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(54) Title: CHROMOPHORE COMBINATIONS FOR BIOPHOTONIC USES

(57) Abstract: The present disclosure provides biophotonic compositions and methods useful in phototherapy. In particular, the biophotonic compositions of the present disclosure comprise at least two xanthene dyes. The biophotonic compositions and the methods of the present disclosure are useful for promoting wound healing and skin rejuvenation, as well as treating acne and other skin disorders.

CHROMOPHORE COMBINATIONS FOR BIOPHOTONIC USES

BACKGROUND OF THE DISCLOSURE

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, to treat skin conditions, to disinfect target sites as an antimicrobial treatment, and to promote wound healing.

Known phototherapy techniques include photodynamic therapy which involves systemic administration or uptake of a photosensitive agent or chromophore into the diseased or injured tissue, followed by site-specific application of activating light. Other types of phototherapy include the use of light alone at specific wavelengths to target tissue using light-emitting diode (LED) or fluorescent lamps, or lasers.

It is an object of the present disclosure to provide new and improved compositions and methods useful in phototherapy.

SUMMARY OF THE DISCLOSURE

The present disclosure provides topical biophotonic compositions and methods of using the biophotonic compositions for the biophotonic treatment of living tissue. Biophotonic treatment may include skin rejuvenation; tissue repair including wound healing, scar removal and scar minimization; treatment of skin conditions such as acne; and treatment of periodontitis.

The biophotonic composition of the present disclosure comprises a gelling agent and at least two xanthene dyes, wherein a first xanthene dye has an emission spectrum that overlaps at least 5%, 10%, 20%, 25%, 30%, 40%, 50%, 60%, 70% with an absorption spectrum of a second xanthene dye. In some embodiments, the first xanthene dye 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 xanthene dye.

Particularly useful combinations of xanthene dyes include but are not limited to: Fluorescein + Eosin Y; Fluorescein + Eosin Y + Rose Bengal; Fluorescein + Eosin Y + Phloxine B; Eosin Y + Rose Bengal; Eosin Y + Phloxine B; Fluorescein + Erythrosine B + Eosin Y; Eosin Y + Erythrosine; Eosin Y + Erythrosine B + Rose Bengal; Eosin Y + Erythrosine B + Phloxine B; Fluorescein + Eosin Y + Erythrosine B + Rose Bengal; and Fluorescein + Eosin Y + Erythrosine B + Phloxine B.

The gelling agent may comprise a hygroscopic substance. In addition or in the alternative, the gelling agent may also be a hydrophilic polymer, a hydrated polymer or a lipid. In certain embodiments, the gelling agent comprises one or more of glycerin, glycols such as propylene glycol, polyacrylic acid polymers, hyaluronic acid, glucosamine sulphate or gelatin.

In certain embodiments, the gelling agent is a high molecular weight, cross-linked polyacrylic acid polymer having a viscosity in the range of about 20,000-80,000, 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 embodiments, the cross-linked polyacrylic acid polymer is a carbomer selected from the group consisting of, but not limited to, Carbopol® 71G NF, 971P NF, 974P NF, 980 NF, 981 NF, 5984 EP, ETD 2020NF, Ultrez 10 NF, 934 NF, 934P NF, 940 NF, 941 NF, or 1342 NF.

In certain embodiments, the biophotonic composition is substantially translucent and/or transparent. In certain embodiment, the biophotonic composition has a translucency of at least 70% at 460 nm. In other embodiments, the composition has a translucency of at least 20%, 30%, 40%, 50%, 60%, 70%, 75%, 85%, 90%, 95% or 100% at 460 nm.

In certain embodiments, the biophotonic composition is a liquid, a gel, a semi-solid, cream, foam, lotion, oil, ointment, paste, suspension, or aerosol spray.

In certain embodiments, the biophotonic composition is encapsulated in a transparent, impermeable membrane, or a breathable membrane which allows permeation of gases but not liquids. The membrane may comprise a lipid.

In certain embodiments, the biophotonic composition further comprises an oxygen-generating agent. In some embodiments, the oxygen-generating agent comprises hydrogen peroxide, carbamide peroxide, benzoyl peroxide, molecular Oxygen or water. When the oxygen-releasing agent is a peroxide, it may be present in values less than 6% H_2O_2, from 0.5-6wt% H_2O_2 (or its equivalent), 0.5-5.5%, 0.5-5.0%, 0.5-4.5%, 0.5-4.0%, 0.5-3.5%, 0.5-3.0%, 0.5-2.5%, 0.5-2%, 0.5-1.5%, or 0.5-1.0%.

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, the first and second xanthene dyes are present in the composition in the amount of about 0.001-0.5% per weight of the composition.

In certain embodiments, 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, 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, the composition does not comprise silica, tanning agents, or non-fluorescent dyes.

The present disclosure also provides uses of the present composition and methods for biophotonic treatment of living tissue.

Accordingly, in some aspects, there is provided a method for providing biophotonic therapy to a wound, comprising: applying to a wound a biophotonic composition comprising at least a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye; and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye.

In some embodiments of the method for providing biophotonic therapy to a wound, the method promotes wound healing. 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 certain embodiments, 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.

In other aspects, there is provided a method for biophotonic treatment of acne comprising: applying to skin tissue a biophotonic composition comprising at least a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye; and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye. 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.

The disclosed methods for treating acne or wounds 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.

In other aspects, there is provided a method for biophotonic treatment of a skin disorder, comprising: applying to target skin tissue a biophotonic composition comprising at least a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye; and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye.

In other aspects, there is provided a method for promoting skin rejuvenation, comprising: applying to target skin tissue a biophotonic composition comprising at least a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye; and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye.

In other aspects, the present disclosure provides a method for treatment of periodontal disease, comprising: applying to a periodontal pocket a biophotonic composition comprising at least a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye; and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye.

In other aspects, there is provided a method of using a cascade of energy transfer between at least a first and a second fluorescent chromophore to absorb and/or emit light within the visible range of the electromagnetic spectrum for treatment of a skin disorder, treatment of a wound, skin rejuvenation, treatment of periodontitis. The present methods and compositions of the present disclosure may also be used to treat fungal and viral infections.

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. 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, 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.

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 illuminated for a period of at least 3 minutes. 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 biophotonic composition is kept in place for up to one, two or three weeks, and 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).

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.

Figures 5A and **5B** are absorbance and emission spectra, respectively, 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, all in a carbamide gel (Example 1).

Figures 6A and **6B** are absorbance and emission spectra, respectively, (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, all in an aqueous solution (Example 2).

Figures 7A and **7B** are absorbance and emission spectra, respectively, of (i) Phloxine B at 0.25mg/mL final concentration, (ii) Eosin Y at about 0.05 mg/mL, and (iii) a mixture of Phloxine B (0.25mg/mL) and Eosin Y (0.05 mg/mL), all in a 12% carbamide gel (Example 3).

Figures 8A and **8B** are absorbance and emission spectra, respectively, of (i) Phloxine B at 0.25mg/mL final concentration, (ii) Eosin Y at about 0.08 mg/mL, and (iii) a mixture of Phloxine B (0.25mg/mL) and Eosin Y (0.08 mg/mL), all in an aqueous solution (Example 4).

Figures 9A and 9B are absorbance and emission spectra, respectively, of (i) Phloxine B at 100μg/g, (ii) Fluorescein at about 100μg/g, and (iii) a mixture of Phloxine B (100μg/g) and Fluorescein (100μg/g), all in a 12% carbamide gel (Example 5).

Figures 10A and 10B are absorbance and emission spectra, respectively, of (i) Phloxine B at 100μg/g, (ii) Fluorescein at about 100μg/g, and (iii) a mixture of Phloxine B (100μg/g) and Fluorescein (100μg/g), all in a 12% carbamide gel (Example 6).

Figures 11A and **11B** are absorbance and emission spectra, respectively, of (i) Eosin Y at 0.305 mg/mL final concentration, (ii) Rose Bengal at about 0.085 mg/mL, and (iii) a mixture of Eosin Y (0.305mg/mL) and Rose Bengal (0.085 mg/mL), all in a 12% carbamide gel (Example 7).

Figure 12 shows that Eosin Y and Rose Bengal act in a synergistic manner (Example 8).

Figures 13A and 13B show the fluorescence emission (power density) over time of compositions comprising (i) Fluorescein + Eosin Y (Figure 11A), and (ii) Eosin Y + Rose Bengal (Figure 11B) (Example 9).

Figures 14A and 14B are absorbance and emission spectra, respectively, of (i) Rose Bengal at about 0.085 mg/mL, (ii) Fluorescein sodium salt at about 0.44 mg/mL final concentration, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of (i), (ii) and (iii) in a carbamide gel (Example 10).

Figures 15A and 15B are absorbance and emission spectra, respectively, of (i) Rose Bengal at about 0.085 mg/mL, (ii) Fluorescein sodium salt at about 0.44 mg/mL final concentration, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of (i), (ii) and (iii) in an aqueous composition (Example 11).

Figure 16 is an emission spectrum showing the intensity over time of the light being emitted from the composition tested in Examples 12 and 13.

Figures 17A and **17B** show that the energy density of emitted fluorescence from Eosin (top) and Fluorescein (bottom) in a composition increases rapidly with increasing chromophore concentration but slows down to a plateau with further concentration increase, whilst the activating light decreases with increasing concentration (Example 15).

DETAILED DESCRIPTION

(1) Overview

The present disclosure provides compositions including at least two photoactive chromophores which can transfer energy from one to the other and methods useful for treating tissue with these compositions for example to promote tissue repair including wound healing, for cosmetic treatment of skin such as for skin rejuvenation, for treating skin disorders such as acne, and for periodontal treatment.

(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.

"Topical composition" means a composition to be applied to body surfaces, such as the skin, mucous membranes, vagina, oral cavity, wounds, and the like. A topical composition may be in the form of, including, but not limited to, a cream, gel, ointment, lotion, levigate, solution, bioadhesive, salve, milk. The topical composition may impregnate material such as a pad, sheet, fabric or fibres, dressings, spray, suspension, foam, or 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, for example a xanthene dye. The chromophore readily undergoes photoexcitation and can then transfer its energy to other molecules or emit it as light.

"Oxidant", "oxidizing agent" or "oxygen-releasing agent" which terms are used interchangeably herein, means a chemical compound that readily transfers oxygen atoms and oxidizes other compounds. It includes molecular oxygen as well as oxygen containing compounds such as water, peroxide etc..

"Photobleaching" means the photochemical destruction of a chromophore.

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.

"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, surgical wounds, contusions, hematomas, crushing injuries, ulcers (such as for example pressure, venous, pressure or diabetic), wounds caused by periodontitis (inflammation of the periodontium), and gun-shot wounds.

