effects, including cellular damage/destruction and systemic or localized toxicity to the patient. Moreover, many existing photodynamic therapy regimens often demonstrate low therapeutic efficacy due to, for example, the poor update of the photosensitive agents into the skin cells the target site. For this reason, may regimens require a wait time of between about one and 72 hours to allow the internalization of the photosensitizer.

Phototherapy on the other hand utilizes the therapeutic effect of light. However, expensive and sophisticated light sources are often required to provide therapeutic wavelengths and intensities of light.

The present disclosure provides biophotonic compositions which are useful in phototherapy and which include photoactive exogenous chromophores which can emit a therapeutic light or which can promote a therapeutic effect on a treatment site by activating other components of the biophotonic composition. The present disclosure also provides methods useful for promoting wound healing, cosmetic treatment of skin such as skin rejuvenation, treating acne and treating other skin disorders, treating acute inflammation, which are distinguished from conventional photodynamic therapy.

Biophotonic therapy using the present compositions does not rely on internalization of the chromophore into cells or substantial contact with the cells or target tissues. Therefore, the undesired side effects caused by direct contact may be reduced, minimized, or prevented. At most, the chromophore has surface contact with the tissue to which the composition is applied, which is likely to be very short lasting due to short treatment times. Furthermore, unlike photodynamic therapy, biophotonic therapy with embodiments of the present biophotonic compositions does not rely on cell death or damage. In fact, Applicants have shown in *in vitro* studies that a biophotonic composition according to an embodiment of the present disclosure reduced cell necrosis (see Example 10).

(2) Definitions

Before continuing to describe the present disclosure in further detail, it is to be understood that this disclosure is not limited to specific compositions or process steps, as such may vary. It must be noted that, as used in this specification and the appended claims, the singular form "a", "an" and "the" include plural referents unless the context clearly dictates otherwise.

As used herein, the term "about" in the context of a given value or range refers to a value or range that is within 20%, preferably within 10%, and more preferably within 5% of the given value or range.

It is convenient to point out here that "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

"Biophotonic" means the generation, manipulation, detection and application of photons in a biologically relevant context. In other words, biophotonic compositions exert their physiological effects primarily due to the generation and manipulation of photons. "Biophotonic composition" is a composition as described herein that may be activated by light to produce photons for biologically relevant applications.

"Gels" are defined as substantially dilute cross-linked systems. Gels may be semi-solids and exhibit substantially no flow when in the steady state at room temperature (e.g. about 20-25°C). By steady state is meant herein during a treatment time and under treatment conditions. Gels, as defined herein, may be physically or chemically cross-linked. As defined herein, gels also include gel-like compositions such as viscous liquids.

"Topical" means as applied to body surfaces, such as the skin, mucous membranes, vagina, oral cavity, internal surgical wound sites, and the like.

Terms "chromophore", "photoactivating agent" and "photoactivator" are used herein interchangeably. A chromophore means a chemical compound, when contacted by light

irradiation, is capable of absorbing the light. The chromophore readily undergoes photoexcitation and can then transfer its energy to other molecules or emit it as light.

"Photobleaching" means the photochemical destruction of a chromophore.

"Leaching" means the release of one or more components of a biophotonic composition (e.g., the chromophore(s) from the composition to the surrounding environment such as for example the wound site or into the tissue being treated with the composition. The leaching properties of the biophotonic composition can be measured by (i) placing a 2 mm thick layer of the biophotonic composition onto an upper side of a polycarbonate (PC) membrane having a diameter of 2.4 to 3 cm, a thickness of 10 μm and a pore size of 3 μm, a lower side of the membrane being in contact with a phosphate saline buffer solution in a receptor compartment, (ii) allowing the biophotonic composition to rest on the membrane upper surface at room temperature and pressure for a time corresponding to a treatment time using the biophotonic composition, and (iii) removing a sample of the solution from the receptor compartment and measuring the concentration of the chromophore in the solution.

The term "actinic light" is intended to mean light energy emitted from a specific light source (e.g., lamp, LED, or laser) and capable of being absorbed by matter (e.g. the chromophore or photoactivator defined above). In a preferred embodiment, the actinic light is visible light.

As used herein, a "hygroscopic" substance is a substance capable of taking up water, for example, by absorption or adsorption even at relative humidity as low as 50%, at room temperature (e.g. about 20-25°C).

"Impermeable membrane" means that the material contained within the membrane is sufficiently or substantially impermeable to the surrounding environment such that the migration of such material out of the membrane, and/or the migration of the environmental components (such as water) into the membrane, is so low as to having substantially no adverse impact on the function or activity of the materials retained within the membrane. The impermeable membrane may be 'breathable' in that gas flow through the membrane is

permitted whilst the flow of liquid is not permitted. The impermeable membrane may also selectively allow the migration of some of the materials through the membrane but not others.

"Wound" means an injury to any tissue, including for example, acute, subacute, delayed or difficult to heal wounds, and chronic wounds. Examples of wounds may include both open and closed wounds. Wounds include, for example, burns, incisions, excisions, lesions, lacerations, abrasions, puncture or penetrating wounds, gunshot wounds, surgical wounds, contusions, hematomas, crushing injuries, ulcers (such as for example pressure, venous, pressure or diabetic), wounds caused by periodontitis (inflammation of the periodontium).

"Skin rejuvenation" means a process of reducing, diminishing, retarding or reversing one or more signs of skin aging. For instance, common signs of skin aging include, but are not limited to, appearance of fine lines or wrinkles, thin and transparent skin, loss of underlying fat (leading to hollowed cheeks and eye sockets as well as noticeable loss of firmness on the hands and neck), bone loss (such that bones shrink away from the skin due to bone loss, which causes sagging skin), dry skin (which might itch), inability to sweat sufficiently to cool the skin, unwanted facial hair, freckles, age spots, spider veins, rough and leathery skin, fine wrinkles that disappear when stretched, loose skin, or a blotchy complexion. According to the present disclosure, one or more of the above signs of aging may be reduced, diminished, retarded or even reversed by the compositions and methods of the present disclosure.

(3) Biophotonic Topical Compositions

The present disclosure provides biophotonic compositions. Biophotonic compositions are compositions that are, in a broad sense, activated by light (e.g., photons) of specific wavelength. These compositions contain at least one exogenous chromophore which is activated by light and accelerates the dispersion of light energy, which leads to light carrying on a therapeutic effect on its own, and/or to the photochemical activation of other agents contained in the composition (e.g., acceleration in the breakdown process of peroxide (an oxygen-releasing agent) when such compound is present in the composition or at the treatment site, leading to the formation of oxygen radicals, such as singlet oxygen). The composition may comprise an oxygen-releasing agent which, when mixed with the first chromophore and

subsequently activated by light, can be photochemically activated which may lead to the formation of oxygen radicals, such as singlet oxygen.

In some aspects, the present disclosure provides biophotonic compositions comprising at least a first chromophore in a medium, wherein the composition is substantially resistant to leaching such that a low or negligible chromophore amount leaches out of the biophotonic composition into a treatment site (e.g. tissue) onto which the composition is applied during treatment. In certain embodiments, this is achieved by the medium comprising a gelling agent which slows or restricts movement or leaching of the chromophore. In other embodiments, this is achieved by provision of an encapsulating membrane around the first chromophore in the medium. In this way, contact of the chromophore and the tissue can be minimized or avoided.

In some aspects, biophotonic compositions of the present disclosure do not stain the tissue onto which they are topically applied during treatment. Staining is determined by visually assessing whether the biophotonic composition colorizes white test paper saturated with 70% by volume ethanol/30% by volume water solution placed in contact with the biophotonic composition for a period of time corresponding to a desired treatment time. In some embodiments, a biophotonic composition of the present disclosure does not visually colorize white test paper saturated with a 70% by volume ethanol/30% by volume water solution placed in contact with the biophotonic composition under atmospheric pressure for a time corresponding to a desired treatment time. In certain embodiments, the time corresponding to a treatment time is at least about 5 minutes, at least about 10 minutes, 15 minutes, 20 minutes, 25 minutes or 30 minutes.

When a chromophore absorbs a photon of a certain wavelength, it becomes excited. This is an unstable condition and the molecule tries to return to the ground state, giving away the excess energy. For some chromophores, it is favorable to emit the excess energy as light when transforming back to the ground state. This process is called fluorescence. The peak wavelength of the emitted fluorescence is shifted towards longer wavelengths compared to the absorption wavelengths ('Stokes' shift'). The emitted fluorescent energy can then be transferred to the other components of the composition or to a treatment site on to which the

biophotonic composition is topically applied. **Figure 1** illustrates the different penetrative depths of different wavelength of light. Differing wavelengths of light may have different and complementary therapeutic effects on tissue. Stokes' shift is illustrated in **Figure 2**.

Without being bound to theory, it is thought that fluorescent light emitted by photoactivated chromophores may have therapeutic properties due to its femto-, pico- or nano-second emission properties which may be recognized by biological cells and tissues, leading to favorable biomodulation. Furthermore, the emitted fluorescent light has a longer wavelength and hence a deeper penetration into the tissue than the activating light. Irradiating tissue with such a broad range of wavelengths, including in some embodiments the activating light which passes through the composition, may have different and complementary effects on the cells and tissues. Moreover, in embodiments of the composition containing oxygen-releasing agent(s), micro-bubbling within the composition has been observed by the inventor which may be associated with the generation of oxygen species by the photoactivated chromophores. This may have a physical impact on the tissue to which it is applied, for example by dislodging biofilm and debridement of necrotic tissue or providing a pressure stimulation. The biofilm can also be pre-treated with an oxygen-releasing agent to weaken the biofilm before treating with the composition of the present disclosure.

Certain embodiments of the biophotonic compositions of the present disclosure are substantially transparent/translucent and/or have high light transmittance in order to permit light dissipation into and through the composition. In this way, the area of tissue under the composition can be treated both with the fluorescent light emitted by the composition and the light irradiating the composition to activate it, which may benefit from the different therapeutic effects of light having different wavelengths.

The % transmittance of the biophotonic composition can be measured in the range of wavelengths from 250 nm to 800 nm using, for example, a Perkin-Elmer Lambda 9500 series UV-visible spectrophotometer. Alternatively, a Synergy HT spectrophotometer (BioTek Instrument, Inc.) can be used in the range of wavelengths from 380 nm to 900 nm.

Transmittance is calculated according to the following equation:

$$A_\lambda = \log_{10} \frac{I_0}{I} = \log_{10} \frac{1}{T}.$$

where A is absorbance, T is transmittance, I_0 is intensity of radiation before passing through material, I is intensity of light passing through material.

The values can be normalized for thickness. As stated herein, %transmittance (translucency) is as measured for a 2 mm thick sample at a wavelength of 526 nm. It will be clear that other wavelengths can be used.

In some embodiments, the biophotonic composition has a transparency or translucency that exceeds 15%, 20%, 30%, 40%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85%. In some embodiments, the transparency exceeds 70%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99%. All transmittance values reported herein are as measured on a 2mm thick sample using the Synergy HT spectrophotometer at a wavelength of 526 nm.

Embodiments of the biophotonic compositions of the present disclosure are for topical uses. The biophotonic composition can be in the form of a semi-solid or viscous liquid, having properties which limit leaching of the chromophore(s) from the composition to less than 15% by weight. Preferably, the biophotonic compositions are a gel or are gel-like, including viscous liquids, and which have a spreadable consistency at room temperature (e.g. about 20-25°C), prior to illumination. By spreadable is meant that the composition can be topically applied to a treatment site at a thickness of about 2 mm. Spreadable compositions can conform to a topography of a treatment site, e.g. a wound. This can have advantages over a non-conforming material in that a better and/or more complete illumination of the treatment site can be achieved.

These compositions may be described based on the components making up the composition. Additionally or alternatively, the compositions of the present disclosure have functional and structural properties and these properties may also be used to define and

describe the compositions. Individual components of the composition of the present disclosure are detailed as below.

(a) Chromophores

The biophotonic topical compositions of the present disclosure comprise one or more chromophores, which can be considered exogenous, e.g., are not naturally present in skin or tissue. The chromophores are contained or held within the biophotonic composition such that they do not substantially contact the target tissue to which the composition is applied during a treatment time. In this way, the beneficial and therapeutic properties of the chromophore can be harnessed without the possibly damaging effects caused by chromophore-cell contact.

Suitable chromophores can be fluorescent dyes (or stains), although other dye groups or dyes (biological and histological dyes, food colorings, carotenoids, naturally occurring fluorescent and other dyes) can also be used. Suitable chromophores can be those that are Generally Regarded As Safe (GRAS), although chromophores which are not well tolerated by the skin or other tissues can be included in the biophotonic composition as contact with the skin is minimal in use due to the leaching – barrier nature of the composition.

In certain embodiments, the biophotonic topical composition of the present disclosure comprises a first chromophore which undergoes partial or complete photobleaching upon application of light. By photobleaching is meant a photochemical destruction of the chromophore which can generally be visualized as a loss of color.

In some embodiments, the first chromophore absorbs at a wavelength in the range of the visible spectrum, such as at a wavelength of about 380-800 nm, 380-700, or 380-600 nm. In other embodiments, the first chromophore absorbs at a wavelength of about 200-800 nm, 200-700 nm, 200-600 nm or 200-500 nm. In one embodiment, the first chromophore absorbs at a wavelength of about 200-600 nm. In some embodiments, the first chromophore absorbs light at a wavelength of about 200-300 nm, 250-350 nm, 300-400 nm, 350-450 nm, 400-500 nm, 400-600 nm, 450-650 nm, 600-700 nm, 650-750 nm or 700-800 nm.

It will be appreciated to those skilled in the art that optical properties of a particular chromophore may vary depending on the chromophore's surrounding medium. Therefore, as used herein, a particular chromophore's absorption and/or emission wavelength (or spectrum) corresponds to the wavelengths (or spectrum) measured in a biophotonic composition of the present disclosure.

The biophotonic compositions disclosed herein may include at least one additional chromophore. Combining chromophores may increase photo-absorption by the combined dye molecules and enhance absorption and photo-biomodulation selectivity. This creates multiple possibilities of generating new photosensitive, and/or selective chromophores mixtures.

When such multi-chromophore compositions are illuminated with light, energy transfer can occur between the chromophores. This process, known as resonance energy transfer, is a photophysical process through which an excited 'donor' chromophore (also referred to herein as first chromophore) transfers its excitation energy to an 'acceptor' chromophore (also referred to herein as second chromophore). The efficiency and directedness of resonance energy transfer depends on the spectral features of donor and acceptor chromophores. In particular, the flow of energy between chromophores is dependent on a spectral overlap reflecting the relative positioning and shapes of the absorption and emission spectra. For energy transfer to occur the emission spectrum of the donor chromophore overlap with the absorption spectrum of the acceptor chromophore (**Figure 3**).

Energy transfer manifests itself through decrease or quenching of the donor emission and a reduction of excited state lifetime accompanied also by an increase in acceptor emission intensity. **Figure 4** is a Jablonski diagram that illustrates the coupled transitions involved between a donor emission and acceptor absorbance.

To enhance the energy transfer efficiency, the donor chromophore should have good abilities to absorb photons and emit photons. Furthermore, it is thought that the more overlap there is between the donor chromospheres' emission spectra and the acceptor chromophore's absorption spectra, the better a donor chromophore can transfer energy to the acceptor chromophore.

In certain embodiments, the biophotonic topical composition of the present disclosure further comprises a second chromophore. In some embodiments, the first chromophore has an emission spectrum that overlaps at least about 80%, 50%, 40%, 30%, 20%, 10% with an absorption spectrum of the second chromophore. In one embodiment, the first chromophore has an emission spectrum that overlaps at least about 20% with an absorption spectrum of the second chromophore. In some embodiments, the first chromophore has an emission spectrum that overlaps at least 1-10%, 5-15%, 10-20%, 15-25%, 20-30%, 25-35%, 30-40%, 35-45%, 50-60%, 55-65% or 60-70% with an absorption spectrum of the second chromophore.

% spectral overlap, as used herein, means the % overlap of a donor chromophore's emission wavelength range with an acceptor chromophore's absorption wavelength rage, measured at spectral full width quarter maximum (FWQM). For example, Figure 3 shows the normalized absorption and emission spectra of donor and acceptor chromophores. The spectral FWQM of the acceptor chromophore's absorption spectrum is from about 60 nm (515 nm to about 575 nm). The overlap of the donor chromophore's spectrum with the absorption spectrum of the acceptor chromophore is about 40 nm (from 515 nm to about 555 nm). Thus, the % overlap can be calculated as 40nm / 60nm x 100 = 66.6%.

In some embodiments, the second chromophore absorbs at a wavelength in the range of the visible spectrum. In certain embodiments, the second chromophore has an absorption wavelength that is relatively longer than that of the first chromophore within the range of about 50-250, 25-150 or 10-100 nm.

As discussed above, the application of light to the compositions of the present disclosure can result in a cascade of energy transfer between the chromophores. In certain embodiments, such a cascade of energy transfer provides photons that penetrate the epidermis, dermis and/or mucosa at the target tissue, including, such as, a site of wound, or a tissue afflicted with acne or a skin disorder. In some embodiments, such a cascade of energy transfer is not accompanied by concomitant generation of heat. In some other embodiments, the cascade of energy transfer does not result in tissue damage.

Optionally, when the biophotonic topical composition comprises a first and a second chromophore, the first chromophore is present in an amount of about 0.005-40% per weight of the composition, and the second chromophore is present in an amount of about 0.001-40% per weight of the composition. In certain embodiments, the total weight per weight of chromophore or combination of chromophores may be in the amount of about 0.005-40.001% per weight of the composition. In certain embodiments, the first chromophore is present in an amount of about 0.005-1%, 0.01-2%, 0.02-1%, 0.02-2%, 0.05-1%, 0.05-2%, 0.05-1%, 0.05-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the second chromophore is present in an amount of about 0.001-1%, 0.001-2%, 0.001-0.01%, 0.01-0.1%, 0.1-1.0%, 1-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the total weight per weight of chromophore or combination of chromophores may be in the amount of about 0.005-1%, 0.01-2%, 0.05-2%, 0.5-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40.05% per weight of the composition.

In some embodiments, the chromophore or chromophores are selected such that their emitted fluorescent light, on photoactivation, is within one or more of the green, yellow, orange, red and infrared portions of the electromagnetic spectrum, for example having a peak wavelength within the range of about 490 nm to about 800 nm. In certain embodiments, the emitted fluorescent light has a power density of between 0.005 to about 10 mW/cm^2, about 0.5 to about 5 mW/cm^2.

Suitable chromophores that may be used in the biophotonic topical compositions of the present disclosure include, but are not limited to the following:

Chlorophyll dyes

Exemplary chlorophyll dyes include but are not limited to chlorophyll a; chlorophyll b; oil soluble chlorophyll; bacteriochlorophyll a; bacteriochlorophyll b; bacteriochlorophyll c;

bacteriochlorophyll d; protochlorophyll; protochlorophyll a; amphiphilic chlorophyll derivative 1; and amphiphilic chlorophyll derivative 2.

Xanthene derivatives

Exemplary xanthene dyes include but are not limited to Eosin B (4',5'-dibromo,2',7'-dinitr- o-fluorescein, dianion); eosin Y; eosin Y (2',4',5',7'-tetrabromo-fluoresc- ein, dianion); eosin (2',4',5',7'-tetrabromo-fluorescein, dianion); eosin (2',4',5',7'-tetrabromo-fluorescein, dianion) methyl ester; eosin (2',4',5',7'-tetrabromo-fluorescein, monoanion) p-isopropylbenzyl ester; eosin derivative (2',7'-dibromo-fluorescein, dianion); eosin derivative (4',5'-dibromo-fluorescein, dianion); eosin derivative (2',7'-dichloro-fluorescein, dianion); eosin derivative (4',5'-dichloro-fluorescein, dianion); eosin derivative (2',7'-diiodo-fluorescein, dianion); eosin derivative (4',5'-diiodo-fluorescein, dianion); eosin derivative (tribromo-fluorescein, dianion); eosin derivative (2',4',5',7'-tetrachlor- o-fluorescein, dianion); eosin; eosin dicetylpyridinium chloride ion pair; erythrosin B (2',4',5',7'-tetraiodo-fluorescein, dianion); erythrosin; erythrosin dianion; erythiosin B; fluorescein; fluorescein dianion; phloxin B (2',4',5',7'-tetrabromo-3,4,5,6-tetrachloro-fluorescein, dianion); phloxin B (tetrachloro-tetrabromo-fluorescein); phloxine B; rose bengal (3,4,5,6-tetrachloro-2',4',5',7'-tetraiodofluorescein, dianion); pyronin G, pyronin J, pyronin Y; Rhodamine dyes such as rhodamines include 4,5-dibromo-rhodamine methyl ester; 4,5-dibromo-rhodamine n-butyl ester; rhodamine 101 methyl ester; rhodamine 123; rhodamine 6G; rhodamine 6G hexyl ester; tetrabromo-rhodamine 123; and tetramethyl-rhodamine ethyl ester.

Methylene blue dyes

Exemplary methylene blue derivatives include but are not limited to 1-methyl methylene blue; 1,9-dimethyl methylene blue; methylene blue; methylene blue (16 .mu.M); methylene blue (14 .mu.M); methylene violet; bromomethylene violet; 4-iodomethylene violet; 1,9-dimethyl-3-dimethyl-amino-7-diethyl-a- mino-phenothiazine; and 1,9-dimethyl-3-diethylamino-7-dibutyl-amino-phenot- hiazine.

Azo dyes

Exemplary azo (or diazo-) dyes include but are not limited to methyl violet, neutral red, para red (pigment red 1), amaranth (Azorubine S), Carmoisine (azorubine, food red 3, acid red 14), allura red AC (FD&C 40), tartrazine (FD&C Yellow 5), orange G (acid orange 10), Ponceau 4R (food red 7), methyl red (acid red 2), and murexide-ammonium purpurate.

In some aspects of the disclosure, the one or more chromophores of the biophotonic composition disclosed herein can be independently selected from any of Acid black 1, Acid blue 22, Acid blue 93, Acid fuchsin, Acid green, Acid green 1, Acid green 5, Acid magenta, Acid orange 10, Acid red 26, Acid red 29, Acid red 44, Acid red 51, Acid red 66, Acid red 87, Acid red 91, Acid red 92, Acid red 94, Acid red 101, Acid red 103, Acid roseine, Acid rubin, Acid violet 19, Acid yellow 1, Acid yellow 9, Acid yellow 23, Acid yellow 24, Acid yellow 36, Acid yellow 73, Acid yellow S, Acridine orange, Acriflavine, Alcian blue, Alcian yellow, Alcohol soluble eosin, Alizarin, Alizarin blue 2RC, Alizarin carmine, Alizarin cyanin BBS, Alizarol cyanin R, Alizarin red S, Alizarin purpurin, Aluminon, Amido black 10B, Amidoschwarz, Aniline blue WS, Anthracene blue SWR, Auramine O, Azocannine B, Azocarmine G, Azoic diazo 5, Azoic diazo 48, Azure A, Azure B, Azure C, Basic blue 8, Basic blue 9, Basic blue 12, Basic blue 15, Basic blue 17, Basic blue 20, Basic blue 26, Basic brown 1, Basic fuchsin, Basic green 4, Basic orange 14, Basic red 2 (Saffranin O), Basic red 5, Basic red 9, Basic violet 2, Basic violet 3, Basic violet 4, Basic violet 10, Basic violet 14, Basic yellow 1, Basic yellow 2, Biebrich scarlet, Bismarck brown Y, Brilliant crystal scarlet 6R, Calcium red, Carmine, Carminic acid (acid red 4), Celestine blue B, China blue, Cochineal, Coelestine blue, Chrome violet CG, Chromotrope 2R, Chromoxane cyanin R, Congo corinth, Congo red, Cotton blue, Cotton red, Croceine scarlet, Crocin, Crystal ponceau 6R, Crystal violet, Dahlia, Diamond green B, DiOC6, Direct blue 14, Direct blue 58, Direct red, Direct red 10, Direct red 28, Direct red 80, Direct yellow 7, Eosin B, Eosin Bluish, Eosin, Eosin Y, Eosin yellowish, Eosinol, Erie garnet B, Eriochrome cyanin R, Erythrosin B, Ethyl eosin, Ethyl green, Ethyl violet, Evans blue, Fast blue B, Fast green FCF, Fast red B, Fast yellow, Fluorescein, Food green 3, Gallein, Gallamine blue, Gallocyanin, Gentian violet, Haematein, Haematine, Haematoxylin, Helio fast rubin BBL, Helvetia blue, Hematein, Hematine, Hematoxylin, Hoffman's violet, Imperial red, Indocyanin green, Ingrain blue, Ingrain blue 1, Ingrain yellow 1, INT, Kermes, Kermesic acid, Kernechtrot, Lac, Laccaic acid, Lauth's violet,

Light green, Lissamine green SF, Luxol fast blue, Magenta 0, Magenta I, Magenta II, Magenta III, Malachite green, Manchester brown, Martius yellow, Merbromin, Mercurochrome, Metanil yellow, Methylene azure A, Methylene azure B, Methylene azure C, Methylene blue, Methyl blue, Methyl green, Methyl violet, Methyl violet 2B, Methyl violet 10B, Mordant blue 3, Mordant blue 10, Mordant blue 14, Mordant blue 23, Mordant blue 32, Mordant blue 45, Mordant red 3, Mordant red 11, Mordant violet 25, Mordant violet 39 Naphthol blue black, Naphthol green B, Naphthol yellow S, Natural black 1, Natural red, Natural red 3, Natural red 4, Natural red 8, Natural red 16, Natural red 25, Natural red 28, Natural yellow 6, NBT, Neutral red, New fuchsin, Niagara blue 3B, Night blue, Nile blue, Nile blue A, Nile blue oxazone, Nile blue sulphate, Nile red, Nitro BT, Nitro blue tetrazolium, Nuclear fast red, Oil red O, Orange G, Orcein, Pararosanilin, Phloxine B, phycobilins, Phycocyanins, Phycoerythrins. Phycoerythrincyanin (PEC), Phthalocyanines, Picric acid, Ponceau 2R, Ponceau 6R, Ponceau B, Ponceau de Xylidine, Ponceau S, Primula, Purpurin, Pyronin B, Pyronin G, Pyronin Y, Rhodamine B, Rosanilin, Rose bengal, Saffron, Safranin O, Scarlet R, Scarlet red, Scharlach R, Shellac, Sirius red F3B, Solochrome cyanin R, Soluble blue, Solvent black 3, Solvent blue 38, Solvent red 23, Solvent red 24, Solvent red 27, Solvent red 45, Solvent yellow 94, Spirit soluble eosin, Sudan III, Sudan IV, Sudan black B, Sulfur yellow S, Swiss blue, Tartrazine, Thioflavine S, Thioflavine T, Thionin, Toluidine blue, Toluyline red, Tropaeolin G, Trypaflavine, Trypan blue, Uranin, Victoria blue 4R, Victoria blue B, Victoria green B, Water blue I, Water soluble eosin, Xylidine ponceau, or Yellowish eosin.

In certain embodiments, the composition of the present disclosure includes any of the chromophores listed above, or a combination thereof, so as to provide a biophotonic impact at the application site. This is a distinct application of these agents and differs from the use of chromophores as simple stains or as a catalyst for photo-polymerization.

Chromophores can be selected, for example, on their emission wavelength properties in the case of fluorophores, on the basis of their energy transfer potential, their ability to generate reactive oxygen species, or their antimicrobial effect. These needs may vary depending on the condition requiring treatment. For example, chlorophylls may have an antimicrobial effect on bacteria found on the face.

In some embodiments, the composition includes Eosin Y as a first chromophore and any one or more of Rose Bengal, Erythrosin, Phloxine B as a second chromophore. It is believed that these combinations have a synergistic effect as Eosin Y can transfer energy to Rose Bengal, Erythrosin or Phloxine B when activated. This transferred energy is then emitted as fluorescence or by production of reactive oxygen species. This absorbed and re-emitted light is thought to be transmitted throughout the composition, and also to be transmitted into the site of treatment.

In further embodiments, the composition includes the following synergistic combinations: Eosin Y and Fluorescein; Fluorescein and Rose Bengal; Erythrosine in combination with Eosin Y, Rose Bengal or Fluorescein; Phloxine B in combination with one or more of Eosin Y, Rose Bengal, Fluorescein and Erythrosine. Other synergistic chromophore combinations are also possible.

By means of synergistic effects of the chromophore combinations in the composition, chromophores which cannot normally be activated by an activating light (such as a blue light from an LED) can be activated through energy transfer from chromophores which are activated by the activating light. In this way, the different properties of photoactivated chromophores can be harnessed and tailored according to the cosmetic or the medical therapy required.

For example, Rose Bengal can generate a high yield of singlet oxygen when photoactivated in the presence of molecular oxygen, however it has a low quantum yield in terms of emitted fluorescent light. Rose Bengal has a peak absorption around 540 nm and so is normally activated by green light. Eosin Y has a high quantum yield and can be activated by blue light. By combining Rose Bengal with Eosin Y, one obtains a composition which can emit therapeutic fluorescent light and generate singlet oxygen when activated by blue light. In this case, the blue light photoactivates Eosin Y which transfers some of its energy to Rose Bengal as well as emitting some energy as fluorescence.

Chromophore combinations can also have a synergistic effect in terms of their photoactivated state. For example, two chromophores may be used, one of which emits fluorescent light when activated in the blue and green range, and the other which emits

fluorescent light in the red, orange and yellow range, thereby complementing each other and irradiating the target tissue with a broad wavelength of light having different depths of penetration into target tissue and different therapeutic effects.

(b) Gelling Agent

The present disclosure provides biophotonic compositions that comprise at least a first chromophore and a gelling agent, wherein the gelling agent provides a barrier such that the chromophore(s) of the biophotonic topical compositions are substantially not in contact with the target tissue. The gelling agent, when present in the biophotonic compositions of the present disclosure, can render the compositions substantially resistant to leaching such that the chromophore(s) or photosensitive agent(s) of the biophotonic topical compositions are not in substantial contact with the target tissue.

In certain embodiments, the biophotonic topical composition allows less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1%, or essentially none of said chromophore content to leach out of the biophotonic composition.

In some embodiments, the biophotonic composition limits leaching of the first chromophore such that less than 15% of total chromophore amount can leach out into tissue during a treatment time in which the composition is topically applied onto tissue and illuminated with light. In some embodiments, the biophotonic composition limits leaching of the first chromophore such that less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of total chromophore amount can leach out into tissue during a treatment time in which the composition is topically applied onto tissue and illuminated with light. In some embodiments, the treatment time is at least about 5 minutes, at least about 10 minutes, at least about 15 minutes, at least about 20 minutes, at least about 25 minutes or at least about 30 minutes.

Leaching can be determined as described in Example 6 (see **Figure 5**). In some embodiments, a biophotonic composition of the present disclosure allows less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore

amount to leach out of the biophotonic composition as through a porous membrane into an aqueous solution when the biophotonic composition is placed in contact with the aqueous solution through the porous membrane for a time corresponding to a desired treatment time. In certain embodiments, the time corresponding to a treatment time is at least about 5 minutes, at least about 10 minutes, 15 minutes, 20 minutes, 25 minutes or 30 minutes.

A gelling agent for use according to the present disclosure may comprise any ingredient suitable for use in a topical biophotonic formulation as described herein. The gelling agent may be an agent capable of forming a cross-linked matrix, including physical and/or chemical cross-links. The gelling agent is preferably biocompatible, and may be biodegradable. In some embodiments, the gelling agent is able to form a hydrogel or a hydrocolloid. An appropriate gelling agent is one that can form a viscous liquid or a semisolid. In preferred embodiments, the gelling agent and/or the composition has appropriate light transmission properties. It is also important to select a gelling agent which will allow biophotonic activity of the chromophore(s). For example, some chromophores require a hydrated environment in order to fluoresce. The gelling agent may be able to form a gel by itself or in combination with other ingredients such as water or another gelling agent, or when applied to a treatment site, or when illuminated with light.

The gelling agent according to various embodiments of the present disclosure may include, but not be limited to, polyalkylene oxides, particularly polyethylene glycol and poly(ethylene oxide)-poly(propylene oxide) copolymers, including block and random copolymers; polyols such as glycerol, polyglycerol (particularly highly branched polyglycerol), propylene glycol and trimethylene glycol substituted with one or more polyalkylene oxides, e.g., mono-, di- and tri-polyoxyethylated glycerol, mono- and di-polyoxy-ethylated propylene glycol, and mono- and di-polyoxyethylated trimethylene glycol; polyoxyethylated sorbitol, polyoxyethylated glucose; acrylic acid polymers and analogs and copolymers thereof, such as polyacrylic acid per se, polymethacrylic acid, poly(hydroxyethylmethacrylate), poly(hydroxyethylacrylate), poly(methylalkylsulfoxide methacrylate), poly(methylalkylsulfoxide acrylate) and copolymers of any of the foregoing, and/or with additional acrylate species such as aminoethyl acrylate and mono-2-(acryloxy)-ethyl succinate;

polymaleic acid; poly(acrylamides) such as polyacrylamide per se, poly(methacrylamide), poly(dimethylacrylamide), and poly(N-isopropyl-acrylamide); poly(olefinic alcohol)s such as poly(vinyl alcohol); poly(N-vinyl lactams) such as poly(vinyl pyrrolidone), poly(N-vinyl caprolactam), and copolymers thereof, polyoxazolines, including poly(methyloxazoline) and poly(ethyloxazoline); and polyvinylamines.