"Wound healing" means promotion or acceleration of tissue repair including closure of a wound, activation of a chronic wound, or minimizing scar formation.

"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 Compositions

The present disclosure provides biophotonic compositions. Biophotonic compositions are compositions that, in a broad sense, comprise chromophore(s) which are activated by light and accelerate 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., the break-down of an oxygen-releasing agent when such agent is present in the composition or at the treatment site, leading to the formation of oxygen radicals, such as singlet oxygen). The biophotonic compositions of the present disclosure comprise at least two xanthene dyes as chromophores.

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 due to loss of energy in the conversion process. This is called the Stokes' shift and is illustrated in **Figure 2**. In the proper environment (e.g., in a biophotonic composition) much of this energy is transferred to the other components of the composition or to the treatment site directly.

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, the generation of oxygen species (e.g. singlet oxygen) by photoactivated chromophores has been observed by the inventors to cause micro-bubbling within the composition which can 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.

Furthermore, it is thought that use of chromophores in a composition to emit fluorescent light provides the ability to fine-tune the emitted light to a far greater degree than using a light source such as an LED or a laser. For example, according to the therapy or treatment required, chromophores may be chosen according to their emitted light wavelength, and appropriate concentrations used to control the power density of the emitted light.

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. 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. In some embodiments, transmittance of the compositions disclosed herein is measured at 460 nm.

As transmittance is dependent upon thickness, the thickness of each sample can be measured with calipers prior to loading in the spectrophotometer. Transmittance values can be normalized to a thickness of 100 µm (or any thickness) according to:

$$F_{T-corr}(\lambda, t_2) = [e^{-\sigma_t}(\lambda)t_1]^{\frac{t_2}{t_1}} = [F_{T-corr}(\lambda, t_1)]^{\frac{t_2}{t_1}},$$

where, t_1=actual specimen thickness, t_2=thickness to which transmittance measurements can be normalized.

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% at 460 nm. In some embodiments, the transparency exceeds 70% at 460 nm, 86% at 460 nm, 87% at 460 nm, 88% at 460 nm, 89% at 460 nm, 90% at 460 nm, 91% at 460 nm, 92% at 460 nm, 93% at

460 nm, 94% at 460 nm, 95% at 460 nm, 96% at 460 nm, 97% at 460 nm, 98% at 460 nm or 99% at 460 nm.

The biophotonic compositions of the present disclosure are for topical uses. 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 at least two xanthene dyes as the chromophores. Combining xanthene dyes may increase photo-absorption by the combined dye molecules and enhance absorption and photobiomodulation selectivity. This creates multiple possibilities of generating new photosensitive, and/or selective xanthene dye mixtures.

When such multi-xanthene dye compositions are illuminated with light of an appropriate wavelength to activate at least one of the xanthene dyes, energy transfer can occur between the xanthene dyes. This process, known as resonance energy transfer, is a photophysical process through which an excited 'donor' xanthene dye (also referred to herein as first xanthene dye) transfers its excitation energy to an 'acceptor' xanthene dye (also referred to herein as second xanthene dye). The efficiency and directedness of resonance energy transfer depends on the spectral features of donor and acceptor xanthene dyes. In particular, the flow of energy between xanthene dyes 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 xanthene dye must preferably overlap with the absorption spectrum of the acceptor xanthene dye (**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 xanthene dye should have good abilities to absorb photons and emit photons. Furthermore, it is thought that the more overlap there is between the donor xanthene dye's emission spectra and the acceptor xanthene dye's absorption spectra, the better a donor xanthene dye can transfer energy to the acceptor xanthene dye.

In some embodiments, the first xanthene dye has an emission spectrum that overlaps at least about 80%, 50%, 40%, 30%, 20%, 10% with an absorption spectrum of the xanthene dye chromophore. In one embodiment, the first xanthene dye has an emission spectrum that overlaps at least about 20% with an absorption spectrum of the second xanthene dye. In some embodiments, the first xanthene dye 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 xanthene dye.

% spectral overlap, as used herein, means the % overlap of a donor xanthene dye's emission wavelength range with an acceptor xanthene dye'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 xanthene dyes. The spectral FWQM of the acceptor xanthene dye's absorption spectrum is from about 60 nm (515 nm to about 575 nm). The overlap of the donor xanthene dye's spectrum with the absorption spectrum of the acceptor xanthene dye 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 xanthene dye absorbs at a wavelength in the range of the visible spectrum. In certain embodiments, the second xanthene dye has an absorption wavelength that is relatively longer than that of the first xanthene dye 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 xanthene dyes. 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 another 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.

In some embodiments, the first xanthene dye 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 xanthene dye absorbs at a wavelength of about 200-800 nm, 200-700 nm, 200-600 nm or 200-500 nm. In one embodiment, the first xanthene dye absorbs at a wavelength of about 200-600 nm. In some embodiments, the first xanthene dye 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 by those skilled in the art that optical properties of a particular xanthene dye may vary depending on the xanthene dye's surrounding medium. Therefore, as used herein, a particular xanthene dye's absorption and/or emission wavelength (or spectrum) corresponds to the wavelengths (or spectrum) measured in a biophotonic composition of the present disclosure.

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.

In certain embodiments, the first xanthene dye is present in an amount of about 0.01-40% per weight of the composition, and the second xanthene dye is present in an amount of about 0.001-40% per weight of the composition. In certain embodiments, the total weight per weight of xanthene dyes is in the amount of about 0.01-40.001% per weight of the composition. In certain embodiments, the first xanthene dye is present in an amount of about 0.01-1%, 0.01-2%, 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 xanthene dye 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 xanthene dyes is in the amount of about less than 0.5%, less than 0.1%, 0.001-0.1%, 0.01-1%, 0.01-2%, 0.05-2%, 0.001-0.5%, 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. All amounts are given as weight percentages per weight of the total concentration, and the equivalent weight or volume amounts.

In certain embodiments, the ratio of the concentrations of the first and second xanthene dyes in the composition range from 1:1 to 1:1000. In certain embodiments, the relative concentration of Eosin Y: Fluorescein may be such that there is less Eosin Y than Fluorescein such as 1000:1 or 100:1 or 10:1 or 60-80%: 20-40%. In certain embodiments, the ratio of Eosin Y to Rose Bengal is 1:1 or 70-90%:10-30%. In certain embodiments, the ratio of Fluorescein to Eosin Y to Rose Bengal can be 20-40%: 30-60%: 10-20%. The ratio can be tailored according to the emitted light spectrum desired for a given treatment or therapy.

In some embodiments, the xanthene dye combinations 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, or about 0.05 to about 2 mW/cm^2.

Particularly useful combinations of xanthene dyes include but are not limited to: Fluorescein + Eosin Y; Fluorescein + Eosin Y + Rose Bengal; Fluorescein + Eosin Y + Phloxine B; Eosin Y + Rose Bengal; Eosin Y + Phloxine B; Eosin Y + Erythrosine; Fluorescein + Erythrosine B + Eosin Y; Eosin Y + Erythrosine B + Rose Bengal; Eosin Y + Erythrosine B + Phloxine B; Fluorescein + Eosin Y + Erythrosine B + Rose Bengal; and Fluorescein + Eosin Y + Erythrosine B + Phloxine B.

It is thought that at least some of these combinations have a synergistic effect at certain concentration ratios within the composition. For example, at certain concentration ratios and with an appropriate activating light, Eosin Y can transfer energy to Rose Bengal, Erythrosin B or Phloxine B when activated. This transferred energy is then emitted as fluorescence and/or by production of reactive oxygen species (such as singlet oxygen).

The synergistic effect may be apparent by the composition having a light absorption spectrum which spans a broader range of wavelengths compared to an individual light absorption spectrum of one of the individual chromophores in the composition, when the individual chromophores and the composition are activated by the same activating light (light having substantially the same emission spectra). This may confer on the composition the ability to be activated by a broader range of activating light wavelengths, for example by white light avoiding the need for a precise wavelength of activating light.

The synergistic effect may also be evident through the composition having a light emission spectrum which spans a broader range of wavelengths compared to an individual light absorption spectrum of one of the individual chromophores in the composition, when the individual chromophores and the composition are activated by the same activating light. This absorbed and re-emitted light spectrum is thought to be transmitted throughout the composition, and also to be transmitted into the site of treatment. This emitted spectrum will then illuminate the target tissue with different penetration depths (**Figure 1**), which may confer on the target tissue beneficial therapeutic effects. For example green light has been reported to have wound healing properties. By emitting a broader range of wavelengths, a broader range of therapeutic effects can be achieved. The emitted wavelength can be fine-tuned using different chromophore combinations and concentrations.

The synergistic effect may also be evident through the composition having a higher light absorption or emission peak compared to an individual light absorption/emission peak of one of the individual chromophores in the composition, when the individual chromophores and the composition are activated by the same activating light. The ability to absorb and emit higher levels of photons may have a therapeutic effect in certain applications. Furthermore, less concentration of an individual chromophore may be required to achieve a certain power density. Higher power densities can equate to shorter treatment times.

The synergistic effect may also be evident through the composition producing more oxygen species, in the presence of an oxygen-releasing agent, compared to oxygen species produced by an individual chromophores in the composition, when the individual chromophores and the composition are activated by the same activating light. The ability to produce higher levels of oxygen species without the need to extend treatment time or increase the power density of the activating light may be advantageous in certain situations.

By means of synergistic effects of the xanthene dye combinations in the composition, xanthene dyes which cannot normally be activated by an activating light (such as a blue light) can be activated through energy transfer from xanthene dyes which are activated by the activating light. In this way, the different properties of photoactivated xanthene dyes 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 fluorescence 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 is thought to photoactivate Eosin Y which transfers some of its energy to Rose Bengal as well as emitting some energy as fluorescence.

One or more of the chromophores may photobleach during illumination. This can be a visible confirmation of 'dose' delivery. As the chromophores photobleach, they emit less fluorescence over time. At the same time, they also absorb less of the activating light over time and so the tissues receive increasingly higher amounts of the activating light. In this way, the chromophores modulate exposure of the tissue to the light which may provide a somewhat protective effect.

(b) Additional Chromophores

In addition to the xanthene dye combination, the biophotonic topical compositions of the present disclosure may also 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.

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 additional 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 additional chromophores listed above in addition to the xanthene dyes, 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.

(c) Gelling Agent

The composition may optionally comprise a gelling agent. 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 chromophores. 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.

In some embodiments the composition is in the form of a gel, cream, ointment, lotion, paste, spray or foam.

The gelling agent according to various embodiments of the present disclosure may comprise 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 comprise 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 15,000-100,000, 15,000-90,000, 15,000-80,000, 20,000-80,000, 20,000-70,000, 20,000-40,000 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 15,000-80,000 cP.