The gelling agent according to certain embodiments of the present disclosure may include a polymer selected from any of synthetic or semi-synthetic polymeric materials, polyacrylate copolymers, cellulose derivatives and polymethyl vinyl ether/maleic anhydride copolymers. In some embodiments, the hydrophilic polymer comprises a polymer that is a high molecular weight (i.e., molar masses of more than about 5,000, and in some instances, more than about 10,000, or 100,000, or 1,000,000) and/or cross-linked polyacrylic acid polymer. In some embodiments, the polymer is a polyacrylic acid polymer and has a viscosity in the range of about 10,000-100,000; 10,000-80,000; 15,000-80,000; 10,000-70,000; 15,000-70,000; 10,000-60,000; 10,000-50,000; 10,000-40,000; 20,000-100,000; 25,000-90,000; 30,000-80,000; 30,000-70,000; 30,000-60,000; 25,000-40,000 cP. In certain embodiment, the polymer is a high molecular weight, and/or cross-linked polyacrylic acid polymer, where the polyacrylic acid polymer has a viscosity in the range of about 10,000-80,000 cP.

In some embodiments, the gelling agent comprises a carbomer. Carbomers are synthetic high molecular weight polymer of acrylic acid that are cross-linked with either allylsucrose or allylethers of pentaerythritol having a molecular weight of about 3×10^6. The gelation mechanism depends on neutralization of the carboxylic acid moiety to form a soluble salt. The polymer is hydrophilic and produces sparkling clear gels when neutralized. Carbomer gels possess good thermal stability in that gel viscosity and yield value are essentially unaffected by temperature. As a topical product, carbomer gels possess optimum rheological properties. The inherent pseudoplastic flow permits immediate recovery of viscosity when shear is terminated and the high yield value and quick break make it ideal for dispensing. Aqueous solution of Carbopol® is acidic in nature due to the presence of free carboxylic acid residues. Neutralization of this solution cross-links and gelatinizes the polymer to form a viscous integral structure of desired viscosity.

Carbomers are available as fine white powders which disperse in water to form acidic colloidal suspensions (a 1% dispersion has approx. pH 3) of low viscosity. Neutralization of these suspensions using a base, for example sodium, potassium or ammonium hydroxides, low molecular weight amines and alkanolamines, results in the formation of translucent gels. Nicotine salts such as nicotine chloride form stable water-soluble complexes with carbomers at about pH 3.5 and are stabilized at an optimal pH of about 5.6.

In some embodiments of the disclosure, the carbomer is Carbopol. Such polymers are commercially available from B.F. Goodrich or Lubrizol under the designation Carbopol® 71G NF, 420, 430, 475, 488, 493, 910, 934, 934P, 940, 971PNF, 974P NF, 980 NF, 981 NF and the like. Carbopols are versatile controlled-release polymers, as described by Brock (Pharmacotherapy, 14:430-7 (1994)) and Durrani (Pharmaceutical Res. (Supp.) 8:S-135 (1991)), and belong to a family of carbomers which are synthetic, high molecular weight, non-linear polymers of acrylic acid, crosslinked with polyalkenyl polyether. In some embodiments, the carbomer is Carbopol® 974P NF, 980 NF, 5984 EP, ETD 2020NF, Ultrez 10 NF, 934 NF, 934P NF or 940 NF. In certain embodiments, the carbomer is Carbopol® 980 NF, ETD 2020 NF, Ultrez 10 NF, Ultrez 21 or 1382 Polymer, 1342 NF, 940 NF. For example, 0.05 to 10%, preferably 0.5 to 5%, more preferably 1 to 3% by weight of the final composition of a high molecular weight carbopol can be present as the gelling agent and which can form a gel having a viscosity of more than about 10,000cP, or preferably more than about 15,000cP.

In certain embodiments, the gelling agent comprises a hygroscopic and/or a hydrophilic material which may be used for their water attracting properties, which may also prevent or limit leaching of the chromophore. The hygroscopic or hydrophilic material may include, but is not limited to, glucosamine, polysaccharides, glycosaminoglycan, poly(vinyl alcohol), poly(2-hydroxyethylmethylacrylate), polyethylene oxide, collagen, chitosan, alginate, a poly(acrylonitrile)-based hydrogel, poly(ethylene glycol)/poly(acrylic acid) interpenetrating polymer network hydrogel, polyethylene oxide-polybutylene terephthalate, hyaluronic acid, high-molecular-weight polyacrylic acid, poly(hydroxy ethylmethacrylate), poly(ethylene glycol), tetraethylene glycol diacrylate, polyethylene glycol methacrylate, and poly(methyl acrylate-co-hydroxyethyl acrylate).

The one or more gelling agents can be selected according to their ability to prevent chromophore leaching. For example, gelling agents which increase the viscosity of the biophotonic composition can be selected. In some embodiments, the viscosity of the biophotonic composition is 15,000-100,000, 15,000-90,000, 15,000-80,000, 20,000-80,000, 20,000-70,000, 20,000-50,000, 10,000-50,000, 15,000-50,000, 10,000-40,000, 15,000-40,000 cP. A composition with sufficiently high viscosity parameters can prevent or limit the leaching of chromophores from the composition. Viscosity of the biophotonic compositions of the present disclosure is as measured using a cone/plate viscometer (Wells-Brookfield) using a CP-51 and measuring viscosity at a speed of 2 rpm and making sure that the torque is >10%. Spindle must rotate at least 5 times before a viscosity reading is taken. Alternatively a Brookfield DV-II+Pro viscometer with Spindle 7, 50 rpm, 1 minute can be used.

Gelling agents which include lipids or other coating agents which can coat the chromophores can also be used to limit or prevent leaching. The gelling agent may be protein-based/naturally derived material such as sodium hyaluronate, gelatin or collagen, or the like. The gelling agent may be a polysaccharide such as starch, chitosan, chitin, agarose, agar, locust bean gum, carrageenan, gellan gum, pectin, alginate, xanthan, guar gum, and the like.

In one embodiment, the composition can include up to about 2% by weight of the final composition of sodium hyaluronate as the single gelling agent. In another embodiment, the composition can include more than about 4%, preferably more than about 5%, by weight of the final composition of gelatin as the single gelling agent. In another embodiment, the composition can include up to about 10%, preferably up to about 8%, starch as the single gelling agent. In yet another embodiment, the composition can include more than about 5%, preferably more than about 10%, by weight of the final composition of collagen as the gelling agent. In further embodiments, about 0.1-10%, or about 0.5-3%, by weight of the final composition of chitin can be used as the gelling agent. In other embodiments, 0.5%-5% by weight of the final composition of corn starch, or 5-10% by weight of the final composition of starch can be used as the gelling agent. In certain other embodiments, more than 2.5wt% by weight of the final composition of alginate can be used in the composition as the gelling agent. In other embodiments, the percentages by weight percent of the final composition of the gelling

agents can be as follows: cellulose gel (about 0.3-2.0%), konjac gum (0.5-0.7%), carrageenan gum (0.02-2.0%), xanthan gum (0.01-2.0%), acacia gum (3-30%), agar (0.04-1.2%), guar gum (0.1-1%), locust bean gum (0.15-0.75%), pectin (0.1-0.6%), tara gum (0.1-1.0%), polyvinylypyrrolidone (1-5%), sodium polyacrylate (1-10%). Other gelling agents can be used in amounts sufficient to gel the composition or to sufficiently thicken the composition to avoid or minimize leaching of the chromophore(s). It will be appreciated that lower amounts of the above gelling agents may be used in the presence of another gelling agent or a thickener.

The biophotonic composition of the present disclosure may be further encapsulated, e.g., in a membrane. Such a membrane may be transparent, and/or substantially, or fully impermeable. The membrane may be impermeable to liquid but permeable to gases such as air. In certain embodiments, the composition may form a membrane that encapsulates the chromophore(s) of the biophotonic topical composition, where the membrane may be substantially impermeable to liquid and/or gas.

In certain embodiments, the retention of the chromophore in the composition during the treatment time can be achieved by providing a membrane around a chromophore(s) in a carrier medium. In this case, it is the membrane which limits or stops leaching of the chromophore such as by providing a barrier. The carrier medium can be a liquid encapsulated by the membrane, wherein the membrane is sufficiently resistant to chromophore leaching such that less than 15% of the total chromophore amount leaches out of the encapsulated composition. The membrane may be formed of one or more lipidic agents, polymers, gelatin, cellulose or cyclodextrins, or the like. Preferably, the membrane is translucent or transparent to allow light to infiltrate to and from the chromophore(s). In one embodiment, the composition is a dendrimer with an outer membrane comprising poly(propylene amine). In another embodiment, the outer membrane comprises gelatin.

(c) Oxygen-releasing agents

According to certain embodiments, the compositions of the present disclosure may optionally further comprise one or more additional components, such as oxygen-releasing agents. For instance, the biophotonic topical composition of the present disclosure may

optionally comprise oxygen-releasing agents as a source of oxygen. Peroxide compounds are oxygen-releasing agents that contain the peroxy group (R-O-O-R), which is a chainlike structure containing two oxygen atoms, each of which is bonded to the other and a radical or some element.

When a biophotonic composition of the present disclosure comprising an oxygen-releasing agent is illuminated with light, the chromophore(s) are excited to a higher energy state. When the chromophore(s)' electrons return to a lower energy state, they emit photons with a lower energy level, thus causing the emission of light of a longer wavelength (Stokes' shift). In the proper environment, some of this energy release is transferred to oxygen or the reactive hydrogen peroxide and causes the formation of oxygen radicals, such as singlet oxygen. The singlet oxygen and other reactive oxygen species generated by the activation of the biophotonic composition are thought to operate in a hormetic fashion. That is, a health beneficial effect that is brought about by the low exposure to a normally toxic stimuli (e.g. reactive oxygen), by stimulating and modulating stress response pathways in cells of the targeted tissues. Endogenous response to exogenous generated free radicals (reactive oxygen species) is modulated in increased defense capacity against the exogenous free radicals and induces acceleration of healing and regenerative processes. Furthermore, activation of the composition can also produce an antibacterial effect. The extreme sensitivity of bacteria to exposure to free radicals makes the composition of the present disclosure a de facto bactericidal composition.

As stated above, the generation of oxygen species by the composition in some embodiments is accompanied by the micro-bubbling which can contribute to debridement or dislodging of biofilm at the site of application. This can allow for the improved penetration of the activating and/or fluorescence light to the treatment site for example to deactivate bacterial colonies leading to their reduction in number.

Suitable oxygen-releasing agents that may be included in the composition include, but are not limited to:

Hydrogen peroxide (H_2O_2) is the starting material to prepare organic peroxides. H_2O_2 is a powerful oxygen-releasing agent, and the unique property of hydrogen peroxide is that it breaks down into water and oxygen and does not form any persistent, toxic residual compound. Hydrogen peroxide for use in this composition can be used in a gel, for example with 6% hydrogen peroxide. A suitable range of concentration over which hydrogen peroxide can be used in the present composition is from about 0.1% to about 6%.

Urea hydrogen peroxide (also known as urea peroxide, carbamide peroxide or percarbamide) is soluble in water and contains approximately 35% hydrogen peroxide. Carbamide peroxide for use in this composition can be used as a gel, for example with 16% carbamide peroxide that represents 5.6 % hydrogen peroxide, or 12 % carbamide peroxide. A suitable range of concentration over which urea peroxide can be used in the present composition is from about 0.3% to about 16%. Urea peroxide breaks down to urea and hydrogen peroxide in a slow-release fashion that can be accelerated with heat or photochemical reactions. The released urea [carbamide, $(NH_2)CO_2$)], is highly soluble in water and is a powerful protein denaturant. It increases solubility of some proteins and enhances rehydration of the skin and/or mucosa.

Benzoyl peroxide consists of two benzoyl groups (benzoic acid with the H of the carboxylic acid removed) joined by a peroxide group. It is found in treatments for acne, in concentrations varying from 2.5% to 10%. The released peroxide groups are effective at killing bacteria. Benzoyl peroxide also promotes skin turnover and clearing of pores, which further contributes to decreasing bacterial counts and reduce acne. Benzoyl peroxide breaks down to benzoic acid and oxygen upon contact with skin, neither of which is toxic. A suitable range of concentration over which benzoyl peroxide can be used in the present composition is from about 2.5% to about 5%.

Specific oxygen-releasing agents that that are preferably used in the materials or methods of this disclosure include, but are not limited to hydrogen peroxide, carbamide peroxide, or benzoyl peroxide. Peroxy acid, alkali metal peroxides, alkali metal percarbonates, peroxyacetic acid, and alkali metal perborates can also be included as the oxygen-releasing

agent. . Oxygen-releasing agents can be provided in powder, liquid or gel form. Alternatively, the oxygen-releasing agents may also be applied to the tissue site separately to the composition. Alternatively, the composition may include an amount of oxygen-releasing agent, which is augmented by the separate application of oxygen-releasing agents to the treatment site.

In the compositions and methods of the present disclosure, additional components may optionally be included, or used in combination with the biophotonic compositions as described herein. Such additional components include, but are not limited to, healing factors, growth factors, antimicrobials, wrinkle fillers (e.g. botox, hyaluronic acid or polylactic acid), collagens, anti-virals, anti-fungals, antibiotics, drugs, and/or agents that promote collagen synthesis. These additional components may be applied to the wound, skin or mucosa in a topical fashion, prior to, at the same time of, and/or after topical application of the biophotonic composition of the present disclosure, and may also be systemically administered. Suitable healing factors, antimicrobials, collagens, and/or agents that promote collagen synthesis are discussed below:

(d) Healing Factors

Healing factors comprise compounds that promote or enhance the healing or regenerative process of the tissues on the application site of the composition. During the photoactivation of the composition of the present disclosure, there is an increase of the absorption of molecules at the treatment site by the skin, wound or the mucosa. An augmentation in the blood flow at the site of treatment is observed for an extent period of time. An increase in the lymphatic drainage and a possible change in the osmotic equilibrium due to the dynamic interaction of the free radical cascades can be enhanced or even fortified with the inclusion of healing factors. Suitable healing factors include, but are not limited to:

Hyaluronic acid (Hyaluronan, hyaluronate): is a non-sulfated glycosaminoglycan, distributed widely throughout connective, epithelial and neural tissues. It is one of the primary components of the extracellular matrix, and contributes significantly to cell proliferation and migration. Hyaluronan is a major component of the skin, where it is involved in tissue repair. While it is abundant in extracellular matrices, it contributes to tissues hydrodynamics,

movement and proliferation of cells and participates in a wide number of cell surface receptor interactions, notably those including primary receptor CD44. The hyaluronidases enzymes degrade hyaluronan. There are at least seven types of hyaluronidase-like enzymes in humans, several of which are tumor suppressors. The degradation products of hyaluronic acid, the oligosaccharides and the very-low molecular weight hyaluronic acid, exhibit pro-angiogenic properties. In addition, recent studies show that hyaluronan fragments, but not the native high molecular mass of hyaluronan, can induce inflammatory responses in macrophages and dendritic cells in tissue injury. Hyaluronic acid is well suited to biological applications targeting the skin. Due to its high biocompatibility, it is used to stimulate tissue regeneration. Studies have shown hyaluronic acid appearing in the early stages of healing to physically create room for white blood cells that mediate the immune response. It is used in the synthesis of biological scaffolds for wound healing applications and in wrinkle treatment. A suitable range of concentration over which hyaluronic acid can be used in the present composition is from about 0.001% to about 3%.

Glucosamine: is one of the most abundant monosaccharides in human tissues and a precursor in the biological synthesis of glycosilated proteins and lipids. It is commonly used in the treatment of osteoarthritis. The common form of glucosamine used is its sulfate salt and including glucosamine sulfate sodium chloride. Glucosamine shows a number of effects including an anti-inflammatory activity, stimulation of the synthesis of proteoglycans and the synthesis of proteolytic enzymes. A suitable range of concentration over which glucosamine can be used in the present composition is from about 0.01% to about 3%.

Allantoin: is a diureide of glyosilic acid. It has keratolytic effect, increases the water content of the extracellular matrix, enhances the desquamation of the upper layers of dead (apoptotic) skin cells, and promotes skin proliferation and wound healing.

Also, saffron can act as both a chromophore and a healing factor, and as a potentiator. Other healing agents can also be included such as growth factors.

(e) Antimicrobials

Antimicrobials kill microbes or inhibit their growth or accumulation. Exemplary antimicrobials (or antimicrobial agent) are recited in U.S. Patent Application Publications 20040009227 and 20110081530. Suitable antimicrobials for use in the methods of the present disclosure include, but not limited to, phenolic and chlorinated phenolic and chlorinated phenolic compounds, resorcinol and its derivatives, bisphenolic compounds, benzoic esters (parabens), halogenated carbonilides, polymeric antimicrobial agents, thazolines, trichloromethylthioimides, natural antimicrobial agents (also referred to as "natural essential oils"), metal salts, and broad-spectrum antibiotics.

Specific phenolic and chlorinated phenolic antimicrobial agents that can be used in the disclosure include, but are not limited to: phenol; 2-methyl phenol; 3-methyl phenol; 4-methyl phenol; 4-ethyl phenol; 2,4-dimethyl phenol; 2,5-dimethyl phenol; 3,4-dimethyl phenol; 2,6-dimethyl phenol; 4-n-propyl phenol; 4-n-butyl phenol; 4-n-amyl phenol; 4-tert-amyl phenol; 4-n-hexyl phenol; 4-n-heptyl phenol; mono- and poly-alkyl and aromatic halophenols; p-chlorophenyl; methyl p-chlorophenol; ethyl p-chlorophenol; n-propyl p-chlorophenol; n-butyl p-chlorophenol; n-amyl p-chlorophenol; sec-amyl p-chlorophenol; n-hexyl p-chlorophenol; cyclohexyl p-chlorophenol; n-heptyl p-chlorophenol; n-octyl; p-chlorophenol; o-chlorophenol; methyl o-chlorophenol; ethyl o-chlorophenol; n-propyl o-chlorophenol; n-butyl o-chlorophenol; n-amyl o-chlorophenol; tert-amyl o-chlorophenol; n-hexyl o-chlorophenol; n-heptyl o-chlorophenol; o-benzyl p-chlorophenol; o-benxyl-m-methyl p-chlorophenol; o-benzyl-m,m-dimethyl p-chlorophenol; o-phenylethyl p-chlorophenol; o-phenylethyl-m-methyl p-chlorophenol; 3-methyl p-chlorophenol 3,5-dimethyl p-chlorophenol, 6-ethyl-3-methyl p-chlorophenol, 6-n-propyl-3-methyl p-chlorophenol; 6-iso-propyl-3-methyl p-chlorophenol; 2-ethyl-3,5-dimethyl p-chlorophenol; 6-sec-butyl-3-methyl p-chlorophenol; 2-iso-propyl-3,5-dimethyl p-chlorophenol; 6-diethylmethyl-3-methyl p-chlorophenol; 6-iso-propyl-2-ethyl-3-methyl p-chlorophenol; 2-sec-amyl-3,5-dimethyl p-chlorophenol; 2-diethylmethyl-3,5-dimethyl p-chlorophenol; 6-sec-octyl-3-methyl p-chlorophenol; p-chloro-m-cresol p-bromophenol; methyl p-bromophenol; ethyl p-bromophenol; n-propyl p-bromophenol; n-butyl p-bromophenol; n-amyl p-bromophenol; sec-amyl p-bromophenol; n-hexyl p-bromophenol; cyclohexyl p-bromophenol; o-bromophenol; tert-amyl o-bromophenol; n-hexyl o-bromophenol; n-propyl-m,m-dimethyl o-bromophenol; 2-phenyl phenol; 4-chloro-2-methyl

phenol; 4-chloro-3-methyl phenol; 4-chloro-3,5-dimethyl phenol; 2,4-dichloro-3,5-dimethylphenol; 3,4,5,6-tetabromo-2-methylphenol- ; 5-methyl-2-pentylphenol; 4-isopropyl-3-methylphenol; para-chloro-metaxylenol (PCMX); chlorothymol; phenoxyethanol; phenoxyisopropanol; and 5-chloro-2-hydroxydiphenylmethane.

Resorcinol and its derivatives can also be used as antimicrobial agents. Specific resorcinol derivatives include, but are not limited to: methyl resorcinol; ethyl resorcinol; n-propyl resorcinol; n-butyl resorcinol; n-amyl resorcinol; n-hexyl resorcinol; n-heptyl resorcinol; n-octyl resorcinol; n-nonyl resorcinol; phenyl resorcinol; benzyl resorcinol; phenylethyl resorcinol; phenylpropyl resorcinol; p-chlorobenzyl resorcinol; 5-chloro-2,4-dihydroxydiphenyl methane; 4'-chloro-2,4-dihydroxydiphenyl methane; 5-bromo-2,4-dihydroxydiphenyl methane; and 4'-bromo-2,4-dihydroxydiphenyl methane.

Specific bisphenolic antimicrobial agents that can be used in the disclosure include, but are not limited to: 2,2'-methylene bis-(4-chlorophenol); 2,4,4'trichloro-2'-hydroxy-diphenyl ether, which is sold by Ciba Geigy, Florham Park, N.J. under the tradename Triclosan®; 2,2'-methylene bis-(3,4,6-trichlorophenol); 2,2'-methylene bis-(4-chloro-6-bromophenol); bis-(2-hydroxy-3,5-dichlorop- henyl) sulphide; and bis-(2-hydroxy-5-chlorobenzyl)sulphide.

Specific benzoie esters (parabens) that can be used in the disclosure include, but are not limited to: methylparaben; propylparaben; butylparaben; ethylparaben; isopropylparaben; isobutylparaben; benzylparaben; sodium methylparaben; and sodium propylparaben.

Specific halogenated carbanilides that can be used in the disclosure include, but are not limited to: 3,4,4'-trichlorocarbanilides, such as 3-(4-chlorophenyl)-1-(3,4-dichlorphenyl)urea sold under the tradename Triclocarban® by Ciba-Geigy, Florham Park, N.J.; 3-trifluoromethyl-4,4'-dichlorocarbanilide; and 3,3',4-trichlorocarbanilide.

Specific polymeric antimicrobial agents that can be used in the disclosure include, but are not limited to: polyhexamethylene biguanide hydrochloride; and poly(iminoimidocarbonyl iminoimidocarbonyl iminohexamethylene hydrochloride), which is sold under the tradename Vantocil® IB.

Specific thazolines that can be used in the disclosure include, but are not limited to that sold under the tradename Micro-Check®; and 2-n-octyl-4-isothiazolin-3-one, which is sold under the tradename Vinyzene® IT-3000 DIDP.

Specific trichloromethylthioimides that can be used in the disclosure include, but are not limited to: N-(trichloromethylthio)phthalimide, which is sold under the tradename Fungitrol®; and N-trichloromethylthio-4-cyclohexene-1,2-dicarboximide, which is sold under the tradename Vancide®.

Specific natural antimicrobial agents that can be used in the disclosure include, but are not limited to, oils of: anise; lemon; orange; rosemary; wintergreen; thyme; lavender; cloves; hops; tea tree; citronella; wheat; barley; lemongrass; cedar leaf; cedarwood; cinnamon; fleagrass; geranium; sandalwood; violet; cranberry; eucalyptus; vervain; peppermint; gum benzoin; basil; fennel; fir; balsam; menthol; ocmea origanuin; hydastis; carradensis; Berberidaceac daceae; Ratanhiae longa; and Curcuma longa. Also included in this class of natural antimicrobial agents are the key chemical components of the plant oils which have been found to provide antimicrobial benefit. These chemicals include, but are not limited to: anethol; catechole; camphene; thymol; eugenol; eucalyptol; ferulic acid; farnesol; hinokitiol; tropolone; limonene; menthol; methyl salicylate; carvacol; terpineol; verbenone; berberine; ratanhiae extract; caryophellene oxide; citronellic acid; curcumin; nerolidol; and geraniol.

Specific metal salts that can be used in the disclosure include, but are not limited to, salts of metals in groups 3a-5a, 3b-7b, and 8 of the periodic table. Specific examples of metal salts include, but are not limited to, salts of: aluminum; zirconium; zinc; silver; gold; copper; lanthanum; tin; mercury; bismuth; selenium; strontium; scandium; yttrium; cerium; praseodymiun; neodymium; promethum; samarium; europium; gadolinium; terbium; dysprosium; holmium; erbium; thalium; ytterbium; lutetium; and mixtures thereof. An example of the metal-ion based antimicrobial agent is sold under the tradename HealthShield®, and is manufactured by HealthShield Technology, Wakefield, Mass. [give other examples here e.g. smith and nephew]

Specific broad-spectrum antimicrobial agents that can be used in the disclosure include, but are not limited to, those that are recited in other categories of antimicrobial agents herein.

Additional antimicrobial agents that can be used in the methods of the disclosure include, but are not limited to: pyrithiones, and in particular pyrithione-including zinc complexes such as that sold under the tradename Octopirox®; dimethyidimethylol hydantoin, which is sold under the tradename Glydant®; methylchloroisothiazolinone/ methylisothiazolinone, which is sold under the tradename Kathon CG®; sodium sulfite; sodium bisulfite; imidazolidinyl urea, which is sold under the tradename Germall 115®; diazolidinyl urea, which is sold under the tradename Germall 11®; benzyl alcohol v2-bromo-2-nitropropane-1,3-diol, which is sold under the tradename Bronopol®; formalin or formaldehyde; iodopropenyl butylcarbamate, which is sold under the tradename Polyphase P100®; chloroacetamide; methanamine; methyldibromonitrile glutaronitrile (1,2-dibromo-2,4-dicyanobutane), which is sold under the tradename Tektamer®; glutaraldehyde; 5-bromo-5-nitro-1,3-dioxane, which is sold under the tradename Bronidox®; phenethyl alcohol; o-phenylphenol/sodium o-phenylphenol sodium hydroxymethylglycinate, which is sold under the tradename Suttocide A®; polymethoxy bicyclic oxazolidine; which is sold under the tradename Nuosept C®; dimethoxane; thimersal; dichlorobenzyl alcohol; captan; chlorphenenesin; dichlorophene; chlorbutanol; glyceryl laurate; halogenated diphenyl ethers; 2,4,4'-trichloro-2'-hydroxy-diphenyl ether, which is sold under the tradename Triclosan® and is available from Ciba-Geigy, Florham Park, N.J.; and 2,2'-dihydroxy-5,5'-dibromo-diphenyl ether.

Additional antimicrobial agents that can be used in the methods of the disclosure include those disclosed by U.S. Pat. Nos. 3,141,321; 4,402,959; 4,430,381; 4,533,435; 4,625,026; 4,736,467; 4,855,139; 5,069,907; 5,091,102; 5,639,464; 5,853,883; 5,854,147; 5,894,042; and 5,919,554, and U.S. Pat. Appl. Publ. Nos. 20040009227 and 20110081530.

(f) Collagens and Agents that Promote Collagen Synthesis

Collagen is a fibrous protein produced in dermal fibroblast cells and forming 70% of the dermis. Collagen is responsible for the smoothing and firming of the skin. Therefore, when the synthesis of collagen is reduced, skin aging will occur, and so the firming and

smoothing of the skin will be rapidly reduced. As a result, the skin will be flaccid and wrinkled. On the other hand, when metabolism of collagen is activated by the stimulation of collagen synthesis in the skin, the components of dermal matrices will be increased, leading to effects, such as wrinkle improvement, firmness improvement and skin strengthening. Thus, collagens and agents that promote collagen synthesis may also be useful in the present disclosure. Agents that promote collagen synthesis (i.e., pro-collagen synthesis agents) include amino acids, peptides, proteins, lipids, small chemical molecules, natural products and extracts from natural products.

For instance, it was discovered that intake of vitamin C, iron, and collagen can effectively increase the amount of collagen in skin or bone. See, e.g., U.S. Patent Application Publication 20090069217. Examples of the vitamin C include an ascorbic acid derivative such as L-ascorbic acid or sodium L-ascorbate, an ascorbic acid preparation obtained by coating ascorbic acid with an emulsifier or the like, and a mixture containing two or more of those vitamin Cs at an arbitrary rate. In addition, natural products containing vitamin C such as acerola and lemon may also be used. Examples of the iron preparation include: an inorganic iron such as ferrous sulfate, sodium ferrous citrate, or ferric pyrophosphate; an organic iron such as heme iron, ferritin iron, or lactoferrin iron; and a mixture containing two or more of those irons at an arbitrary rate. In addition, natural products containing iron such as spinach or liver may also be used. Moreover, examples of the collagen include: an extract obtained by treating bone, skin, or the like of a mammal such as bovine or swine with an acid or alkaline; a peptide obtained by hydrolyzing the extract with a protease such as pepsine, trypsin, or chymotrypsin; and a mixture containing two or more of those collagens at an arbitrary rate. Collagens extracted from plant sources may also be used.

Additional pro-collagen synthesis agents are described, for example, in U.S. Patent Patents 7598291, 7722904, 6203805 , 5529769, etc, and U.S. Patent Application Publications 20060247313, 20080108681, 20110130459, 20090325885, 20110086060, etc.

The composition can also include other ingredients such as humectants (e.g. glycerine and propylene glycol), preservatives such as parabens, and pH adjusters such as sodium hydroxide.

(4) Methods of Use

The biophotonic compositions of the present disclosure have numerous uses. Without being bound by theory, the biophotonic compositions of the present disclosure may promote wound healing or tissue repair. The biophotonic compositions of the present disclosure may also be used to treat a skin disorder. The biophotonic compositions of the present disclosure may also be used to treat acne. The biophotonic compositions of the present disclosure may also be used for skin rejuvenation. The biophotonic compositions of the present disclosure may also be used for treating acute inflammation. Therefore, it is an objective of the present disclosure to provide a method for providing biophotonic therapy to a wound, where the method promotes wound healing. It is also an objective of the present disclosure to provide a method for providing biophotonic therapy to a skin tissue afflicted with acne, wherein the method is used to treat acne. It is also an objective of the present disclosure to provide a method for providing biophotonic therapy to a skin tissue afflicted with a skin disorder, wherein the method is used to treat the skin disorder. It is also an objective of the present disclosure to provide a method for providing biophotonic therapy to skin tissue, wherein the method is used for promoting skin rejuvenation.

In certain embodiments, the present disclosure provides a method for providing a biophotonic therapy to a wound, the method comprising: applying (e.g., by topical application) a biophotonic composition of the present disclosure to a site of a wound, and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the chromophore(s) of the biophotonic composition.

In one aspect, the present disclosure provides a method for providing biophotonic therapy to a wound, comprising: topically applying a biophotonic composition comprising a first chromophore; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the

biophotonic composition is substantially resistant to leaching such that it limits leaching of the chromophore into the tissue during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment.

In another aspect, the present disclosure provides a method for treating a wound or providing biophotonic therapy to a wound, comprising: topically applying a biophotonic composition comprising a first chromophore and a gelling agent to a site of a wound; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the gelling agent blocks substantial leaching of the chromophores into the site of a wound during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment.

In yet another aspect, the present disclosure provides a method for promoting skin rejuvenation. In certain embodiments, the present disclosure provides a method for providing skin rejuvenation, the method comprising: applying (e.g., by topical application) a biophotonic composition of the present disclosure to the skin, and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the chromophore(s) of the biophotonic composition.

In other embodiments, the present disclosure provides a method for promoting skin rejuvenation comprising: topically applying a biophotonic composition comprising a first chromophore to skin; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it limits leaching of the chromophore into the skin during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment.

In another aspect, the present disclosure provides a method for promoting skin rejuvenation, comprising: topically applying a biophotonic composition comprising a first chromophore and a gelling agent to skin; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it blocks substantial leaching of the chromophores into the skin during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the skin during treatment.

In yet another aspect, the present disclosure to provide a method for providing biophotonic therapy to a target skin tissue afflicted with a skin disorder. In certain embodiments, the present disclosure provides a method for providing a biophotonic therapy to a target skin tissue, the method comprising: applying (e.g., by topical application) a biophotonic composition of the present disclosure to a target skin tissue, and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the chromophore(s) of the biophotonic composition.