Carbomers may be used. Carbomers are synthetic high molecular weight polymer of acrylic acid that are crosslinked 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.

In certain embodiments, the gelling agent comprises a hygroscopic material. By hygroscopic material is meant 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 25°C). The hygroscopic material may include, but is not limited to, glucosamine, 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 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.

The composition may include any other carrier.

(d) Oxygen-releasing agents

According to certain embodiments, the compositions of the present disclosure may optionally further comprise an oxygen-releasing agent, for example, as a source of oxygen.

When a biophotonic composition of the present disclosure comprising an oxygen-releasing agent is illuminated with light, the xanthene dyes are excited to a higher energy state.

When the xanthene dyes' 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 peroxides such as hydrogen peroxide, urea hydrogen peroxide and benzoyl peroxide. 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.

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%.

Other oxygen-releasing agents include molecular oxygen, water, perbonates and carbonates. Oxygen-releasing agents can be provided in powder, liquid or gel form within the composition. 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.

Alternatively, oxygen-releasing agents may also be applied to the tissue site separately to the composition.

(e) Healing Factors

The composition of the present disclosure may comprise 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. 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.

(f) Antimicrobials

The composition of the present disclosure may comprise antimicrobial agents. 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.

(g) Collagens and Agents that Promote Collagen Synthesis

The compositions of the present disclosure may include 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.

(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 first xanthene dye (e.g., donor xanthene dye) of the biophotonic composition.

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 first xanthene dye (e.g., donor xanthene dye) of the biophotonic composition.

In yet another aspect, the present disclosure provides 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 first xanthene dye (e.g., donor xanthene dye) of the biophotonic composition.

In yet another aspect, the present disclosure provides 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 first xanthene dye (e.g., donor xanthene dye) of the biophotonic composition.

In other embodiments, the present disclosure provides a method for treating acute inflammation, the method comprising: topically applying a biophotonic composition of the present disclosure to a target skin tissue with acute inflammation, and illuminating the biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye (e.g., donor xanthene dye) of the biophotonic composition.

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 xanthene dye that undergoes at least partial photobleaching upon application of light. The first xanthene dye 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 xanthene dye absorbs at a wavelength of about 200-600 nm. In some embodiments, the first xanthene dye 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. The absorption spectrum of the second xanthene dye should overlap at least about 80%, 50%, 40%, 30%, or 20% with the emission spectrum of the first xanthene dye. In some embodiments, the first xanthene dye 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 xanthene dye.

Illumination of the biophotonic composition with light may cause a transfer of energy from the first xanthene dye to the second xanthene dye. Subsequently, the second xanthene dye 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 can be formulated with any carrier. In certain embodiments, the carrier is 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.

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 chromophores 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 donor xanthene dye 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 the donor xanthene dye in the biophotonic composition.

In certain embodiments, the first and/or the second xanthene dye 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 xanthene dye 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, gun shots, 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 a 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, viral infections, 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 such as insect bites e.g.; mosquito, bees, wasps, poison ivy, 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, 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 of the present disclosure. 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. The first xanthene dye may be present in an amount of about 0.01-40% per weight of the composition, and a second xanthene dye may be present in an amount of about 0.01-40% per weight of the composition. In certain embodiments, the first xanthene dye 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 second xanthene dye 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 xanthene dyes may be in the amount of about 0.05-40.05% per weight of the composition. In certain embodiments, the amount of xanthene dyes 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.

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 xanthene dyes in a liquid or as a powder. In some embodiments, the kit includes containers comprising the compositions of the present disclosure.

The composition (s) may be contained in containers. The containers may be light impermeable, air-tight and/or leak resistant. Exemplary containers include, but are not limited to, syringes, vials, or pouches. 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 container may be a spray can which may or may not be pressurized. The composition may be in liquid and/or gaseous form.

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 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 – Absorption/emission spectra of Fluorescein and Eosin Y in a gel

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, all in a gel (comprising about 12% carbamide peroxide), were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 5A** and **5B** which indicate an energy transfer between the chromophores in the combination. In particular a broader absorption and emission spectra was achieved with the Eosin Y and chromophore combination, compared with the individual chromophores. This means that the multiple chromophore composition can be activated with a broader bandwidth of light, and that the multiple chromophore light can emit a broader bandwidth of light after illumination. In other words, emission from the multi-chromophore composition occured in a broader range of wavelengths compared to the individual chromophores. In this example, the composition emitted light in the green, yellow and orange wavelengths of the visible spectra. Photobleaching of Eosin Y was observed during illumination. Furthermore, results (not shown) indicate that the presence of peroxide in the gel does not affect the absorbance and emission spectra. Peroxide is optional in compositions and methods of the present disclosure.

Example 2 - Absorption/emission spectra of a Fluorescein and Eosin Y aqueous solution

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, all in an aqueous solution were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 6A** and **6B** which indicate an energy transfer between the chromophores in the combination. Also, as with **Figures 5A** and **5B**, a broader emission spectra was achieved with the Eosin Y and chromophore combination, compared with the individual chromophores. The composition emitted light in the green, yellow and orange wavelengths of the visible spectra. The difference in the absorption and emission spectra between Examples 1 and 2 may be explained by the optical difference in the media (gel in Example 1 and aqueous solution in this example) as well as possibly the effect of doubling the fluorescein concentration. It can be seen that adding Fluorescein to Eosin Y, broadens the bandwidth of the absorption and emission peaks of Eosin Y. This confers on the multiple chromophore combination, the ability to absorb a broader range of wavelengths for photoactivation and to emit a wider range of wavelengths which may confer different therapeutic effects at the same time. Photobleaching of Eosin Y was observed during illumination.

Example 3 - Absorption/emission spectra of Phloxine B and Eosin Y in a gel

The photodynamic properties of (i) Phloxine B at 0.25mg/mL final concentration, (ii) Eosin Y at about 0.05 mg/mL, and (iii) a mixture of Phloxine B (0.25mg/mL) and Eosin Y (0.05 mg/mL), all in a 12% carbamide gel were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 7A** and **7B** which indicate an energy transfer between the chromophores in the combination. As before, broader absorption and emission spectra were achieved with the Phloxine B and Eosin Y chromophore combination, compared with the individual chromophores. The composition emitted light in the green, yellow, orange and red wavelengths of the visible spectra.

Example 4 - Absorption/emission spectra of an aqueous solution of Phloxine B and Eosin Y

The photodynamic properties of (i) Phloxine B at 0.25mg/mL final concentration, (ii) Eosin Y at about 0.08 mg/mL, and (iii) a mixture of Phloxine B (0.25mg/mL) and Eosin Y (0.08 mg/mL), all in an aqueous solution were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 8A** and **8B** which indicate an energy transfer between the chromophores in the combination. Broader absorption and emission spectra were achieved with the Phloxine B and Eosin Y chromophore combination, compared with the individual chromophores. The composition emitted light in the green, yellow, orange and red wavelengths of the visible spectra.

Example 5 - Absorption/emission spectra of Phloxine B and Fluorescein in a gel

The photodynamic properties of (i) Fluorescein at about 100μg/g final concentration, (ii) Phloxine B at about 100μg/g, and (iii) a mixture of Fluorescein (100μg/g) and Phloxine B (100μg/g), all in a 12% carbamide gel were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 9A** and **9B** which indicate an energy transfer between the chromophores in the combination. For this particular combination of chromophores and at this concentration, for the chromophore combination two peaks corresponding to fluorescein and phloxine B emission was absorved, with a higher peak at around 577 nm absorption, compared with the individual chromophores.

Example 6 - Absorption/emission spectra of Fluorescein and Rose Bengal in a gel

The photodynamic properties of (i) Fluorescein at about 100μg/g final concentration, (ii) Rose Bengal at about 100μg/g, and (iii) a mixture of Fluorescein (100μg/g) and Phloxine B (100μg/g), all in a 12% carbamide gel were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 10A** and **10B** which indicate an energy transfer between the chromophores in the combination. For this particular combination of chromophores and at this concentration, two emission peaks were observed in the combined chromophore composition with the combined composition having a higher peak at around 580 nm, compared with the individual chromophores.

Example 7 - Absorption/emission spectra of Rose Bengal and Eosin Y in a gel

The photodynamic properties of (i) Eosin Y at 0.305 mg/mL final concentration, (ii) Rose Bengal at about 0.085 mg/mL, and (iii) a mixture of Eosin Y (0.305mg/mL) and Rose Bengal (0.085 mg/mL), all in a 12% carbamide gel were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorption and emission spectra are shown in **Figures 11A** and **11B** which indicate an energy transfer between the chromophores in the combination. For this particular combination of chromophores and at this concentration, a higher absorption was achieved with the chromophore combination, compared with the individual chromophores. The emission spectra of this specific combination had a lower power density than for Eosin Y alone. In the absence of a temperature rise in the composition during or after illumination, this apparent loss of energy may be attributed to reactive Oxygen species generation (see Example 8 below).

Example 8 - Eosin and Rose Bengal generate oxygen species

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 singlet oxygen production in the presence of oxygen-releasing agents when photoactivated by green light (a singlet oxygen quantum yield of approximately 75% in water [*Murasecco-Suardi et al, Helvetica Chimica Acta, Vol. 70, pp.1760-73, 1987*]). 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 has a much lower quantum yield in terms of singlet oxygen production in the presence of oxygen-releasing agents (a singlet oxygen quantum yield when fully activated of approximately 4% [*Gandin et al, Photochemistry and Photobiology, Vol.37, pp.271-8, 1983*]).

When Eosin Y and Rose Bengal are combined, it appears that both chromophores are activated by the same blue light as evidenced by **Figure 12**.

Figure 12, left panel, shows a photograph of the composition when viewed under a light microscope (x250) before exposure to an activating blue light. Very few bubbles were seen in both compositions. Following illumination with blue light 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 or Eosin Y alone (not shown). This suggests that there is a transfer of energy from Eosin Y to Rose Bengal leading to the formation oxygen species. Eosin Y alone in a carbamide gel presented similar properties to Rose Bengal. A similar effect was observed with Fluorescein and Rose Bengal.

Example 9 – Variation of the chromophore concentration ratios

The effect of varying the concentrations of the individual chromophores in multiple chromophore compositions, according to embodiments of the present disclosure, were investigated. The fluorescence emission over time of compositions comprising (i) Fluorescein – Eosin Y, and (ii) Eosin Y – Rose Bengal, are presented in **Figures 13A** and **13B** respectively.