In other embodiments, the present disclosure provides a method for treating a skin disorder, comprising: topically applying a biophotonic composition to a target skin tissue afflicted with the skin disorder, wherein the biophotonic composition comprises a first chromophore; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it limits leaching of the chromophore into the skin during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the skin during treatment.

In another aspect, the present disclosure provides a method for treating a skin disorder, comprising: topically applying a biophotonic composition comprising a first chromophore and a gelling agent to skin afflicted with the skin disorder; and illuminating said biophotonic

composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it blocks substantial leaching of the chromophores into the skin during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the skin during treatment.

In yet another aspect, the present disclosure to provide a method for providing biophotonic therapy to a target skin tissue afflicted with acne. In certain embodiments, the present disclosure provides a method for providing a biophotonic therapy to a target skin tissue afflicted with acne, the method comprising: applying (e.g., by topical application) a biophotonic composition of the present disclosure to a target skin tissue, and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the chromophore(s) of the biophotonic composition.

In other embodiments, the present disclosure provides a method for treating acne, comprising: topically applying a biophotonic composition to a target skin tissue afflicted with acne, wherein the biophotonic composition comprises a first chromophore; illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it limits leaching of the chromophore into tissue during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the tissue during treatment.

In another aspect, the present disclosure provides a method for treating acne, comprising: topically applying a biophotonic composition comprising a first chromophore to skin afflicted with acne; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it blocks substantial leaching of the chromophores into the skin during treatment. In some embodiments, less than

30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment.

In other embodiments, the present disclosure provides a method for treating acute inflammation, comprising: topically applying a biophotonic composition to a target skin tissue with acute inflammation, wherein the biophotonic composition comprises a first chromophore; illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it limits leaching of the chromophore into tissue during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the tissue during treatment.

In another aspect, the present disclosure provides a method for treating acute inflammation, comprising: topically applying a biophotonic composition comprising a first chromophore to skin afflicted with acute inflammation; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it blocks substantial leaching of the chromophores into the skin during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%, 0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment.

In another aspect, the present disclosure provides a method for treating fungal infections, comprising: topically applying a biophotonic composition comprising a first chromophore to a target site afflicted with acute inflammation; and illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore; wherein the biophotonic composition is substantially resistant to leaching such that it blocks substantial leaching of the chromophores into the target site during treatment. In some embodiments, less than 30%, 25%, 20%, 15%, 10%, 5%, 1%,

0.8%, 0.5% or 0.1% or essentially 0% of the total chromophore amount leaches out of the biophotonic composition into the wound or tissue during treatment. In some eboidments, the target site may be skin or nails.

The biophotonic compositions suitable for use in the methods of the present disclosure may be selected from any of the embodiments of the biophotonic compositions described above. For instance, the biophotonic compositions useful in the method of the present disclosure may comprise a first chromophore that undergoes at least partial photobleaching upon application of light. The first chromophore may absorb at a wavelength of about 200-800 nm, 200-700 nm, 200-600 nm or 200-500 nm. In one embodiment, the first chromophore absorbs at a wavelength of about 200-600 nm. In some embodiments, the first chromophore absorbs light at a wavelength of about 200-300 nm, 250-350 nm, 300-400 nm, 350-450 nm, 400-500 nm, 450-650 nm, 600-700 nm, 650-750 nm or 700-800 nm. In other examples, suitable biophotonic compositions for the methods of the present disclosure may further comprise at least one additional chromophore (e.g., a second chromophore). The absorption spectrum of the second chromophore overlaps at least about 80%, 50%, 40%, 30%, or 20% with the emission spectrum of the first chromophore. In some embodiments, the first chromophore has an emission spectrum that overlaps at least 1-10%, 5-15%, 10-20%, 15-25%, 20-30%, 25-35%, 30-40%, 35-45%, 50-60%, 55-65% or 60-70% with an absorption spectrum of the second chromophore.

Illumination of the biophotonic composition with light may cause a transfer of energy from the first chromophore to the second chromophore. Subsequently, the second chromophore may emit energy as fluorescence and/or generate reactive oxygen species. In certain embodiments of the methods the present disclosure, energy transfer caused by the application of light is not accompanied by concomitant generation of heat, or does not result in tissue damage.

The biophotonic compositions useful for the present methods comprise a gelling agent. The gelling agent may include, but is not limited to, lipids such as glycerin, glycols such as propylene glycol, hyaluronic acid, glucosamine sulfate, cellulose derivatives (hydroxypropyl

methylcellulose, hydroxyethyl cellulose, hydroxypropyl cellulose, methylcellulose and the like), noncellulose polysaccharides (galactomannans, guar gum, carob gum, gum arabic, sterculia gum, agar, alginates and the like) and acrylic acid polymers.

When the method involves a biophotonic composition having at least two chromophores, the first chromophore is present in an amount of about 0.01-40% per weight of the composition, and the second chromophore is present in an amount of about 0.001-40% per weight of the composition. In certain embodiments, the first chromophore is present in an amount of about 0.01-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the second chromophore is present in an amount of about 0.001-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the total weight per weight of chromophore or combination of chromophores may be in the amount of about 0.01-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40.05% per weight of the composition.

In the methods of the present disclosure, any source of actinic light can be used. Any type of halogen, LED or plasma arc lamp or laser may be suitable. The primary characteristic of suitable sources of actinic light will be that they emit light in a wavelength (or wavelengths) appropriate for activating the one or more photoactivators present in the composition. In one embodiment, an argon laser is used. In another embodiment, a potassium-titanyl phosphate (KTP) laser (e.g. a GreenLight™ laser) is used. In another embodiment, sunlight may be used. In yet another embodiment, a LED photocuring device is the source of the actinic light. In yet another embodiment, the source of the actinic light is a source of light having a wavelength between about 200 to 800 nm. In another embodiment, the source of the actinic light is a source of visible light having a wavelength between about 400 and 600 nm. Furthermore, the source of actinic light should have a suitable power density. Suitable power density for non-collimated light sources (LED, halogen or plasma lamps) are in the range from about 1 mW/cm^2 to about

200 mW/cm^2. Suitable power density for laser light sources are in the range from about 0.5 mW/cm^2 to about 0.8 mW/cm^2.

In some embodiments of the methods of the present disclosure, the light has an energy at the subject's skin, wound or mucosa surface of between about 1 mW/cm^2 and about 500 mW/cm^2, 1-300 mW/cm^2, or 1-200 mW/cm^2, wherein the energy applied depends at least on the condition being treated, the wavelength of the light, the distance of the subject's skin from the light source, and the thickness of the biophotonic compsoition. In certain embodiments, the light at the subject's skin is between about 1-40 mW/cm^2, or 20-60 mW/cm^2, or 40-80 mW/cm^2, or 60-100 mW/cm^2, or 80-120 mW/cm^2, or 100-140 mW/cm^2, or 120-160 mW/cm^2, or 140-180 mW/cm^2, or 160-200 mW/cm^2, or 110-240 mW/cm^2, or 110-150 mW/cm^2, or 190-240 mW/cm^2.

In some embodiments, a mobile device can be used to activate embodiments of the biophotonic composition of the present disclosure, wherein the mobile device can emit light having an emission spectra which overlaps an absorption spectra of the chromophore in the biophotonic composition. The mobile device can have a display screen through which the light is emitted and/or the mobile device can emit light from a flashlight which can photoactivate the biophotonic composition.

In some embodiments, a display screen on a television or a computer monitor can be used to activate the biophotonic composition, wherein the display screen can emit light having an emission spectra which overlaps an absorption spectra of a photoactive agent in the photoactivatable composition.

In certain embodiments, the first and/or the second chromophore (when present) can be photoactivated by ambient light which may originate from the sun or other light sources. Ambient light can be considered to be a general illumination that comes from all directions in a room that has no visible source. In certain embodiments, the first and/or the second chromophore (when present) can be photoactivated by light in the visible range of the electromagnetic spectrum. Exposure times to ambient light may be longer than that to direct light.

In certain embodiments, different sources of light can be used to activate the biophotonic compositions, such as a combination of ambient light and direct LED light.

The duration of the exposure to actinic light required will be dependent on the surface of the treated area, the type of lesion, trauma or injury that is being treated, the power density, wavelength and bandwidth of the light source, the thickness of the biophotonic composition, and the treatment distance from the light source. The illumination of the treated area by fluorescence may take place within seconds or even fragment of seconds, but a prolonged exposure period is beneficial to exploit the synergistic effects of the absorbed, reflected and reemitted light on the composition of the present disclosure and its interaction with the tissue being treated. In one embodiment, the time of exposure to actinic light of the tissue, skin or wound on which the biophotonic composition has been applied is a period between 1 minute and 5 minutes. In another embodiment, the time of exposure to actinic light of the tissue, skin or wound on which the biophotonic composition has been applied is a period between 1 minute and 5 minutes. In some other embodiments, the biophotonic composition is illuminated for a period between 1 minute and 3 minutes. In certain embodiments, light is applied for a period of 1-30 seconds, 15-45 seconds, 30-60 seconds, 0.75-1.5 minutes, 1-2 minutes, 1.5-2.5 minutes, 2-3 minutes, 2.5-3.5 minutes, 3-4 minutes, 3.5-4.5 minutes, 4-5 minutes, 5-10 minutes, 10-15 minutes, 15-20 minutes, 20-25 minutes, or 20-30 minutes. In yet another embodiment, the source of actinic light is in continuous motion over the treated area for the appropriate time of exposure. In yet another embodiment, multiple applications of the biophotonic composition and actinic light are performed. In some embodiments, the tissue, skin or wound is exposed to actinic light at least two, three, four, five or six times. In some embodiments, a fresh application of the biophotonic composition is applied before exposure to actinic light.

In the methods of the present disclosure, the biophotonic composition may be optionally removed from the site of treatment following application of light. In certain embodiments, the biophotonic composition is left on the treatment site for more than 30 minutes, more than one hour, more than 2 hours, more than 3 hours. It can be illuminated with ambient light. To prevent drying, the composition can be covered with a transparent or

translucent cover such as a polymer film, or an opaque cover which can be removed before illumination.

(5) Wounds and Wound Healing

The biophotonic compositions and methods of the present disclosure may be used to treat wounds and promote wound healing. Wounds that may be treated by the biophotonic compositions and methods of the present disclosure include, for example, injuries to the skin and subcutaneous tissue initiated in different ways (e.g., pressure ulcers from extended bed rest, wounds induced by trauma, wounds induced by conditions such as periodontitis) and with varying characteristics. In certain embodiments, the present disclosure provides biophotonic compositions and methods for treating and/or promoting the healing of, for example, burns, incisions, excisions, lacerations, abrasions, puncture or penetrating wounds, surgical wounds, contusions, hematomas, crushing injuries, sores and ulcers.

Biophotonic compositions and methods of the present disclosure may be used to treat and/or promote the healing of chronic cutaneous ulcers or wounds, which are wounds that have failed to proceed through an orderly and timely series of events to produce a durable structural, functional, and cosmetic closure. The vast majority of chronic wounds can be classified into three categories based on their etiology: pressure ulcers, neuropathic (diabetic foot) ulcers and vascular (venous or arterial) ulcers.

In certain other embodiments, the present disclosure provides biophotonic compositions and methods for treating and/or promoting healing, Grade I-IV ulcers. In certain embodiments, the application provides compositions suitable for use with Grade II ulcers in particular. Ulcers may be classified into one of four grades depending on the depth of the wound: i) Grade I: wounds limited to the epithelium; ii) Grade II: wounds extending into the dermis; iii) Grade III: wounds extending into the subcutaneous tissue; and iv) Grade IV (or full-thickness wounds): wounds wherein bones are exposed (e.g., a bony pressure point such as the greater trochanter or the sacrum).

For example, the present disclosure provides biophotonic compositions and methods for treating and/or promoting healing of a diabetic ulcer. Diabetic patients are prone to foot and other ulcerations due to both neurologic and vascular complications. Peripheral neuropathy can cause altered or complete loss of sensation in the foot and/or leg. Diabetic patients with advanced neuropathy lose all ability for sharp-dull discrimination. Any cuts or trauma to the foot may go completely unnoticed for days or weeks in a patient with neuropathy. A patient with advanced neuropathy loses the ability to sense a sustained pressure insult, as a result, tissue ischemia and necrosis may occur leading to for example, plantar ulcerations. Microvascular disease is one of the significant complications for diabetics which may also lead to ulcerations. In certain embodiments, compositions and methods of treating a chronic wound are provided here in, where the chronic wound is characterized by diabetic foot ulcers and/or ulcerations due to neurologic and/or vascular complications of diabetes.

In other examples, the present disclosure provides biophotonic compositions and methods for treating and/or promoting healing of a pressure ulcer. Pressure ulcer includes bed sores, decubitus ulcers and ischial tuberosity ulcers and can cause considerable pain and discomfort to a patient. A pressure ulcer can occur as a result of a prolonged pressure applied to the skin. Thus, pressure can be exerted on the skin of a patient due to the weight or mass of an individual. A pressure ulcer can develop when blood supply to an area of the skin is obstructed or cut off for more than two or three hours. The affected skin area can turns red, becomes painful and can become necrotic. If untreated, the skin breaks open and can become infected. An ulcer sore is therefore a skin ulcer that occurs in an area of the skin that is under pressure from e.g. lying in bed, sitting in a wheelchair, and/or wearing a cast for a prolonged period of time. Pressure ulcer can occur when a person is bedridden, unconscious, unable to sense pain, or immobile. Pressure ulcer often occur in boney prominences of the body such as the buttocks area (on the sacrum or iliac crest), or on the heels of foot.

In other examples, the present disclosure provides biophotonic compositions and methods for treating and/or promoting healing of acute wounds.

Additional types of wound that can be treated by the biophotonic compositions and methods of the present disclosure include those disclosed by U.S. Pat. Appl. Publ. No. 20090220450, which is incorporated herein by reference.

Wound healing in adult tissues is a complicated reparative process. For example, the healing process for skin involves the recruitment of a variety of specialized cells to the site of the wound, extracellular matrix and basement membrane deposition, angiogenesis, selective protease activity and re-epithelialization.

There are three distinct phases in the wound healing process. First, in the inflammatory phase, which typically occurs from the moment a wound occurs until the first two to five days, platelets aggregate to deposit granules, promoting the deposit of fibrin and stimulating the release of growth factors. Leukocytes migrate to the wound site and begin to digest and transport debris away from the wound. During this inflammatory phase, monocytes are also converted to macrophages, which release growth factors for stimulating angiogenesis and the production of fibroblasts.

Second, in the proliferative phase, which typically occurs from two days to three weeks, granulation tissue forms, and epithelialization and contraction begin. Fibroblasts, which are key cell types in this phase, proliferate and synthesize collagen to fill the wound and provide a strong matrix on which epithelial cells grow. As fibroblasts produce collagen, vascularization extends from nearby vessels, resulting in granulation tissue. Granulation tissue typically grows from the base of the wound. Epithelialization involves the migration of epithelial cells from the wound surfaces to seal the wound. Epithelial cells are driven by the need to contact cells of like type and are guided by a network of fibrin strands that function as a grid over which these cells migrate. Contractile cells called myofibroblasts appear in wounds, and aid in wound closure. These cells exhibit collagen synthesis and contractility, and are common in granulating wounds.

Third, in the remodeling phase, the final phase of wound healing which can take place from three weeks up to several years, collagen in the scar undergoes repeated degradation and re-synthesis. During this phase, the tensile strength of the newly formed skin increases.

However, as the rate of wound healing increases, there is often an associated increase in scar formation. Scarring is a consequence of the healing process in most adult animal and human tissues. Scar tissue is not identical to the tissue which it replaces, as it is usually of inferior functional quality. The types of scars include, but are not limited to, atrophic, hypertrophic and keloidal scars, as well as scar contractures. Atrophic scars are flat and depressed below the surrounding skin as a valley or hole. Hypertrophic scars are elevated scars that remain within the boundaries of the original lesion, and often contain excessive collagen arranged in an abnormal pattern. Keloidal scars are elevated scars that spread beyond the margins of the original wound and invade the surrounding normal skin in a way that is site specific, and often contain whorls of collagen arranged in an abnormal fashion.

In contrast, normal skin consists of collagen fibers arranged in a basket-weave pattern, which contributes to both the strength and elasticity of the dermis. Thus, to achieve a smoother wound healing process, an approach is needed that not only stimulates collagen production, but also does so in a way that reduces scar formation.

The biophotonic compositions and methods of the present disclosure promote the wound healing by promoting the formation of substantially uniform epithelialization; promoting collagen synthesis; promoting controlled contraction; and/or by reducing the formation of scar tissue. In certain embodiments, the biophotonic compositions and methods of the present disclosure may promote wound healing by promoting the formation of substantially uniform epithelialization. In some embodiments, the biophotonic compositions and methods of the present disclosure promote collagen synthesis. In some other embodiments, the biophotonic compositions and methods of the present disclosure promote controlled contraction. In certain embodiments, the biophotonic compositions and methods of the present disclosure promote wound healing, for example, by reducing the formation of scar tissue or by speeding up the wound closure process. In certain embodiments, the biophotonic compositions and methods of the present disclosure promote wound healing, for example, by reducing inflammation. In certain embodiments, the biophotonic composition can be used following wound closure to optimize scar revision. In this case, the biophotonic composition may be applied at regular intervals such as once a week, or at an interval deemed appropriate by the physician.

The biophotonic composition may be soaked into a woven or non-woven material or a sponge and applied as a wound dressing. A light source, such as LEDs or waveguides, may be provided within or adjacent the wound dressing or the composition to illuminate the composition. The waveguides can be optical fibres which can transmit light, not only from their ends, but also from their body. For example, made of polycarbonate or polymethylmethacrylate.

Adjunct therapies which may be topical or systemic such as antibiotic treatment may also be used. Negative pressure assisted wound closure can also be used to assist wound closure and/or to remove the composition.

(6) Acne and Acne Scars

The biophotonic compositions and methods of the present disclosure may be used to treat acne. As used herein, "acne" means a disorder of the skin caused by inflammation of skin glands or hair follicles. The biophotonic compositions and methods of the disclosure can be used to treat acne at early pre-emergent stages or later stages where lesions from acne are visible. Mild, moderate and severe acne can be treated with embodiments of the biophotonic compositions and methods. Early pre-emergent stages of acne usually begin with an excessive secretion of sebum or dermal oil from the sebaceous glands located in the pilosebaceous apparatus. Sebum reaches the skin surface through the duct of the hair follicle. The presence of excessive amounts of sebum in the duct and on the skin tends to obstruct or stagnate the normal flow of sebum from the follicular duct, thus producing a thickening and solidification of the sebum to create a solid plug known as a comedone. In the normal sequence of developing acne, hyperkeratinazation of the follicular opening is stimulated, thus completing blocking of the duct. The usual results are papules, pustules, or cysts, often contaminated with bacteria, which cause secondary infections. Acne is characterized particularly by the presence of comedones, inflammatory papules, or cysts. The appearance of acne may range from slight skin irritation to pitting and even the development of disfiguring scars. Accordingly, the biophotonic compositions and methods of the present disclosure can be used to treat one or more of skin

irritation, pitting, development of scars, comedones, inflammatory papules, cysts, hyperkeratinazation, and thickening and hardening of sebum associated with acne.

The composition may be soaked into or applied to a woven or non-woven material or a sponge and applied as a mask to body parts such as the face, body, arms, legs etc. A light source, such as LEDs or waveguides, may be provided within or adjacent the mask or the composition to illuminate the composition. The waveguides can be optical fibres which can transmit light, not only from their ends, but also from their body. For example, made of polycarbonate or polymethylmethacrylate.

The biophotonic compositions and methods of the present disclosure may be used to treat various types of acne. Some types of acne include, for example, acne vulgaris, cystic acne, acne atrophica, bromide acne, chlorine acne, acne conglobata, acne cosmetica, acne detergicans, epidemic acne, acne estivalis, acne fulminans, halogen acne, acne indurata, iodide acne, acne keloid, acne mechanica, acne papulosa, pomade acne, premenstral acne, acne pustulosa, acne scorbutica, acne scrofulosorum, acne urticata, acne varioliformis, acne venenata, propionic acne, acne excoriee, gram negative acne, steroid acne, and nodulocystic acne.

(7) Skin Aging and Rejuvenation

The dermis is the second layer of skin, containing the structural elements of the skin, the connective tissue. There are various types of connective tissue with different functions. Elastin fibers give the skin its elasticity, and collagen gives the skin its strength.

The junction between the dermis and the epidermis is an important structure. The dermal-epidermal junction interlocks forming finger-like epidermal ridges. The cells of the epidermis receive their nutrients from the blood vessels in the dermis. The epidermal ridges increase the surface area of the epidermis that is exposed to these blood vessels and the needed nutrients.

The aging of skin comes with significant physiological changes to the skin. The generation of new skin cells slows down, and the epidermal ridges of the dermal-epidermal

junction flatten out. While the number of elastin fibers increases, their structure and coherence decrease. Also the amount of collagen and the thickness of the dermis decrease with the ageing of the skin.

Collagen is a major component of the skin's extracellular matrix, providing a structural framework. During the aging process, the decrease of collagen synthesis and insolubilization of collagen fibers contribute to a thinning of the dermis and loss of the skin's biomechanical properties.

The physiological changes to the skin result in noticeable aging symptoms often referred to as chronological-, intrinsic- and photo-ageing. The skin becomes drier, roughness and scaling increase, the appearance becomes duller, and most obviously fine lines and wrinkles appear. Other symptoms or signs of skin aging include, but are not limited to, thinning and transparent skin, loss of underlying fat (leading to hollowed cheeks and eye sockets as well as noticeable loss of firmness on the hands and neck), bone loss (such that bones shrink away from the skin due to bone loss, which causes sagging skin), dry skin (which might itch), inability to sweat sufficiently to cool the skin, unwanted facial hair, freckles, age spots, spider veins, rough and leathery skin, fine wrinkles that disappear when stretched, loose skin, a blotchy complexion.

The dermal-epidermal junction is a basement membrane that separates the keratinocytes in the epidermis from the extracellular matrix, which lies below in the dermis. This membrane consists of two layers: the basal lamina in contact with the keratinocytes, and the underlying reticular lamina in contact with the extracellular matrix. The basal lamina is rich in collagen type IV and laminin, molecules that play a role in providing a structural network and bioadhesive properties for cell attachment.

Laminin is a glycoprotein that only exists in basement membranes. It is composed of three polypeptide chains (alpha, beta and gamma) arranged in the shape of an asymmetric cross and held together by disulfide bonds. The three chains exist as different subtypes which result in twelve different isoforms for laminin, including Laminin-1 and Laminin-5.

The dermis is anchored to hemidesmosomes, specific junction points located on the keratinocytes, which consist of α-integrins and other proteins, at the basal membrane keratinocytes by type VII collagen fibrils. Laminins, and particularly Laminin-5, constitute the real anchor point between hemidesmosomal transmembrane proteins in basal keratinocytes and type VII collagen.

Laminin-5 synthesis and type VII collagen expression have been proven to decrease in aged skin. This causes a loss of contact between dermis and epidermis, and results in the skin losing elasticity and becoming saggy.

Recently another type of wrinkles generally referred to as expression wrinkles, got general recognition. These wrinkles require loss of resilience, particularly in the dermis, because of which the skin is no longer able to resume its original state when facial muscles which produce facial expressions exert stress on the skin, resulting in expression wrinkles.

The compositions and methods of the present disclosure promote skin rejuvenation. In certain embodiments, the compositions and methods of the present disclosure promote collagen synthesis. In certain other embodiments, the compositions and methods of the present disclosure may reduce, diminish, retard or even reverse one or more signs of skin aging including, but not limited to, appearance of fine lines or wrinkles, thin and transparent skin, loss of underlying fat (leading to hollowed cheeks and eye sockets as well as noticeable loss of firmness on the hands and neck), bone loss (such that bones shrink away from the skin due to bone loss, which causes sagging skin), dry skin (which might itch), inability to sweat sufficiently to cool the skin, unwanted facial hair, freckles, age spots, spider veins, rough and leathery skin, fine wrinkles that disappear when stretched, loose skin, or a blotchy complexion. In certain embodiments, the compositions and methods of the present disclosure may induce a reduction in pore size, enhance sculpturing of skin subsections, and/or enhance skin translucence.

(8) Skin Disorders

The biophotonic compositions and methods of the present disclosure may be used to treat skin disorders that include, but are not limited to, erythema, telangiectasia, actinic telangiectasia, psoriasis, skin cancer, pemphigus, sunburn, dermatitis, eczema, rashes, impetigo, lichen simplex chronicus, rhinophyma, perioral dermatitis, pseudofolliculitis barbae, drug eruptions, erythema multiforme, erythema nodosum, granuloma annulare, actinic keratosis, purpura, alopecia areata, aphthous stomatitis, drug eruptions, dry skin, chapping, xerosis, ichthyosis vulgaris, fungal infections, parasitic infection, herpes simplex, intertrigo, keloids, keratoses, milia, moluscum contagiosum, pityriasis rosea, pruritus, urticaria, and vascular tumors and malformations. Dermatitis includes contact dermatitis, atopic dermatitis, seborrheic dermatitis, nummular dermatitis, generalized exfoliative dermatitis, and statis dermatitis. Skin cancers include melanoma, basal cell carcinoma, and squamous cell carcinoma.

Some skin disorders present various symptoms including redness, flushing, burning, scaling, pimples, papules, pustules, comedones, macules, nodules, vesicles, blisters, telangiectasia, spider veins, sores, surface irritations or pain, itching, inflammation, red, purple, or blue patches or discolorations, moles, and/or tumors. Accordingly, the biophotonic compositions and methods of the present disclosure can be used to treat redness, flushing, burning, scaling, pimples, papules, pustules, comedones, macules, nodules, vesicles, blisters, telangiectasia, spider veins, sores, surface irritations or pain, itching, acute inflammation, red, purple, or blue patches or discolorations, moles, and/or tumors. Acute inflammation can present itself as pain, heat, redness, swelling and loss of function. It includes those seen in allergic reactions e.g.; such as insect bites (mosquito, bees, wasps, ants, spiders etc), reaction to poison ivy or stinging nettle or the like, post-ablative treatment.

The composition may be soaked into or applied to a woven or non-woven material or a sponge and applied as a mask to body parts to treat skin disorders. A light source, such as LEDs or waveguides, may be provided within or adjacent the mask or the composition to illuminate the composition. The waveguides can be optical fibres which can transmit light, not only from their ends, but also from their body. For example, the waveguides can be made of polycarbonate or polymethylmethacrylate.

(9) Kits

The present disclosure also provides kits for preparing and/or applying any of the compositions of the present disclosure. The kit may include a biophotonic topical composition, as defined above, together with one or more of a light source, devices for applying or removing the composition, instructions of use for the composition and/or light source. In some embodiments, the composition comprises at least a first chromophore in a gelling agent. The chromophore may be present in an amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In embodiments where the composition comprises more than one chromophore, the first chromophore may be present in an amount of about 0.01-40% per weight of the composition, and a second chromophore may be present in an amount of about 0.0001-40% per weight of the composition. In certain embodiments, the first chromophore is present in an amount of about 0.01-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the second chromophore is present in an amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the composition. In certain embodiments, the amount of chromophore or combination of chromophores may be in the amount of about 0.05-40.05% per weight of the composition. In certain embodiments, the amount of chromophore or combination of chromophores may be in the amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40.05% per weight of the composition. The composition may include an oxygen-releasing agent present in amount about 0.01% - 40%, 0.01% - 1.0%, 0.5% - 10.0%, 5% - 15%, 10% - 20%, 15% - 25%, 20% - 30%, 15.0% - 25%, 20% - 30%, 25% - 35%, or 30% - 40% by weight to weight of the composition. Alternatively, the kit may include the oxygen-releasing agent as a separate component to the chromophore containing composition.

In some embodiments, the kit includes more than one composition, for example, a first and a second composition. The first composition may include the oxygen-releasing agent and the second composition may include the first chromophore in the gelling agent. The first chromophore may have an emission wavelength between about 400 nm and about 570 nm. The oxygen-releasing agent may be present in the first composition in an amount of about 0.01% - 1.0%, 0.5% - 10.0%, 5% - 15%, 10% - 20%, 15% - 25%, 20% - 30%, 15.0% - 25%, 20% - 30%, 25% - 35%, 30% - 40% or 35% - 45% by weight to weight of the first composition. The chromophore may be present in the second composition in an amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the second composition. In embodiments where the second composition comprises more than one chromophore, the first chromophore may be present in an amount of about 0.01-40% per weight of the second composition, and a second chromophore may be present in an amount of about 0.0001-40% per weight of the second composition. In certain embodiments, the first chromophore is present in an amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the second composition. In certain embodiments, the second chromophore is present in an amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40% per weight of the second composition. In certain embodiments, the amount of chromophore or combination of chromophores may be in the amount of about 0.05-40.05% per weight of the second composition. In certain embodiments, the amount of chromophore or combination of chromophores may be in the amount of about 0.001-0.1%, 0.05-1%, 0.5-2%, 1-5%, 2.5-7.5%, 5-10%, 7.5-12.5%, 10-15%, 12.5-17.5%, 15-20%, 17.5-22.5%, 20-25%, 22.5-27.5%, 25-30%, 27.5-32.5%, 30-35%, 32.5-37.5%, or 35-40.05% per weight of the second chromophore.

In some other embodiments, the first composition may comprise the first chromophore in a liquid or as a powder, and the second composition may comprise a gelling composition for thickening the first composition. The oxygen-releasing agent may be contained in the second composition or in a third composition in the kit. In some embodiments, the kit includes

containers comprising the compositions of the present disclosure. In some embodiments, the kit includes a first container comprising a first composition that includes the oxygen-releasing agent, and a second container comprising a second composition that includes at least one chromophore. The containers may be light impermeable, air-tight and/or leak resistant. Exemplary containers include, but are not limited to, syringes, vials, or pouches. The first and second compositions may be included within the same container but separated from one another until a user mixes the compositions. For example, the container may be a dual-chamber syringe where the contents of the chambers mix on expulsion of the compositions from the chambers. In another example, the pouch may include two chambers separated by a frangible membrane. In another example, one component may be contained in a syringe and injectable into a container comprising the second component.

The biophotonic composition may also be provided in a container comprising one or more chambers for holding one or more components of the biophotonic composition, and an outlet in communication with the one or more chambers for discharging the biophotonic composition from the container. In one embodiment, discharging the biophotonic compositions causes the components of the composition to mix to form a biophotonic composition which has less than 15% leaching properties.

In other embodiments, the kit comprises a systemic or topical drug for augmenting the treatment of the composition. For example, the kit may include a systemic or topical antibiotic or hormone treatment for acne treatment or wound healing.

Written instructions on how to use the biophotonic composition in accordance with the present disclosure may be included in the kit, or may be included on or associated with the containers comprising the compositions of the present disclosure.

In certain embodiments, the kit may comprise a further component which is a dressing. The dressing may be a porous or semi-porous structure for receiving the biophotonic composition. The dressing may comprise woven or non-woven fibrous materials.

In certain embodiments of the kit, the kit may further comprise a light source such as a portable light with a wavelength appropriate to activate the chromophore in the biophotonic composition. The portable light may be battery operated or re-chargeable.

In certain embodiments, the kit may further comprise one or more waveguides.

Identification of equivalent compositions, methods and kits are well within the skill of the ordinary practitioner and would require no more than routine experimentation, in light of the teachings of the present disclosure. Practice of the disclosure will be still more fully understood from the following examples, which are presented herein for illustration only and should not be construed as limiting the disclosure in any way.

EXAMPLES

The examples below are given so as to illustrate the practice of various embodiments of the present disclosure. They are not intended to limit or define the entire scope of this disclosure.

Example 1

The photodynamic properties of (i) Fluorescein sodium salt at about 0.09 mg/mL, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of Fluorescein sodium salt at about 0.09 mg/mL and Eosin Y at about 0.305 mg/mL in a gel according to an embodiment of the present disclosure (comprising about 12% carbamide peroxide), were evaluated. A flexstation 384 II spectrometer was used with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorption and emission spectra are shown in **Figures 6a and 6b** which indicate an energy transfer between the chromophores in the combination.

Example 2

The photodynamic properties of (i) Fluorescein sodium salt at 0.18 mg/mL final concentration, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of Fluorescein sodium salt at about 0.18 mg/mL and Eosin Y at about 0.305 mg/mL in an aqueous solution were evaluated. A flexstation 384 II spectrometer was used with the following parameters: mode

fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorption and emission spectra are shown in **Figures 7a and 7b** which indicate an energy transfer between the chromophores in the combination.

Example 3

The photodynamic properties of (i) Rose Bengal at about 0.085 mg/mL, (ii) Fluorescein sodium salt at about 0.44 mg/mL final concentration, (iii) Eosin Y at about 0.305 mg/mL, and (iv) a mixture of (i), (ii) and (iii) in a gel comprising about 12% carbamide peroxide (Set A), according to an embodiment of the invention, were evaluated. A flexstation 384 II spectrometer was used with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance and emission spectra are shown in **Figures 8a and 8b** which indicate an energy transfer between the chromophores in the chromophore combination.