As can be seen in **Figure 13A**, the emission properties of the following were investigated: (i) 109 μg/g of Eosin Y + 10μg/g of fluorescein, (ii) 109 μg/g of Eosin Y + 100μg/g of fluorescein, (iii) 109 μg/g of Eosin Y, (iv) 10μg/g of fluorescein, (v) 100μg/g of fluorescein, all in a carbamide peroxide gel. An SP-100 spectroradiometer was used to measure the power density spectra (mW/cm^2 versus wavelength) of a photonic signal detected from the various compositions when illuminated with blue light (wavelength of about 440 to 480 nm at a power density of less than 150 mW/cm^2 for about 5 minutes). Fluorescence is measured as light within the 519-700nm range.

As can be seen, the emitted fluorescence of all concentrations decay over time. This decay is often accompanied by a photobleaching of one or more of the chromophores in the composition. A higher concentration of fluorescein in a multiple chromophore composition provides a higher initial emitted fluorescence which also lasts longer, i.e. has a longer lifetime. For the Eosin Y (109 μg/g) and Fluorescein (100μg/g) composition, the initial emitted fluorescence is slightly lower than that of a composition comprising 100μg/g fluorescein alone. This may be attributed to use of energy to form oxygen species (as described in Example 6 above). Therefore, the relative concentrations of the chromophores within a multiple chromophore composition can be varied to tailor the resultant fluorescence and oxygen species properties.

In **Figure 13B**, the following compositions were evaluated (i) 109 μg/g of Eosin Y + 1μg/g of rose bengal (ratio of about 10:1), (ii) 109 μg/g of Eosin Y + 100μg/g of rose bengal (ratio of about 1:1), (iii) 109 μg/g of Eosin Y, (iv) 1μg/g of rose bengal, (v) 100μg/g of rose bengal, all in a carbamide peroxide gel. The same decay trend observed in **Figure 13A** was also observed for eosin Y alone, eosin Y-1μg/g rose bengal, as well as eosin Y-10μg/g rose Bengal (not shown). The very low fluorescence levels for both concentrations of rose bengal alone when activated by blue light can also be observed. Surprisingly, for the composition of 109 μg/g of Eosin Y + 100μg/g of rose bengal a sustained fluorescence was observed, albeit at a lower level than that of Eosin Y alone, and Eosin Y + 1μg/g of rose bengal. In this composition, no photobleaching of Eosin Y was observed. Without wishing to be limited by theory, it is believed that Eosin Y is not photobleaching as at this ratio of Eosin Y/rose Bengal, Eosin Y is able to transfer all of its absorbed energy to rose bengal which then emits the energy and thus prevents the photodegradation of the eosin Y molecules. The peak emission wavelength of the 109 μg/g Eosin Y + 100μg/g rose bengal composition is closer to that of rose bengal's peak emission wavelength than that of eosin y.

A similar sustained fluorescence effect was observed for a composition comprising fluorescein, eosin Y and rose bengal at relative concentration ratios of about 1:10:10 (not shown).

Example 10 - Absorption/emission spectra of Fluorescein, Eosin Y and Rose Bengal in a gel

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, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of (i), (ii) and (iii) according to an embodiment of the present disclosure in a gel comprising about 12% carbamide peroxide were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorbance and emission spectra are shown in **Figures 14A** and **14B** which indicate an energy transfer between the chromophores in the chromophore combination. As is clear from **Figure 14B**, the bandwidth of the Fluorescein, Eosin Y and Rose Bengal combination is wider than that of Eosin Y alone.

Example 11 - Absorption/emission spectra of Fluorescein, Eosin Y and Rose Bengal in an aqueous solution

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, (ii) Eosin Y at about 0.305 mg/mL, and (iii) a mixture of (i), (ii) and (iii) in an aqueous solution according to an embodiment of the present disclosure were evaluated. A flexstation 384 II spectrophotometer was used to measure emitted fluorescence with the following parameters: mode fluorescence, excitation 460 nm, emission spectra 465-750 nm. The absorbance was read using a synergy HT microplate reader: mode absorbance; spectra between 300-650nm.

The absorbance and emission spectra are shown in **Figures 15A** and **15B** which indicate an energy transfer between the chromophores in the chromophore combination, in the absence of a peroxide but in the presence of other oxygen-releasing agents (e.g. water).

In reference to the absorption and emission spectra of the compositions of the present disclosure within a carbamide peroxide gel, the same spectra was obtained for the same chromophores in a gel without the peroxide.

Example 12 - Angiogenic potential of a composition of the disclosure

A human skin model was developed to assess the angiogenic potential of compositions of the present disclosure. Briefly, a composition comprising Eosin Y and Erythrosine was placed on top of a human skin model containing fibroblasts and keratinocytes. 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 composition emitted fluorescent light. Since the 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 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 and image analysis after 24 hours. The conditioned media 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 media and conditioned media from untreated skin samples were used as a control, and did not induce endothelial tube formation.

Figure 16 is an emission spectrum showing the intensity over time of the light being emitted from the biophotonic composition as measured using the spectroradiometer of Example 9. It can be reasonably inferred that other chromophore combinations exhibiting a comparable emission spectra would also induce angiogenesis. As can be seen from **Figure 16**, the emitted fluorescence light had a wavelength of about 520-620 nm with a peak at around 560 nm. Similar emission spectra were observed using Eosin Y and Fluorescein (Figure 5B); Eosin Y and Phloxine B (Figure 7B, Figure 8B); Eosin Y and Rose Bengal (Figure 11B); Fluorescein, Eosin Y and Rose bengal (Figure 14B, Figure 15B). Other chromophore combinations with similar emission spectra are also possible, which can be reasonably expected to have angiogenic properties.

Example 13 - Protein secretion and gene expression profiles

Wounded and unwounded 3D human skin models (EpiDermFT, MatTek Corporation) were used to assess the potential of a composition of the present disclosure to trigger distinct protein secretion and gene expression profiles. Briefly, a composition comprising Eosin and Erythrosine were placed on top of wounded and unwounded 3D human skin models cultured under different conditions (with growth factors (1X), 50% growth factors (0.5X) and no growth factors (0X)). The different conditions mimicked non-compromised healing, semi-starvation conditions and starvations conditions, respectively. 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 energy density 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 1 and 2) 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. Cytotoxicity was not observed in the light treatments. The eosin y and erythrosine b composition had the same emission properties as illustrated in **Figure 16**. As stated above, it can be reasonably inferred that other chromophore combinations exhibiting a comparable emission spectra would also induce secretion of proteins or gene expression as seen in this Example.

Table 1 – 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 2 – 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 ↑↑**	**MMP3 p=0.007 ↑↑** LAMA1 p=0.03 ↑ ITGA2 p=0.03 ↑

		THBS1 p=0.03 ↑	
Decrease	HAS1 p=0.009 ↓↓ NCAM1 p=0.05 ↓↓ VCAM1 p=0.03 ↓↓ COL7A1 p=0.04 ↓ CTNNA1 p=0.03 ↓	**NCAM1 p=0.02** ↓↓ VCAN p=0.02 ↓ LAMC1 p=0.002 ↓ COL6A1 p=0.007 ↓ MMP7 p=0.003 ↓	

Example 14 – Eosin Y and Fluorescein induce collagen formation

A composition according to an embodiment of the present invention, comprising 0.01% Eosin Y and 0.01% Fluorescein in a carrier matrix (1.8% carbopol gel) was evaluated for its potential to induce collagen formation. Dermal human fibroblasts were plated in glass-bottomed dishes with wells (MatTek®). There were approximately 4000 cells per well. After 48 hours, the glass-bottomed dishes were inverted and the cells were treated through the glass bottom with (i) a no light (control), (ii) sunlight exposure for about 13 minutes at noon (control), (iii) the composition applied to the glass well bottom on the other side of the cells (no light exposure), (iv) the composition applied to the glass well bottom on the other side of the cells (sun light exposure for about 13 minutes at noon), and (v) the composition applied to the glass well bottom on the other side of the cells (blue light exposure for about 5 minutes). In the case of (iii), (iv) and (v), there was no direct contact between the cells and the composition. In the case of (iv) and (v), the cells were exposed to emitted light from and through the Eosin Y and Fluorescein composition when exposed to sunlight and blue light respectively. An at least partial photobleaching was observed in (iv) and (v). After the treatment, the cells were washed and incubated in regular medium for 48 hours. A collagen assay was then performed on the supernatant using the Picro-Sirius red method. This involved adding Sirius red dye solution in picric acid to the supernatant, incubating with gentle agitation for 30 minutes followed by centrifugation to form a pellet. The pellet was washed first with 0.1N HCl and then 0.5 N NaOH to remove free dye. After centrifugation, the suspension was read at 540 nm for collagen type I. The results are shown in Table 1.

Table 1 – A qualitative comparison of collagen type I concentration in a dermal human fibroblast supernatant exposed to (i) a no light (control), (ii) sunlight exposure for about 13 minutes at noon (control), (iii) any light emitted from a Eosin Y and Fluorescein composition through a glass separation (no light exposure), (iv) any light emitted from a Eosin Y and Fluorescein composition through a glass separation (sun light exposure for about 13 minutes at noon), and (v) the composition applied to the glass well bottom on the other side of the cells (blue light exposure for about 5 minutes). ++ indicates collagen levels about twice as high as +, and +++ indicates collagen levels about three times as high as +.

	No light (control)	Sunlight (control)	Eosin Y + Fluorescein – no light	Eosin and Fluorescein – sunlight	Eosin and Fluorescein – blue light
Collagen concentration	+	+	++	+++	+++

There was a statistical difference between the collagen levels induced by the Eosin Y and Fluorescein composition exposed to sunlight and blue light compared to the no light and sunlight alone controls.

Collagen generation is indicative of a potential for tissue repair including stabilization of granulation tissue and decreasing of wound size. It is also linked to reduction of fine lines, a decrease in pore size, improvement of texture and improvement of tensile strength of intact skin. The emission spectra of the Eosin Y and Fluorescein composition of this example had a single peak emission with a wavelength that ranged from about 480-620 nm. Following illumination with sunlight, the power density of the peak was reduced indicating an at least partial photobleaching in 13 minutes, which was also observed by a change in colour of the composition. The rate of fluorescence emission/photobleaching was slower when illuminated by sunlight (white light) compared to Eosin Y and Fluorescein compositions (e.g. compositions of Examples 5 and 6) when activated by blue light.

Example 15 – Selecting the concentration of chromophore in the biophotonic composition

The fluorescence spectra of compositions with different concentrations of chromophores were investigated using a spectroradiometer and an activating blue light (as in **Example 9**). Exemplary fluorescence spectra of Eosin Y and Fluorescein are presented in **Figures 17A and 17B**. 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 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 17A**).

Therefore, concentration can be selected according to required activating light and fluorescence. In some embodiments, it will be after zone of rapid increase, i.e. between 0.5 and 1 mg/mL for Eosin Y (**Figure 17A**).

Compositions with rose bengal behave slightly differently and become more opaque with increasing concentration which may be due to bubble formation.

Similarly, the relationship between the power density of light received by the tissues with illuminating time was investigated. It was found that the power density of the activating light was low initially and increased with time. This correlates with the light absorbing chromophores photobleaching and more of the activating light passing through the composition to reach tissues. In parallel, the fluorescent light emitted by the composition decreased with time as one or more of the chromophores photobleached. Overall, the total power density of the light treating the tissues increased gradually over illumination time.

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.

What is claimed is:

1. A biophotonic composition for topical application to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye, wherein the first xanthene dye 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 xanthene dye, and wherein the first and second xanthene dyes are present in the composition at a concentration of about 0.001-0.5% per weight of the composition.

2. A biophotonic composition for topical application to a target tissue, the composition comprising Eosin Y and Fluorescein.

3. A biophotonic composition for topical application to a target tissue, the composition comprising Eosin Y and Rose Bengal.

4. A biophotonic composition for topical application to a target tissue, the composition comprising Fluorescein and Rose Bengal.

5. The biophotonic composition of claim 2, further comprising a third xanthene dye which is Rose Bengal.

6. The biophotonic composition of any of claims 1-5, wherein activation by light results in a cascade of energy transfer between the first and second xanthene dye.

7. The biophotonic composition of claim 6, wherein the energy transfer provides photons that penetrate the epidermis and/or dermis at a target skin tissue site, in use.

8. The biophotonic composition of claim 6 or 7, wherein said energy transfer is not accompanied by concomitant generation of heat.

9. The biophotonic composition of any of claims 6-8, wherein the energy transfer does not result in tissue damage.

10. The biophotonic composition of any of claims 1-9, wherein the first xanthene dye absorbs at a wavelength in the range of the visible spectrum.

11. The biophotonic composition of any of claims 1-10, wherein the second xanthene dye absorbs at a wavelength in the range of the visible spectrum.

12. The biophotonic composition of any of claims 1-11, wherein the first xanthene dye absorbs at a wavelength of 400-500 nm.

13. The biophotonic composition of any of claims 1-12, wherein the second xanthene dye absorbs at a wavelength that is relatively longer than that of the first xanthene dye within the range of 10-100 nm.

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

15. The biophotonic composition of claim 14, wherein at least the first or the second xanthene dye undergoes photobleaching upon application of light.

16. The biophotonic composition of any of claims 1-15, wherein the first and second xanthene dyes are present in the composition at a concentration of about 0.001-0.1% or 0.001-0.01% per weight of the composition.

17. The biophotonic composition of any of claims 1-16, wherein said biophotonic composition promotes the healing of a skin disorder or a wound.

18. A method for biophotonic treatment of a skin disorder comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Fluorescein.

19. A method for biophotonic treatment of a skin disorder comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Rose Bengal.

20. A method for biophotonic treatment of a skin disorder comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Fluorescein and the second xanthene dye is Rose Bengal.

21. A method for providing biophotonic therapy to a wound comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Fluorescein.

22. A method for providing biophotonic therapy to a wound comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Rose Bengal.

23. A method for providing biophotonic therapy to a wound comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Fluorescein and the second xanthene dye is Rose Bengal.

24. A method for promoting wound healing comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Fluorescein.

25. A method for promoting wound healing comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Rose Bengal.

26. A method for promoting wound healing comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Fluorescein and the second chromophore is Rose Bengal.

27. A method for providing skin rejuvenation comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second chromophore is Fluorescein.

28. A method for providing skin rejuvenation comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Eosin Y and the second xanthene dye is Rose Bengal.

29. A method for providing skin rejuvenation comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a first xanthene dye and a second xanthene dye; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first xanthene dye, wherein the first xanthene dye is Fluorescein and the second chromophore is Rose Bengal.

30. The method of any of claims 26- 28, wherein said biophotonic composition promotes collagen synthesis.

31. The method of any of claims 18-20, wherein said biophotonic composition promotes the healing of a skin disorder.

32. The method of any one of claims 18-20, wherein said biophotonic composition reduces inflammation.

33. The method of any one of claims 18-26, wherein said biophotonic composition reduces the formation of scar tissue.

34. The method of any of claims 18, 21, 24 and 27, further comprising a third xanthene dye which is Rose Bengal.

35. The method of any of claims 18-34, wherein the biophotonic composition is illuminated for a period of at least 5 minutes.

36. The method of any of claims 18-35, wherein the biophotonic composition is illuminated for a period of at least 3 minutes.

37. The method of any of claims 18-36, wherein the biophotonic composition is removed from the target tissue following application of light.

38. The method of any of claims 18-37, wherein the first and/or the second xanthene dye undergoes photobleaching upon application of light.

39. The method of any one of claims 18-38, wherein the application of light results in a cascade of energy transfer between the chromophores.

40. The method of claim 39, wherein the cascade of energy transfer provides photons that penetrate into epidermis and/or dermis at the target tissue site.

41. The method of claim 39 or 40, wherein said cascade of energy transfer is not accompanied by concomitant generation of heat.

42. The method of claim 39-41, wherein the cascade of energy transfer does not result in tissue damage.

43. The method of any of claims 18-42, wherein the first xanthene dye absorbs at a wavelength of about 200-600 nm.

44. The method of any one of claims 18-43, wherein the first and second xanthene dyes are present in an amount of about 0.01-0.5%, about 0.001-0.1%, or about 0.001-0.01% per weight of the composition.

45. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and the composition has a light absorption spectrum spanning a broader range of wavelengths compared to a light absorption spectrum of at least one of the individual first and second chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

46. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and the composition has a light emission spectrum spanning a broader range of wavelengths compared to a light emission

spectrum of at least one of the individual first and chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

47. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and the composition has a light absorption peak with a higher density compared to a light absorption peak of at least one of the individual first and chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

48. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and the composition has a light emission peak with a higher density compared to a light emission peak of at least one of the individual first and chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

49. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and the composition produces higher levels of Oxygen species compared to a level of Oxygen species generated by at least one of the individual first and chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

50. A biophotonic composition for topical application to a target skin tissue, the composition comprising a first chromophore and a second chromophore, wherein the first and second chromophores are first and second xanthene dyes and an emitted fluorescence of the composition is substantially maintained over an illumination time compared to an emitted fluorescence of at least one of the individual first and chromophores, when the composition and the individual chromophores are illuminated with the same activating light.

51. The biophotonic composition of claim 45, wherein the light emission spectrum spans portions of the visible spectra selected from: green and yellow; yellow and orange; green, yellow and orange; yellow and red; or orange, yellow and red.

52. The biophotonic composition of any of claims 45-51, wherein the first and second xanthene dyes are selected from: Fluorescein and Eosin Y; Eosin Y and Phloxine B; Fluorescein; Eosin Y and Rose Bengal; Fluorescein, Eosin Y and Rose Bengal.

53. The biophotonic composition of any of claims 45-52, wherein the activating light has a single peak emission wavelength.

54. The biophotonic composition of claim 53, wherein the activating light has a peak emission wavelength within the blue and/or violet spectrum.

55. The biophotonic composition of any of claims 45-52, wherein the activating light is sunlight.

56. The biophotonic composition of any of claims 45-55, wherein the composition further comprises oxygen-releasing agents.

57. A method for promoting collagen formation, comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a biophotonic composition according to any of claims 45-56; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first and/or second xanthene dye.

58. A method for promoting healing of a skin disorder, comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a biophotonic composition according to any of claims 45-56; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first and/or second xanthene dye.

59. A method for promoting angiogenesis, comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a biophotonic composition according to any of claims 45-56; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first and/or second xanthene dye.

60. A method for promoting wound healing, comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a biophotonic composition according to any of claims 45-56; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first and/or second xanthene dye.

61. A method for modulating expression of any one of MMP1, MMP3, MMP8, MMP10, MCP-2, IL-1R4/ST2, ENA78 and TNFα to promote tissue repair, comprising:

- topically applying a biophotonic composition to a target tissue, the composition comprising a biophotonic composition according to any of claims 45-56; and

- illuminating said biophotonic composition with light having a wavelength that overlaps with an absorption spectrum of the first and/or second xanthene dye.

62. The method of any of claims 57-61, wherein the biophotonic composition is illuminated for a period of at least 5 minutes.

63. The method of any of claims 57-62, wherein the biophotonic composition is removed from the target tissue following application of light.

64. The method of any of claims 57-63, wherein the first and/or the second xanthene dye undergoes at least partial photobleaching upon application of light.

65. The method of any of claims 57-64, wherein the application of light results in a cascade of energy transfer between the xanthene dye.

66. The method of claim 65, wherein the cascade of energy transfer provides photons that penetrate into the target tissue site.

67. The method of claim 65 or 66, wherein said cascade of energy transfer is not accompanied by concomitant generation of heat.

68. The method of claim 65-67, wherein the cascade of energy transfer does not result in tissue damage.

69. The method of any of claims 57-68, wherein the first xanthene dye absorbs at a wavelength of 400-600 nm.

70. The method of any of claims 57-68, wherein the activating light has a single peak emission wavelength.

71. The method of claim 70, wherein the activating light has a peak emission wavelength within the blue and/or violet spectrum.

72. The method of any of claims 57-69, wherein the activating light is sunlight.

73. A kit comprising:

a first component comprising a biophotonic composition according to any of claims 1-17 or claims 45-56; and

optionally a second component comprising an oxidizing agent.

74. A kit comprising:

a first component comprising the first xanthene dye of the biophotonic composition according to any of claims 1-17 or claims 45-56; and

a second component comprising the second xanthene dye of the biophotonic composition according to any of claims 1-17 or claims 45-56 .

75. The kit of claim 73 or 74, further comprising instructions for application of the biophotonic composition to skin.

76. The kit of claim 75, wherein the instructions are for treatment of a skin disorder, skin rejuvenation or treatment of a wound.

77. Use of a composition according to any of claims 1-15, or 45-56 for collagen formation.

78. The use of claim 77, wherein the collagen formation is in intact or broken skin.

79. Use of a composition according to any of claims 1-17, or 45-56 for modulating expression of any one of MMP1, MMP3, MMP8, MMP10, MCP-2, IL-1R4/ST2, ENA78 and TNFα to promote tissue repair.

80. Use of a composition according to any of claims 1-17, or 45-56 for promoting angiogenesis.

81. A composition comprising:

an oxidizing agent; and

Eosin Y and Fluorescein.

82. A composition comprising:
an oxidizing agent; and
Eosin Y, Fluorescein and Rose Bengal.

83. A composition comprising:
an oxidizing agent; and
Eosin Y and Rose Bengal.

84. A composition comprising:
an oxidizing agent; and
Fluorescein and Rose Bengal.

85. A composition comprising:
an oxidizing agent; and
Eosin Y, Fluorescein and Erythrosine.

86. A composition for use in wound healing, skin rejuvenation or treating skin disorders, the composition comprising:
an oxidizing agent; and
Eosin Y and Fluorescein.