Example 4

The photodynamic properties of (i) Rose Bengal at about 0.085 mg/mL, (ii) Fluorescein sodium salt at about 0.44 mg/mL final concentration, (iii) Eosin Y at about 0.305 mg/mL, and (iv) a mixture of (i), (ii) and (iii) in an aqueous solution (Set A), were evaluated. A flexstation 384 II spectrometer was used with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance and emission spectra are shown in **Figures 9a and 9b** which indicate an energy transfer between the chromophores in the chromophore combination, in the absence of an oxygen-releasing agent.

Energy transfer was also seen between: Eosin Y and Rose Bengal; Phloxine B and Eosin Y; Phloxine B, EosinY and Fluorescein, amongst other combinations. It is to be reasonably inferred that energy transfer can also occur in biophotonic compositions of the present disclosure.

Example 5

A randomized, split-face clinical trial of 12 weeks was performed on 90 patients (ages 14-30) having moderate to severe facial acne. Moderate facial acne was defined as having "an

Investigator's Global Assessment (IGA) of 3 with 20-40 inflammatory lesions and no more than 1 nodule". Severe facial acne was defined as having "an IGA of 4 with more than 40 inflammatory lesions with the presence of more than 2 nodules and/or presence of severe erythema and inflammatory scarring type lesion". For each patient, one randomly selected side of the face was treated twice a week for 6 weeks with a spreadable and translucent biophotonic composition comprising a fluorophore (Eosin Y) in a carbomer polymer-based gel including carbamide peroxide. The biophotonic gel demonstrated less than 15% leaching of the chromophore when tested according to Example 6 for up to 30 minutes. The treatment comprised topically applying the biophotonic composition to the treatment area and exposing the composition to light from an LED light source (peak wavelength range 400-470 nm) for about 5 minutes. Other hemiface remained untreated for the 6 week period. Both the treated and untreated sides of the face were evaluated after 12 weeks. Results are presented in Tables 1-5 below. The fluorescence spectra observed when the biophotonic gel was illuminated during treatment is illustrated in **Figure 10**. The treatment was well tolerated by the patients and there were no serious adverse events. 80% of patients completed the study with no adverse events reported. At week 4, there was a 30% reduction in inflammatory lesions (including papules, pustules and nodules) for the treated group compared to 9.0% reduction for untreated. At week 6, the reduction was 46.8% for treated and 18.4% for untreated, and at week 12, the reduction was 59.2% for treated and 35.6% for untreated.

Table 1. Total reduction from baseline at weeks 2, 4, 6, 8, 10 and 12 of ≥2 IGA grades for treated and untreated hemifaces.

	Reduction from baseline in IGA of ≥ 2 grades (%)	
	Treated (n=89)	**Untreated (n=89)**
Week 2	0 (0%)	0 (0%)
Week 4	6 (6.7%)	1 (1.1%)
Week 6	26 (29.2%)	6 (6.7%)
Week 8	28 (31.5%)	9 (10.1%)
Week 10	32 (36.0%)	10 (11.2%)

Week 12	46 (51.7%)*	16 (18.0%)*

*P<0.0001

Table 2. Total reduction from baseline at weeks 2, 4, 6, 8, 10 and 12 of ≥1 IGA grade for treated and untreated hemifaces.

	Reduction from baseline in IGA of ≥ 1 grade (%)	
	Treated (n=89)	**Untreated (n=89)**
Week 2	9 (10.1%)	5 (5.6%)
Week 4	49 (55.1%)	29 (32.6%)
Week 6*	71 (79.8%)	40 (44.9%)
Week 8	73 (82.0%)	43 (48.3%)
Week 10	76 (85.4%)	47 (52.8%)
Week 12+	79 (88.8%)	62 (69.7%)

*P value < 0.0001; +P value < 0.0001

Table 3. Total reduction from baseline at weeks 2, 4, 6, 8, 10 and 12 to IGA grade 0 or 1 for treated and untreated hemifaces.

	Reduction from baseline in IGA to grade 0 or 1 (%)	
	Treated (n=89)	**Untreated (n=89)**
Week 2	0 (0%)	0 (0%)
Week 4	4 (4.5%)	1 (1.1%)
Week 6+	16 (18.0%)	6 (6.7%)
Week 8	16 (18.0%)	8 (9.0%)
Week 10	18 (20.2%)	9 (10.1%)
Week 12*	29 (32.6%)	10 (11.2%)

+P value = 0.0213; *P value < 0.0001

Table 4. Proportion of patients showing at least 40% reduction from baseline in inflammatory lesion count (includes papules, pustules and nodules) at weeks 6 and 12 for treated and untreated hemifaces.

	Total reduction from baseline in inflammatory lesion count at week 12	
	Treated (n=87)	**Untreated (n=87)**
Week 6+	56 (64.4 %)	27 (31.0%)
Week 12*71	71 (81.6%)	40 (46.0%)

+P value < 0.0001 *P value < 0.0001

Table 5. Summary of inflammatory lesion count and absolute changes by hemiface.

		Treated		**Untreated**		**Difference (Treated - Untreated)**	
Inflammatory Lesion Count*		**Lesion Count**	**Change (%)**	**Lesion Count**	**Change (%)**	**Change (Absolute)**	**Change (%)**
Baseline	n Mean (SD)	90 23.0 (13.79)		90 23.3 (15.41)		90 -0.3 (7.10)	
Week 6	n Mean (SD) p value	87 12.4 (8.35)	87 --45.3 (25.15)	87 19.0 (13.92)	87 -18.6 (33.51)	87 -6.6 (8.34) <0.0001	87 -26.7 (33.86) <0.0001
Week 12	n Mean (SD) p value	87 9.5 (7.10)	87 -55.6 (32.36)	87 15.0 (11.33)	87 -32.0 (36.61)	87 -5.5 (7.37) <0.0001	87 -23.6 (37.55) <0.0001

* Includes papules, pustules and nodules

Figure 10 is an emission spectrum showing the intensity over time of the light being emitted from the biophotonic composition.

Example 6 - Leaching test using polycarbonate membrane

Figure 5 depicts an experimental setup of an *in vitro* release test for evaluating leaching of the chromophore(s) or other components (e.g., oxygen releasing agents) from the biophotonic compositions of the present disclosure. In this *in vitro* test, a 2 mm thick layer of the biophotonic composition is applied on an upper surface of a circular polycarbonate (PC) membrane with a diameter of 2.4 to 3 cm, a thickness of 10 microns, and a pore size of 3 microns. The underside of the membrane is in direct contact with phosphate saline buffer (PBS) contained in a closed compartment (i.e., the receptor compartment). Samples (100 µl x 2) are then taken from the receptor compartment at different time points (e.g., at 5, 10, 20, and 30 min), and evaluated for concentration of the chromophore(s) or any other components of the biophotonic composition using spectrophotometry or any other suitable method.

For example, when the chromophore being tested is eosin, a wavelength of about 517 nm (absorbance) may be used. The concentration of the chromophore may then be calculated based on the chromophore standards of known concentration prepared in PBS and measured at the same time. The presence of peroxide (i.e., an indicator of the oxygen releasing agents) can also be assessed using peroxide test sticks (e.g. Quantofix Peroxide 25, Sigma Aldrich).

Table 6 summarizes leaching data for different biophotonic compositions according to the present disclosure. All compositions were spreadable, translucent gels, having a viscosity of about 10,000-80,000 cP. The amount of hydrogen peroxide found in the receptor compartment was very low for all compositions containing peroxide in Table 6. The detection method of chromophore by spectrophotometry can measure the chromophore concentration from 0.2 µg/ml. For all biophotonic compositions tested, the release of chromophores increased over time. For all compositions, there was less than 15% chromophore leaching after 5 minutes, 10 minutes, 15 minutes, and 25 minutes of incubation. All tested compositions, other than Eosin Y (0.2%) in a carbopol polymer gel including urea peroxide had less than 15% chromophore leaching even after 30 minutes incubation, which is longer than a treatment time according to many embodiments of the present disclosure.

The effect of illumination on chromophore leaching from the biophotonic compositions was also investigated. It was found that illumination of the biophotonic compositions with light for 5 minutes at a distance of 5 cm induced photobleaching of the chormophore(s). In fact, the chomophores photobleached in about 2-3 minutes. In these cases, the chromophore(s) were undetectable in the receptor compartment. Therefore, during treatment involving light illumination, even lower chromophore leaching than the results presented in Table 9 can be reasonably expected.

Table 6: Percentage of chromophores released from biophotonic compositions according to embodiments of the present disclosure, with time of incubation.

Composition	**Percentage chromophore released into receptor compartment from composition with time of incubation (n=3)**			
	5mins	10 mins	20 mins	30 mins
Eosin Y (0.011%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	Not detectable	Not detectable	0.75	0.78
Fluorescein (0.2%), carbopol gel (1.7%), urea peroxide (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.71	4.85	4.72	4.84
Rose Bengal (0.2%), carbopol gel (1.7%), urea peroxide (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.39	3.32	5.26	5.21
Rose Bengal (0.1%) + Fluorescein (0.1%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.91	5.21	8.48	8.43
Phloxin B (0.2%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	0.54	2.39	4.62	4.50
Eosin Y (0.2%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine,	2.77	2.72	6.56	9.08

propylene glycol, hyaluronic acid.				
Phloxin B (0.1%) + Fluorescein (0.1%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.28	4.49	7.56	11.02
Phloxin B (0.1%) + Rose Bengal (0.1%), carbopol gel (1.7%), urea peroxide gel (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.41	2.36	5.14	4.90
Phloxin B (0.1%) + Eosin Y (0.1%) carbopol gel (1.7%), urea peroxide (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	3.84	6.25	10.08	12.00
Rose Bengal (0.1%) + Eosin Y (0.1%), carbopol gel (1.7%), urea peroxide (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	3.04	4.28	6.63	8.12
Fluorescein (0.1%) + Eosin Y (0.1%), carbopol gel (1.7%), urea peroxide (12%), saffron, glycerine, propylene glycol, hyaluronic acid.	2.96	3.99	5.78	7.58
Phloxin B (0.1%) + Eosin Y (0.1%), carbopol gel (1.7%)	1.00	2.3	4.48	5.80
Eosin Y (0.2%), carbopol gel (1.7%), urea peroxide gel (12%)	6.78	8.2	14.38	17.89
Eosin Y (0.2%), carbopol gel (1.7%), saffron, glycerine, propylene glycol	3.34	4.90	7.30	9.26
Phloxin B (0.1%) + Eosin Y (0.1%), gelatin gel (5%)	0.51	0.25	1.79	3.14
Rose Bengal (0.1%) + Eosin Y (0.1%), gelatin gel (5%)	0	0.39	1.39	2.15
Eosin Y (0.2%), starch gel (8%)	2.91	3.72	7.11	9.06
Eosin Y (0.2%), sodium hyaluronate gel	3.41	6.24	9.93	12.77

(2%)				

Example 7 - Angiogenic potential of the biophotonic composition of the disclosure

A human skin model was developed to assess the angiogenic potential of the biophotonic composition of the present disclosure. Briefly, a biophotonic composition comprising fluorophores (Eosin Y and Erythrosine) in a carbomer polymer-based gel including urea peroxide, was placed on top of a human skin model containing fibroblasts and keratinocytes. The spreadable and translucent biophotonic composition had less than 15% leaching of chromophore for up to 30 minutes, when tested separately according to Example 6. The skin model and the composition were separated by a nylon mesh of 20 micron pore size. The composition was then irradiated with blue light ('activating light') for 5 minutes at a distance of 5 cm from the light source. The activating light consisted of light emitted from an LED lamp having an average peak wavelength of about 400-470 nm, and a power intensity measured at 10 cm of 7.7 J/cm^2 to 11.5 J/cm^2. Upon illumination with the activating light, the biophotonic composition emitted fluorescent light (Figure 4). Since the biophotonic composition was in limited contact with the cells, the fibroblasts and keratinocytes were exposed mainly to the activating light and the fluorescent light emitted from the biophotonic composition. Conditioned media from the treated human 3D skin model were then applied to human aortic endothelial cells previously plated in matrigel. The formation of tubes by endothelial cells was observed and monitored by microscopy after 24 hours. The conditioned medium from 3D skin models treated with light illumination induced endothelial tube formation in vitro, suggesting an indirect effect of the light treatment (blue light and fluorescence) on angiogenesis via the production of factors by fibroblasts and keratinocytes. Plain medium and conditioned medium from untreated skin samples were used as a control, and did not induce endothelial tube formation.

Figure 11 is an emission spectrum showing the intensity over time of the light being emitted from the biophotonic composition.

Example 8 - Protein secretion and gene expression profiles

Wounded and unwounded 3D human skin models (EpiDermFT, MatTek Corporation) were used to assess the potential of a biophotonic composition of the present disclosure to trigger distinct protein secretion and gene expression profiles. Briefly, a biophotonic composition comprising Eosin and Erythrosine in a carbomer polymer-based gel including urea peroxide, was placed on top of wounded and unwounded 3D human skin models cultured under different conditions (with growth factors, 50% growth factors and no growth factors). The spreadable and translucent biophotonic gel had less than 15% leaching of chromophore during a 30 minute test time, according to Example 6. The skin models and the composition were separated by a nylon mesh of 20 micron pore size. Each skin model-composition combination was then irradiated with blue light ('activating light') for 5 minutes at a distance of 5 cm from the light source. The activating light consisted of light emitted from an LED lamp having an average peak wavelength of about 440-470 nm, a power density of 60-150mW/cm^2 at 5 cm, and a total intensity after 5 minutes of about 18-39 J/cm^2. The controls consisted of 3D skin models not illuminated with light.

Gene expression and protein secretion profiles were measured 24 hours post-light exposure. Cytokine secretion was analyzed by antibody arrays (RayBio Human Cytokine antibody array), gene expression was analyzed by PCR array (PAHS-013A, SABioscience) and cytotoxicity was determined by GAPDH and LDH release. Results (Tables 7 and 8) showed that the light treatment is capable of increasing the level of protein secreted and gene expression involved in the early inflammatory phase of wound healing in wounded skin inserts and in non-starvation conditions. In starvation conditions mimicking chronic wounds, there was no increase in the level of inflammatory protein secreted when compared to the control. Interestingly, the effect of the light treatment on unwounded skin models has a much lower impact at the cellular level than on wounded skin insert, which suggests an effect at the cellular effect level of the light treatment. It seems to accelerate the inflammatory phase of the wound healing process. Due to the lack of other cell types such as macrophages in the 3D skin model, the anti-inflammatory feed-back is absent and may explain the delay in wound closure. Cytoxicity was not observed in the light treatments.

Table 7 – List of proteins with statistically significant difference secretion ratio between treated and untreated control at day 3. Two arrows mean that the ratio was over 2 folds.

	Medium 1X	**Medium 0.5X**	**Medium 0X**
Increase		**ENA78 p=0.04** ↑↑ **Il-1R4/ST2 p=0.02** ↑↑ **MMP3 p=0.01** ↑↑ **MCP-2 p=0.04** ↑↑	Angiogenin p=0.03 ↑ CXCL16 p=0.04 ↑
Decrease	BMP6 p=0.01 ↓ TNFα p=0.005 ↓	BMP6 p=0.02 ↓	

Table 8 – List of genes with statistically significant difference expression ratio between treated and untreated control during the first 24 hours. Two arrows mean that the ratio was over 2 folds.