87. A composition for use in wound healing, skin rejuvenation or treating skin disorders, the composition comprising:
an oxidizing agent; and
Eosin Y, Fluorescein and Rose Bengal.

88. A composition for use in wound healing, skin rejuvenation or treating skin disorders, the composition comprising:
an oxidizing agent; and
Eosin Y and Rose Bengal.

89. A composition for use in wound healing, skin rejuvenation or treating skin disorders, the composition comprising:
an oxidizing agent; and
Fluorescein and Rose Bengal.

90. A composition for use in wound healing, skin rejuvenation or treating skin disorders, the composition comprising:
an oxidizing agent; and
Eosin Y, Fluorescein and Erythrosine.

91. The composition of claims 81-90, further comprising erythrosine.

92. The composition of any one of claims 81- 91, wherein the oxidizing agent comprises hydrogen peroxide.

93. The composition of claim 92, wherein the hydrogen peroxide content is less than or equal to 6% by weight of hydrogen peroxide in the composition.

94. The composition of any one of claims 81-92, wherein the oxidizing agent comprises carbamide peroxide.

95. The composition of claim 94, wherein the carbamide peroxide content is less than or equal to 22% by weight carbamide peroxide in the composition.

96. The composition of any one of claims 81-95, wherein the total content of the oxidizing agent is equivalent to about 6% by weight of hydrogen peroxide content.

97. The composition of any one of claims 81-96, further comprising a stabilizing agent.

98. The composition of any one of claims 81-97, further comprising a thickening agent.

99. The composition of claim 98, wherein the thickening agent is silicon dioxide and/or fumed silica having a particle size less than one micron.

100. The composition of any one of claims 91-99, further comprising a hydrophilic gelling agent.

101. The composition of claim 100, wherein the hydrophilic gelling agent comprises polypropylene glycol, polyethylene glycol, propylene glycol, glycerol, or a large molecular weight polyol, or any combination thereof.

102. The composition of any one of claims 91-101, further comprising a base.

103. The composition of claim 102, wherein the base is potassium hydroxide.

104. The composition of any one of claims 91-103, wherein the pH of the composition is between 2 and 10.

105. The composition of any one of claims 91-103, wherein the pH of the composition is between 4 and 8, preferably between 6 and 7, more preferably 6.5.

106. The composition of any one of claims 91-105, wherein Eosin Y is present in an amount of 0.001 % to 1 % weight per weight of the composition.

107. The composition of any one of claims 91-105, wherein Fluorescein is present in an amount of 0.001 % to 1 % weight per weight of the composition.

108. The composition of any one of claims 91-105, wherein Rose
Bengal is present in an amount of 0.001 % to 1 % weight per weight of the composition.

109. The composition of any one of claims 91-108, wherein Erythrosine is
present in an amount of 0.001 % to 1 % weight per weight of the composition.

110. A kit comprising:
a first component comprising an oxidizing agent; and
a second component comprising Eosin Y and Fluorescein.

111. A kit comprising:
a first component comprising an oxidizing agent; and
a second component comprising Eosin Y, Fluorescein and Rose Bengal.

112. A kit comprising:
a first component comprising an oxidizing agent; and
a second component comprising Eosin Y and Rose Bengal.

113. A kit comprising:
a first component comprising an oxidizing agent; and
a second component comprising Fluorescein and Rose Bengal.

114. A kit comprising:
a first component comprising an oxidizing agent; and
a second component comprising Eosin Y, Fluorescein and Erythrosine.

115. The kit of claims 110-113, wherein the second component further comprises erythrosine.

116. The kit of any one of claims 110-115, wherein the oxidizing agent comprises hydrogen peroxide or carbamide peroxide.

117. The kit of claim 116, wherein the oxidizing agent is carbamide peroxide.

118. The kit of any one of claims 110-117, wherein one or both of the first and second components further comprises a thickening agent.

119. The kit of claim 118, wherein the thickening agent is silicon dioxide and/or fumed silica having a particle size less than one micron.

120. The kit of any one of claims 110-119, wherein one or both of the first and second components further comprises a hydrophilic gelling agent.

121. The kit of any one of claims 110 to 120, further comprising an applicator.

122. The kit of any one of claims 110 to 121, further comprising instructions for using the kit, apparatus for mixing together the first and second components, a light source, or information for assessing the efficacy of the composition.

123. The kit of any one of claims 110 to 122, further comprising instructions for application of the biophotonic composition to skin.

124. The kit of claim 123, wherein the instructions are for treatment of a skin disorder, for treatment of a wound or for skin rejuvenation.

125. Use of a biophotonic composition for treatment of a skin disorder, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, wherein the first chromophore is Eosin Y and the second chromophore is Fluorescein.

126. Use of a biophotonic composition for treatment of a skin disorder, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Rose Bengal.

127. Use of a biophotonic composition for treatment of a skin disorder, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Fluorescein and the second chromophore is Rose Bengal.

128. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Fluorescein.

129. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Rose Bengal.

130. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Fluorescein and the second chromophore is Rose Bengal.

131. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Fluorescein.

132. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Rose Bengal.

133. Use of a biophotonic composition for providing biophotonic therapy to a wound, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Fluorescein and the second chromophore is Rose Bengal.

134. Use of a biophotonic composition for providing skin rejuvenation, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an

absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Fluorescein.

135. Use of a biophotonic composition for providing skin rejuvenation, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Eosin Y and the second chromophore is Rose Bengal.

136. Use of a biophotonic composition for providing skin rejuvenation, the composition comprising a first chromophore and a second chromophore, wherein the biophotonic composition is suitable for illumination with light having a wavelength that overlaps with an absorption spectrum of the first chromophore, and wherein the first chromophore is Fluorescein and the second chromophore is Rose Bengal.

137. The biophotonic composition of any one of claims 1-17, or claims 45-56 for use in treatment of a skin disorder, for treatment of a wound or for skin rejuvenation.

138. A method of using a cascade of energy transfer between at least a first and a second fluorescent chromophore to absorb and/or emit light within the visible range of the electromagnetic spectrum for treatment of a skin disorder, treatment of a wound or skin rejuvenation.

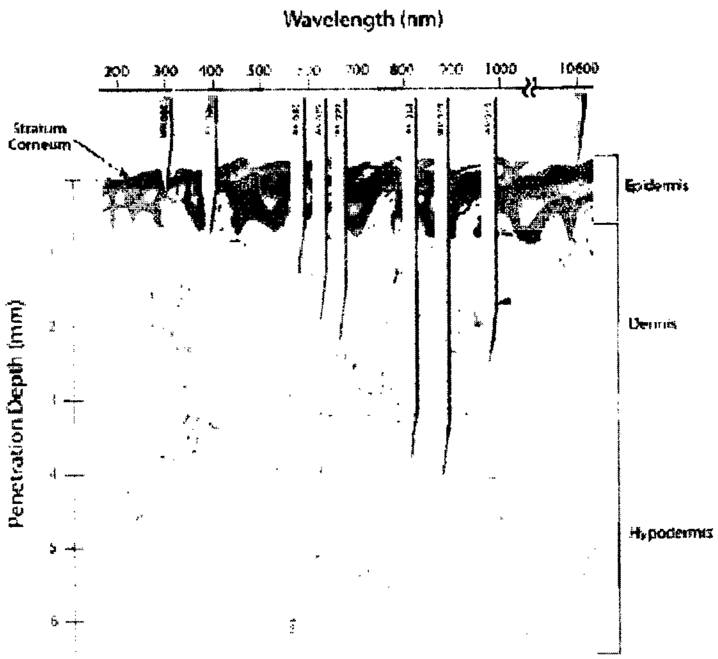

Figure 1

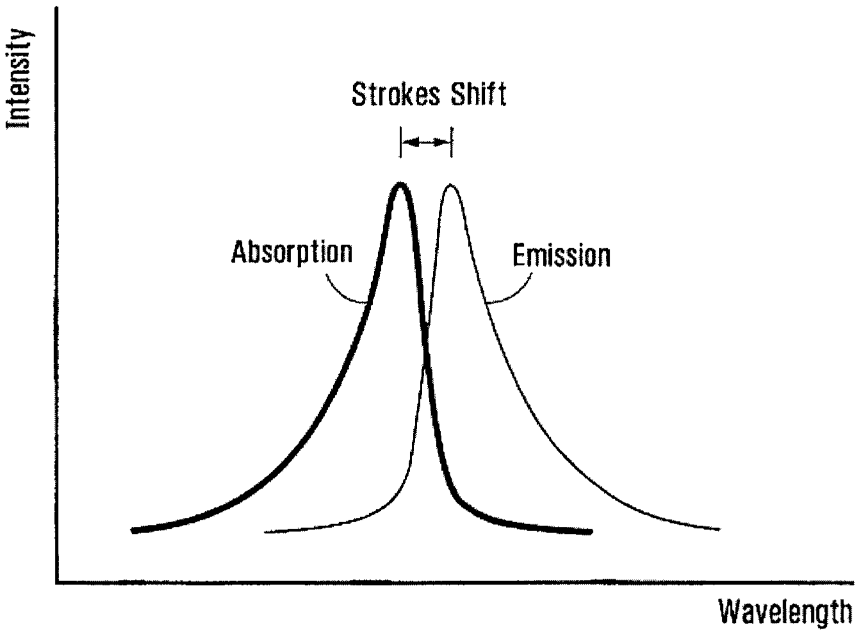

Figure 2

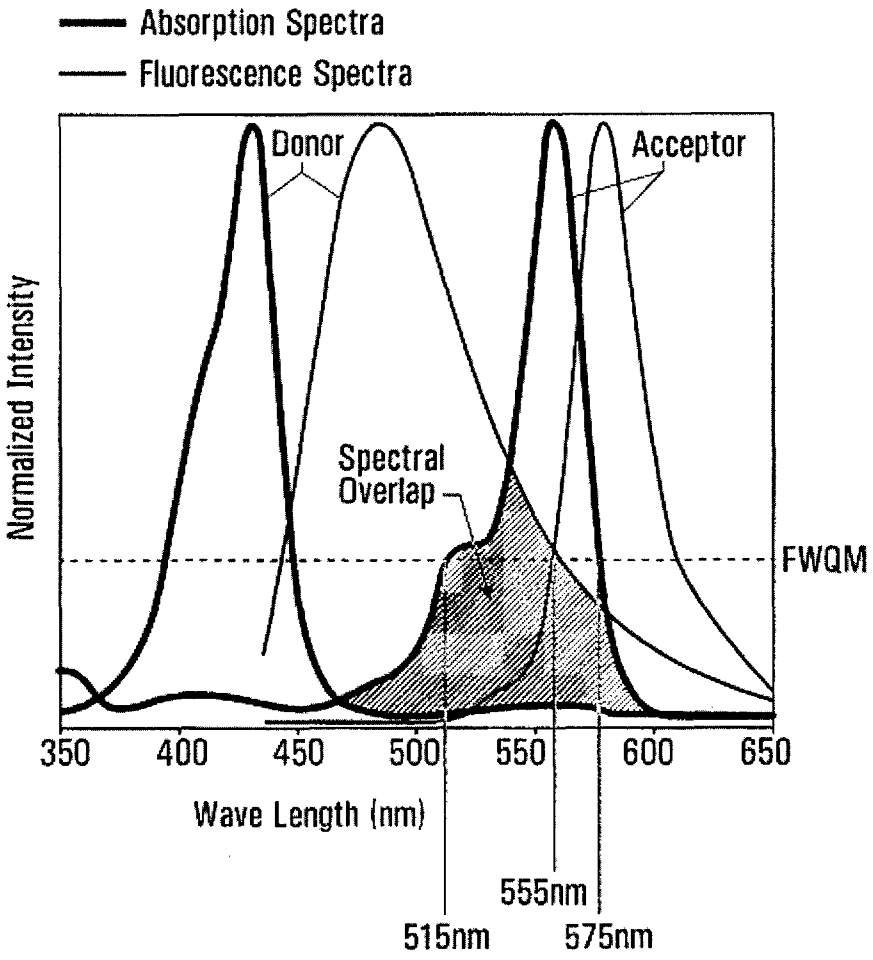
Absorption Spectra
Fluorescence Spectra
Donor
Acceptor
Spectral Overlap
FWQM
Normalized Intensity
350
400
450
500
550
600
650
Wave Length (nm)
515nm
555nm
575nm