	Medium 1X	**Medium 0.5X**	**Medium 0X**
Increase	CTGF p=0.02 ↑ ITGB3 p=0.03 ↑ MMP1 p=0.03 ↑ MMP3 p=0.01 ↑ THBS1 P=0.02 ↑	CTGF P=0.04 ↑ ITGB3 p=0.05 ↑ **MMP1 p=0.02** ↑↑ **MMP10 p=0.003** ↑↑ **MMP3 p=0.007** ↑↑ **MMP8 p=0.02** ↑↑ THBS1 p=0.03 ↑	**MMP3 p=0.007** ↑↑ LAMA1 p=0.03 ↑ ITGA2 p=0.03 ↑
Decrease	HAS1 p=0.009 ↓↓ NCAM1 p=0.05 ↓↓ VCAM1 p=0.03 ↓↓ COL7A1 p=0.04 ↓	**NCAM1 p=0.02** ↓↓ VCAN p=0.02 ↓ LAMC1 p=0.002 ↓ COL6A1 p=0.007 ↓	

	CTNNA1 p=0.03 ↓	MMP7 p=0.003 ↓	

Example 9 - Collagen formation in skin

A randomized, placebo-controlled, single-blinded, split face and single hand study of 32 patients, split into 4 groups (A, B, C and D), assessed the safety and efficacy of treatment once a week for 4 weeks: (A) "light alone" – light, according to an embodiment of the present disclosure, comprising light from an LED source having an average peak wavelength of about 400-490 nm at a power density of less than 150 mW/cm^2 for 5 minutes; and a placebo formulation; (B) "light + gel" - light as in (A) plus biophotonic gel according to an embodiment of the present disclosure); (C) "gel alone" – biophotonic gel as in (B) and a sham light (white LED light); and (D) 0.1% retinoic based cream. The biophotonic gel included a fluorophore in a carbopol gel and urea peroxide, the gel having a viscosity of about 10,000 cP to 50,000 cP, and demonstrating less than 15% leaching when tested for up to 30 minutes according to Example 6. The gel was translucent and spreadable. Skin biopsies were obtained before treatment and 12 weeks after treatment from the treatment site. Histological samples of the skin biopsies were graded by an independent and experienced pathologist blinded to the treatment assignment. The results are presented in Table 9 below and show that the light treatment with and without the biophotonic gel, according to embodiments of the present disclosure, showed a 287% and 400% increase from the baseline, respectively, in collagen clusters as viewed through Gomori Trichome staining, in the treated areas of skin. There were no serious adverse events. There was no reported or observed photosensitivity, inflammation or pain.

Table 9- Semi-quantitative histological collagen evaluation

Treatment	**% increase in collagen**
Photoactivatable composition excited with light having 460nm peak wavelength	400

Placebo composition + light having 460nm peak wavelength	287
Retinol cream with no light	189
Placebo composition with white light	150

Example 10 - Flap Closure

A caudally based rectangular flap was elevated in the back of Wistar rats. A silicone sheet was inserted beneath the skin flap to prevent adhesion and reperfusion of the flap from the underlying tissues. Following flap closure, a biophotonic gel according to an embodiment of the present disclosure was applied onto the dorsal flap in a thin monolayer (2 mm) and exposed to a light, for 5 minutes, from a LED light source having a peak wavelength of about 440-470 nm. The spreadable biophotonic gel included a fluorophore in a carbopol gel and urea peroxide, the gel having a viscosity of about 10,000 cP to 50,000 cP, and demonstrating less than 15% leaching when tested for up to 30 minutes according to Example 6. The biophotonic gel was removed and skin specimens were collected from different areas in the flap for histological analyses nine days post-treatment. The treated group demonstrated a significantly greater number of Ki67-positive-staining events ($P=0.02$) compared to those in the non-treated group these results, suggesting that the treatment may modulate the proliferation of the cells involved in wound healing (**Figure 12**). Following examination by an external pathologist, the treatment group was associated with a significant ($P<0.05$) decrease in the coagulative necrosis in the epidermis and an increase of the fibrillar stroma (dermis) as compared to the control group.

Example 11 - Evaluation of removal of biophotonic composition from ethanol soaked paper

Regular white print paper was soaked in 70% ethanol (EtOH). A 2mm thickness of different embodiment's of biophotonic compositions according to the present disclosure (Table 10) were placed onto the soaked paper and left for 5 minutes. After 5 minutes, the compositions were washed off with 70% EtOH. A composition comprising Eosin (0.017%), silica particles, modified starch, and hydrogen peroxide was also tested.

The results show that biophotonic compositions of the present disclosure including a carbamide gel do not stain white paper. A composition containing Eosin and another hydrophilic polymer (starch) in combination with silica particles did stain the paper.

Table 10. Evaluation of removal of biophotonic composition from paper

Biophotonic composition	**Colour of paper after washing**
Eosin (0.017%), silica particles, modified starch, hydrogen peroxide (included for comparison only).	Orange/red stain on paper observed.
Eosin (0.011%) in a urea peroxide, glycerin, propylene glycol, carbopol, hyaluronic acid, glucosamine gel.	Substantially white – no staining observed.
Eosin (0.011%) +carbamide peroxide+ 1.8% carbopol 940	Substantially white – no staining observed.

Example 12 - Evaluation of heat dissipation during illumination of a biophotonic composition

A 3mm thick layer of a biophotonic composition according to an embodiment of the present disclosure comprising a fluorescent chromophore in a carbopol gel according to an embodiment of the present disclosure was applied on the skin of hands of volunteers with different skin types and illuminated for 5 minutes with a blue LED light having a power density of about 50 to 150 mW/cm^2 at a distance of 5 cm from the light. The biophotonic gel was spreadable and had less than 15% by weight leaching when tested according to Example 6. A thermometer probe was placed within the composition, at the surface of the skin, and the temperature was monitored in real-time during illumination of the composition. The skin temperature with no composition but the same light illumination was also measured for the same volunteers. The skin types tested were, according to Fitzpatrick classification scales, type III (white skin, sometimes burns and gradually tans), type IV (beige to brown skin, rarely burns and easily tans) and type VI (black skin, never burns, very easily tans). The results are shown in table 11.

Table 11. Temperature of skin under biophotonic composition during illumination for 5 minutes compared to temperature skin with no composition and illumination alone

	Minimum-maximum temperature of skin under composition during 5 mins of illumination/°C (Average over 5 mins/ °C)	**Minimum-maximum temperature of skin without composition during 5 mins. of illumination/°C (Average over 5 mins/ °C)**
Skin Type III	26.5-35.1 (32.2)	28.7-39.1 (36.2)
Skin Type IV	27.6-39.9 (36.1)	31.4-39.9 (37.0)
Skin Type VI	28.5-39.9 (35.6)	29.6-40.0 (37.4)

All skin types with biophotonic composition applied demonstrated a slower temperature increase compared to bare skin (no biophotonic composition), and so the biophotonic composition conferred a buffer effect. After 5 minutes of light illumination, the temperature of the skin under the biophotonic composition for all volunteers reached a maximum of 39.9°C, compared to 40°C with light alone and bare skin. Overall no pain, burning or discomfort was felt by the volunteers.

Example 13 – Selecting the concentration of chromophore in the biophotonic composition

The fluorescence spectra of biophotonic compositions with different concentrations of chromophores were investigated using a spectrophotometer and an activating blue light. Exemplary fluorescence spectra of Eosin Y and Fluorescein are presented in **Figure 13**. It was found that emitted fluorescence from the chromophore increases rapidly with increasing concentration but slows down to a plateau with further concentration increase. Activating light passing through the composition decreases with increasing chromophore composition as more is absorbed by the chromophores. Therefore, the concentration of chromophores in biophotonic compositions of the present disclosure can be selected according to a required ratio and level of activating light and fluorescence treating the tissue based on this example. In some

embodiments, it will be after the zone of rapid increase, i.e. between 0.5 and 1 mg/mL for Eosin Y (**Figure 13**).

Therefore, concentration can be selected according to required activating light and fluorescence. In some embodiments, it will be after the zone of rapid increase, i.e. between 0.5 and 1 mg/mL for Eosin Y (**Figure 13**). A person skilled in the art would also take into account the effect on fluorescence of other ingredients in a composition and adapt the concentration of the chromophore accordingly. For example, certain gelling agents bind to certain chromophores which may lower their fluorescence. One example is albumin. In such cases, a higher concentration of the chromophore can be used in the composition.

Example 14 - Eosin and Rose Bengal act in a synergistic manner

The synergy between two chromophores according to various embodiments of the present disclosure was investigated by preparing the following:

1 – Eosin Y (0.035%) + Rose Bengal (0.085%) in a 12% carbamide gel.

2 – Rose Bengal (0.085%) in a 12% carbamide gel

Rose Bengal is known to have a high quantum yield in terms of oxygen production in the presence of oxygen-releasing agents when photoactivated by green light. Eosin Y is known to have a high quantum yield in terms of emitted fluorescent light when photoactivated and can be at least partially activated by blue light when in a gel. Photoactivated Eosin Y does not have a high quantum yield in terms of oxygen production in the presence of oxygen-releasing agents. When Eosin Y and Rose Bengal are combined, it appears that both chromophores are activated by the same blue light as evidenced by **Figure 14**.

Figure 14, left panel, shows a photograph of the composition when viewed under a light microscope (x250) before exposure to an activating light. Very few bubbles were seen in both compositions. Following illumination with blue light, right panel, a dramatic increase in bubbles was seen with the composition comprising a combination of Eosin Y and Rose Bengal,

but not with the composition comprising Rose Bengal alone. This suggests that there is a transfer of energy from Eosin Y to Rose Bengal leading to the formation of oxygen species.

Example 15 – Viscosity of spreadable compositions

Gels, based on carbopol polymers with differing concentrations, were evaluated for their suitability for use in embodiments of biophotonic compositions of the present disclosure. The viscosity, spreadability and ability to stay in place on tissue of the gels were evaluated. The gels comprised carbopol 940, glycerin, propylene glycol, water, as well as small amounts of a chelator, a pH adjuster, healing factors and preservatives. Ten gel compositions were tested having varying amounts of carbopol 940, all other ingredient concentrations remaining the same. Viscosity was evaluated using (1) Brookfield DV-II+Pro viscometer: spindle 7, 50 rpm, 1 minute; and (2) Brookfield HN viscometer: spindle CP51, 2 rpm. Ability to be easily spread was evaluated based on ease of forming a 2mm thick layer on a surface and ability to conform to the surface topography. Ability to stay in place was evaluated by placing a 2 mm thick layer of each gel on a surface of a pork chop having an 8 mm diameter biopsy punch in order to simulate a wound. The steak was then positioned such that the surface having the gel thereon was at about 90° to a horizontal plane (i.e. the gel was substantially vertical) and was then left in place for 5 minutes at room temperature. The results are summarized in Table 12.

Table 12. Evaluation of viscosity, spreadability and ability to stay in place of gels having different carbopol concentrations.

Gel	% by wt Carbopol	Viscosity (cP) (1)	Viscosity (cP) (2)	Ability to be spread	Ability to stay in place
1	0.2	0	0	Too liquid	Too liquid
2	0.5	800	828	Too liquid	Too liquid
3	1.0	11920	11737	Good	Good
4	1.7	33840	38110	Good	Good
5	2.0	71520	74563	Not easy to make gel conform to the wound topography	Good

6	2.5	74080	74770	Very difficult to make gel conform to the wound topography	Good
7	1.1	15840	15948	Good	Good
8	1.3	21280	22783	Good	Good
9	1.5	31360	33346	Good	Good
10	1.85	44320	49295	Good	Good

Gels which did not flow when positioned vertically were obtained with a carbopol wt% of more than 0.5% and higher. Gels with a carbopol wt% of more than 0.5% and less than 2wt% could be spread as a 2 mm thick layer. These gels may be suitable gelling agents for biophotonic compositions of the present invention. Other concentrations of carbopol may also be used in conjunction with thickening agents or diluters in biophotonic compositions of the present disclosure. Also, other carbopol grades, polymers and other gelling agents may also have properties suitable for use as a gelling agent in the present compositions.

It should be appreciated that the invention is not limited to the particular embodiments described and illustrated herein but includes all modifications and variations falling within the scope of the invention as defined in the appended claims.

CLAIMS

1. A biophotonic composition comprising:

a first chromophore; and

a gelling agent present in an amount sufficient to gel the composition and render the biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use, measured by less than 15% by weight of the total chromophore amount leaching out of the biophotonic composition into a phosphate saline buffer solution contained in a receptor compartment, through a 2.4-3 cm diameter polycarbonate (PC) membrane with a thickness of 10 microns and a pore size of 3 microns, having a top side onto which a 2 mm thick layer of the biophotonic composition is placed for 5 minutes at room temperature and pressure, and a bottom side which is in direct contact with the phosphate saline buffer solution.

2. The biophotonic composition of claim 1, wherein the biophotonic composition is translucent.

3. The biophotonic composition of claim 2, wherein the translucency comprises at least 20%, 30%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 85%, 90%, 95% or 100% transmittance of light through a 2mm thick composition.

4. The biophotonic composition of any of claims 1 to 3, having a viscosity of about 10,000-100,000, 10,000-90,000, 10,000-80,000, 10,000-70,000, 15,000-80,000, 15,000-70,000, or 15,000-40,000 cP when measured using a Wells-Brookfield cone/plate viscometer and a CP-51 cone at room temperature at a rotational speed of 2 rpm and a torque >10%.

5. The biophotonic composition of any of claims 1 to 4, wherein the gelling agent is selected from the group of cross-linked polymers.

6. The biophotonic composition of any of claims 1 to 5, wherein the gelling agent is selected from at least one of a hydrophilic material, a hygroscopic material or a hydrated polymer.

7. The biophotonic composition of any of claims 1 to 6, wherein the gelling agent is polyanionic in charge character.

8. The biophotonic composition of any one of claims 1 to 7, wherein the gelling agent is present in an amount of about 0.5% to about 5% by weight of the final composition.

9. The biophotonic composition of any of claims 1 to 6, wherein the gelling agent comprises a protein-based polymer.

10. The biophotonic composition of any of claims 1 to 6, wherein the gelling agent comprises a polysaccharide.

11. The biophotonic composition of any of claims 1 to 10, further comprising a humectant.

12. The biophotonic composition of any of claims 1 to 11, wherein the chromophore is in solution in the composition.

13. The biophotonic composition of claim 12, wherein the chromophore solution and the gelling agent form a hydrocolloid.

14. The biophotonic composition of any of claims 1 to 13, further comprising an oxygen-releasing agent.

15. The biophotonic composition of claim 14, wherein the oxygen-releasing agent is a peroxide or a peroxide-releasing agent.

16. The biophotonic composition of any of claims 1 to 15, wherein the first chromophore is a xanthene dye.

17. The biophotonic composition of claim 16, wherein the first chromophore is selected from Eosin Y, Eosin B, Erythrosin B, Fluorescein, Rose Bengal and Phloxin B.

18. The biophotonic composition of any of claims 1 to 17, wherein the first chromophore is present in an amount of about 0.001% to about 40% by weight of the total composition.

19. The biophotonic composition of any of claims 1 to 18, wherein the composition further comprises a second chromophore.

20. The biophotonic composition of claim 19, wherein the first chromophore has an emission spectrum that overlaps at least 20% with an absorption spectrum of the second chromophore.

21. The biophotonic composition of claim 19 or 20, wherein the first chromophore is Eosin Y, and the second chromophore is one or more selected from Fluorescein, Phloxine B and Erythrosine B.

22. The biophotonic composition of any of claims 19 to 21, wherein the second chromophore is present in an amount of about 0.0001% to about 40% by weight of the total composition.

23. The biophotonic composition of any of claims 1 to 22, wherein the pH of the composition is within the range 4.0 to 7.0.

24. A method for providing cosmetic treatment, comprising:

- applying topically a biophotonic composition according to any of claims 1 to 23 to a skin; and
- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first chromophore.

25. The method of claim 24, wherein the cosmetic treatment promotes skin rejuvenation.

26. The method of claim 24 wherein the skin has acne.

27. The method of any of claims 24 to 26, wherein the biophotonic composition is illuminated for about 1 minute to about 30 minutes, preferably less than 20 minutes, 15 minutes, 10 minutes or about 5 minutes.

28. The method of any of claims 24 to 27, wherein the biophotonic composition is illuminated with visible non-coherent light or with visible non-coherent light in the form of violet and/or blue light.

29. A composition according to any one of claims 1 to 23 for use in therapy.

30. A kit comprising:

a composition according to any of claims 1 to 23, and

one or more of a light source for activating the chromophore, instructions for use of the composition and/or the light source, a dressing, and a device for applying and/or removing the composition from a treatment area.

31. A kit comprising:

a first component comprising a first chromophore; and

a second component comprising a gelling agent present in an amount sufficient to gel or thicken the composition and render the biophotonic composition substantially resistant to leaching such that less than 15% by weight of the total chromophore amount leaches out of the biophotonic composition in use measured by less than 15% by weight of the total chromophore amount leaching out of the biophotonic composition into a phosphate saline buffer solution contained in a receptor compartment, through a 2.4-3 cm diameter polycarbonate (PC) membrane with a thickness of 10 microns and a pore size of 3 microns, having a top side onto which a 2 mm thick layer of the biophotonic composition is placed for 5 minutes at room temperature and pressure, and a bottom side which is in direct contact with the phosphate saline buffer solution.

32. A kit comprising:

a first component comprising a composition according to any of claims 1 to 23, and

a second component comprising an oxygen-releasing agent.