Figure 3

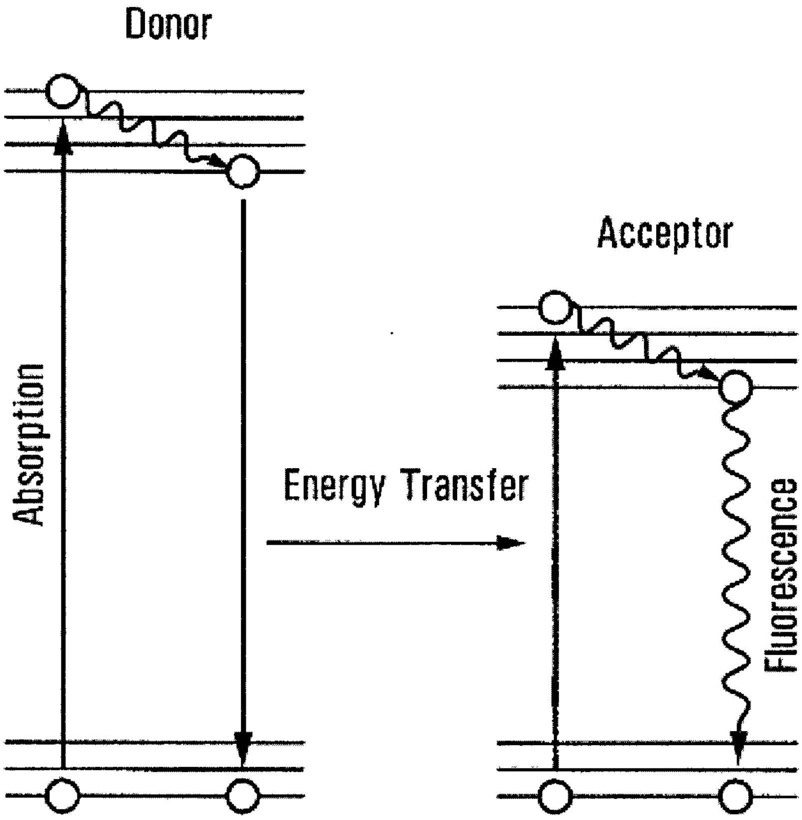

Figure 4

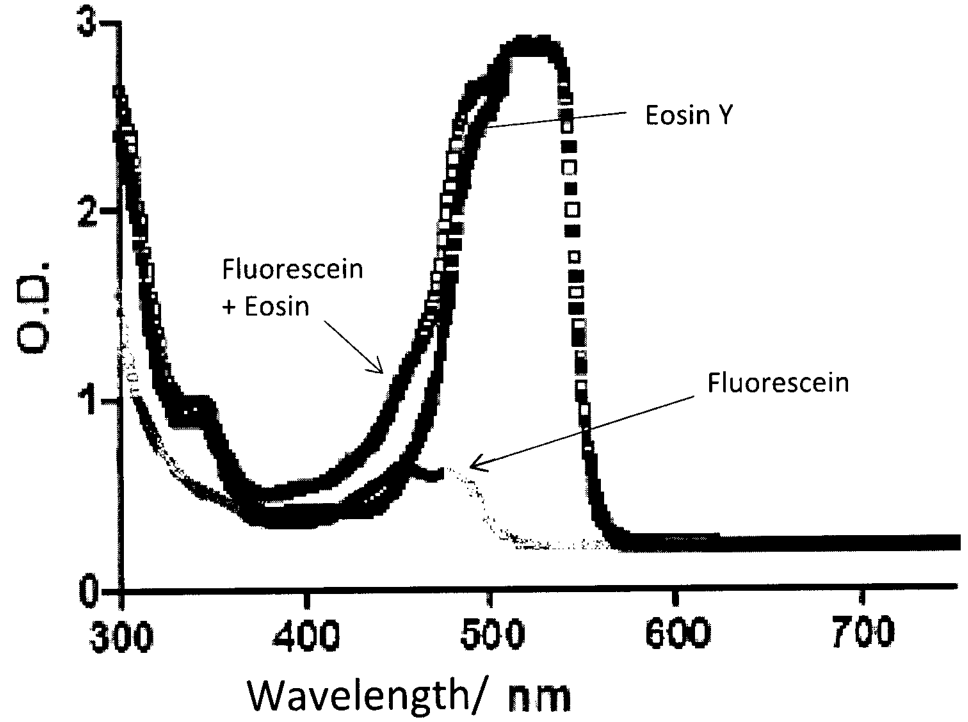

Figure 5A

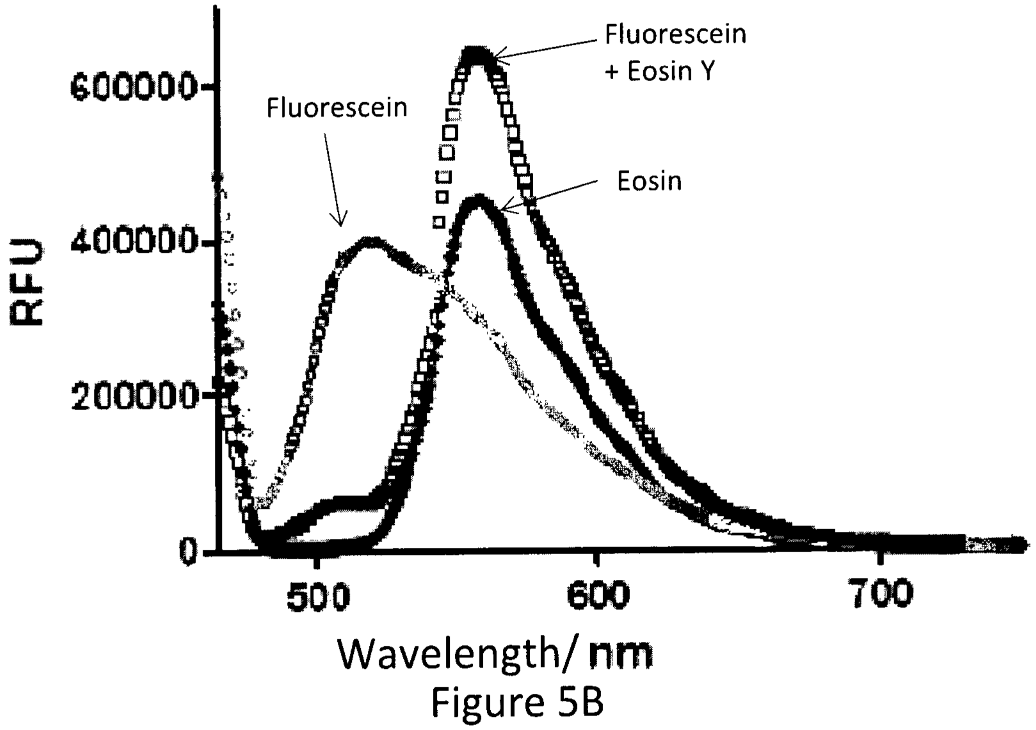

Figure 5B

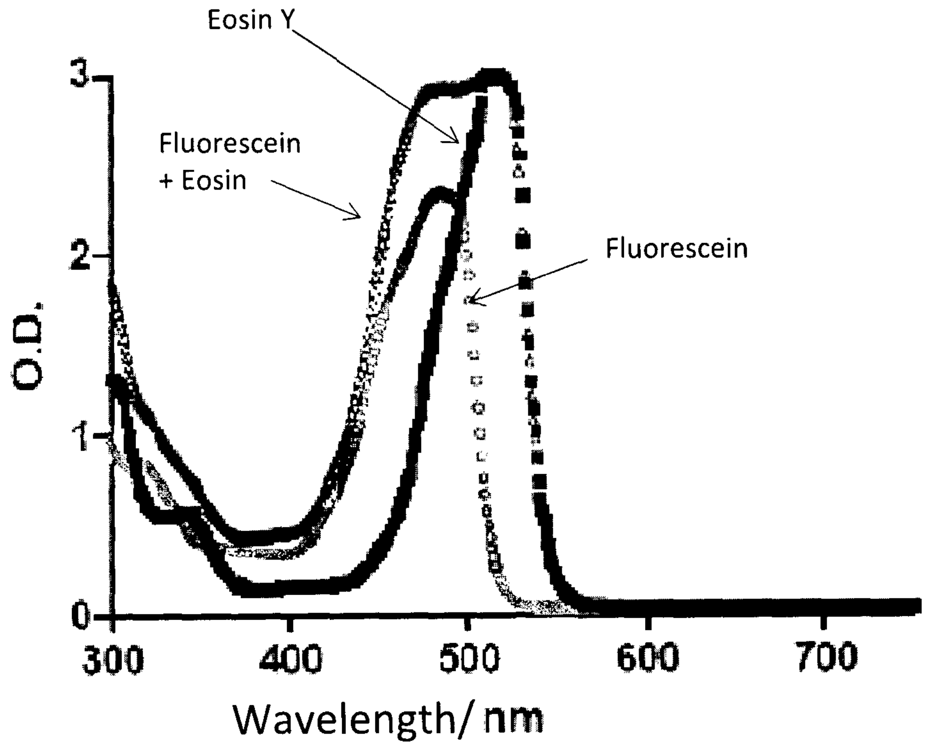

Figure 6A

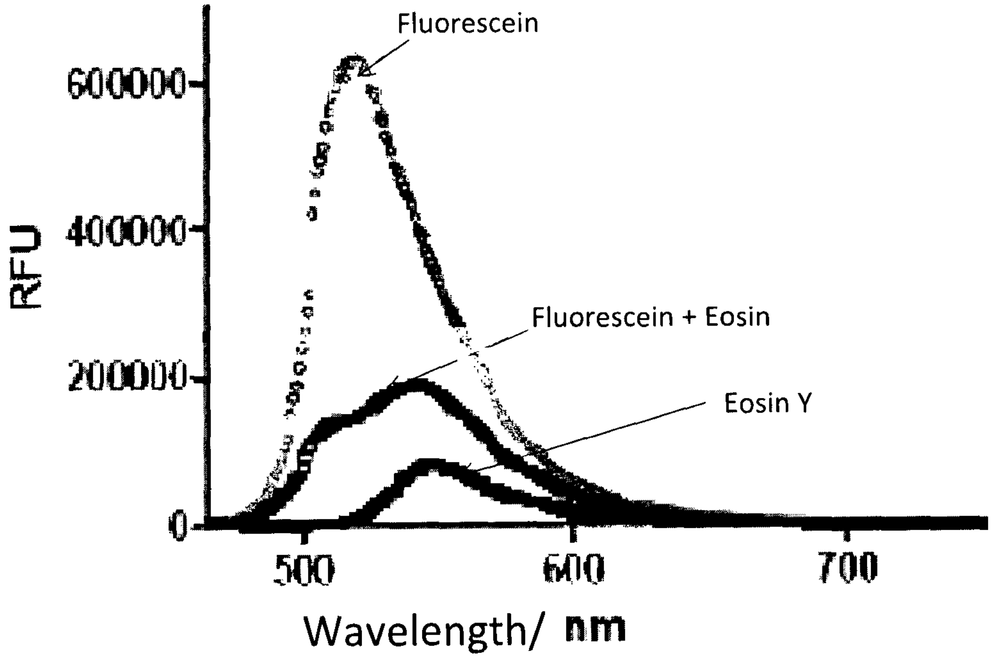

Figure 6b

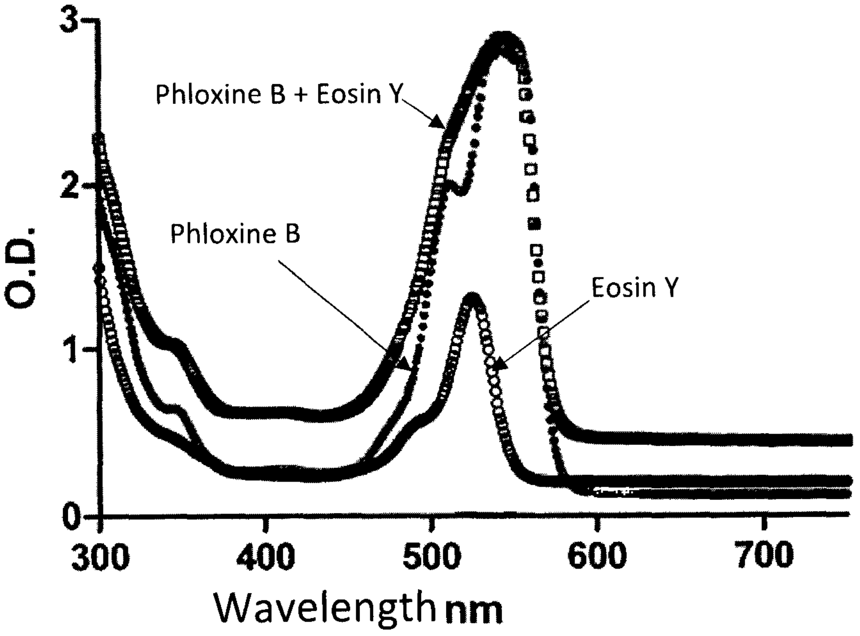

Figure 7A

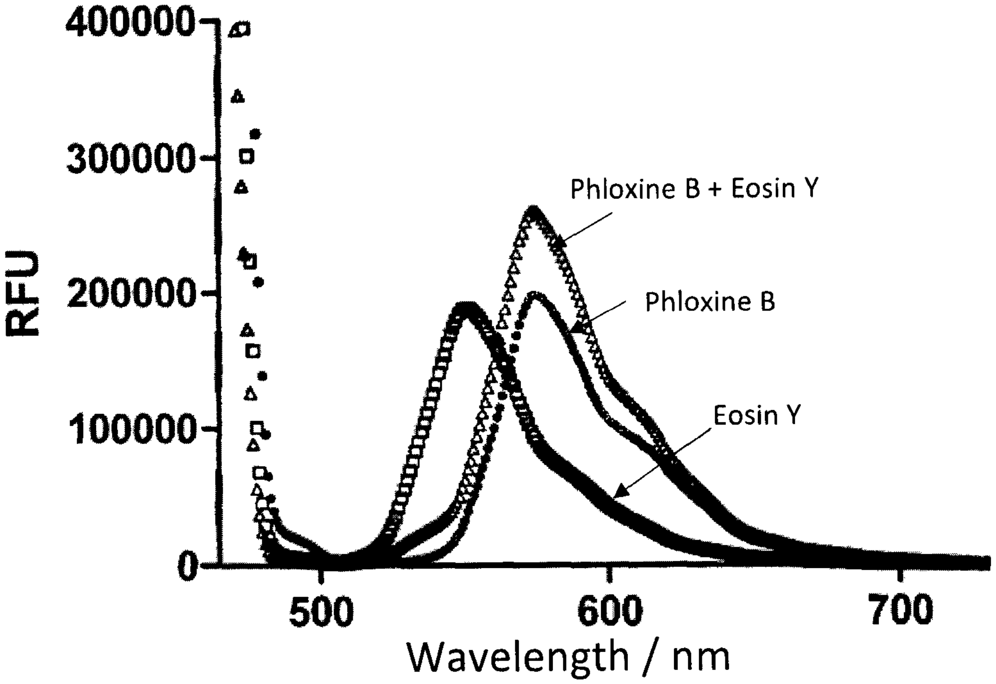

Figure 7B

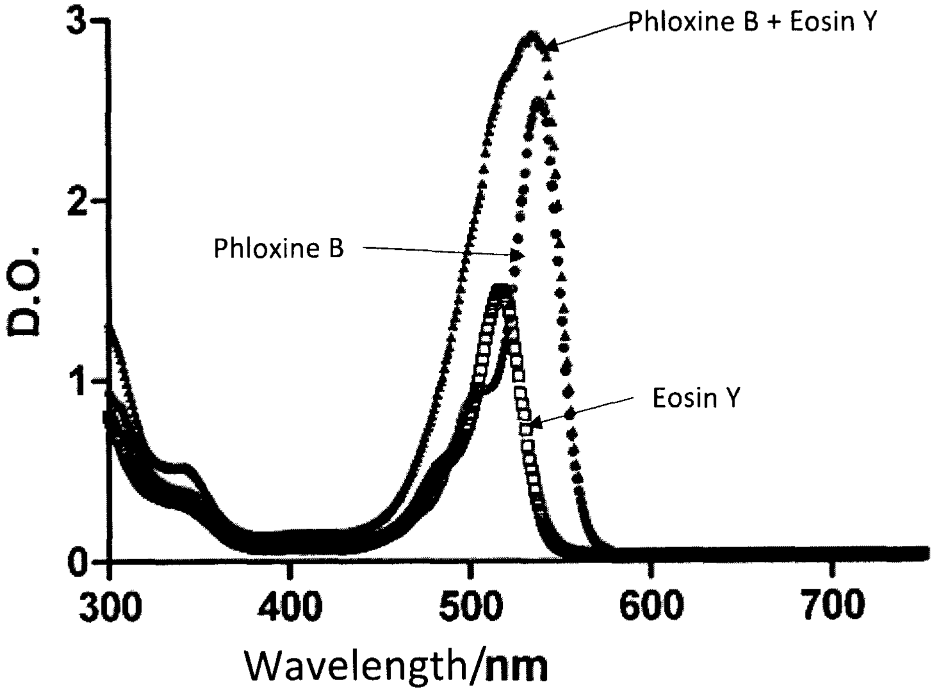

Figure 8A

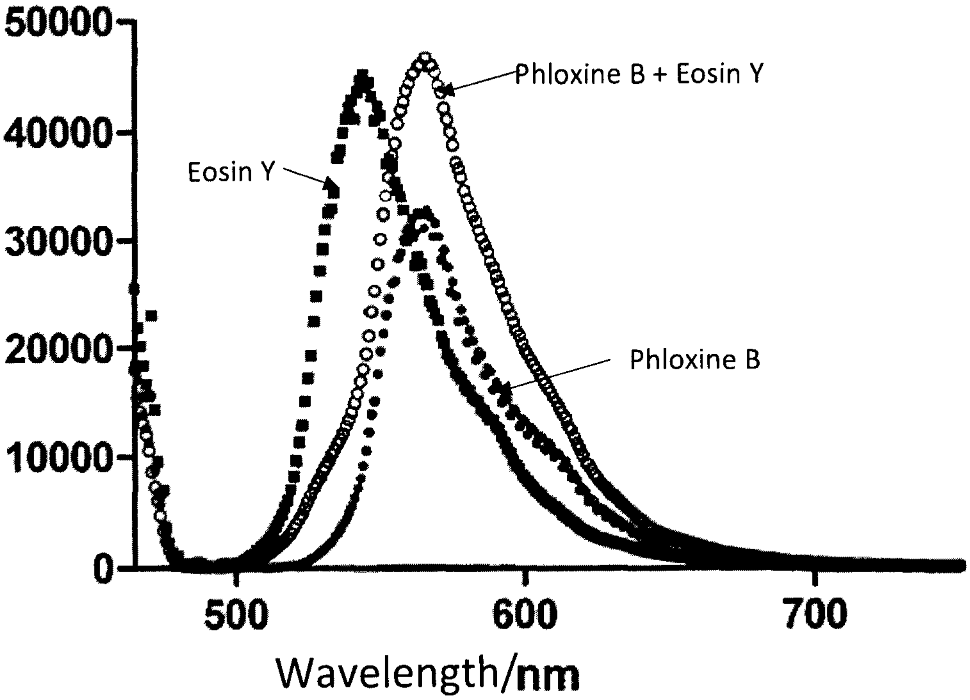

Figure 8B

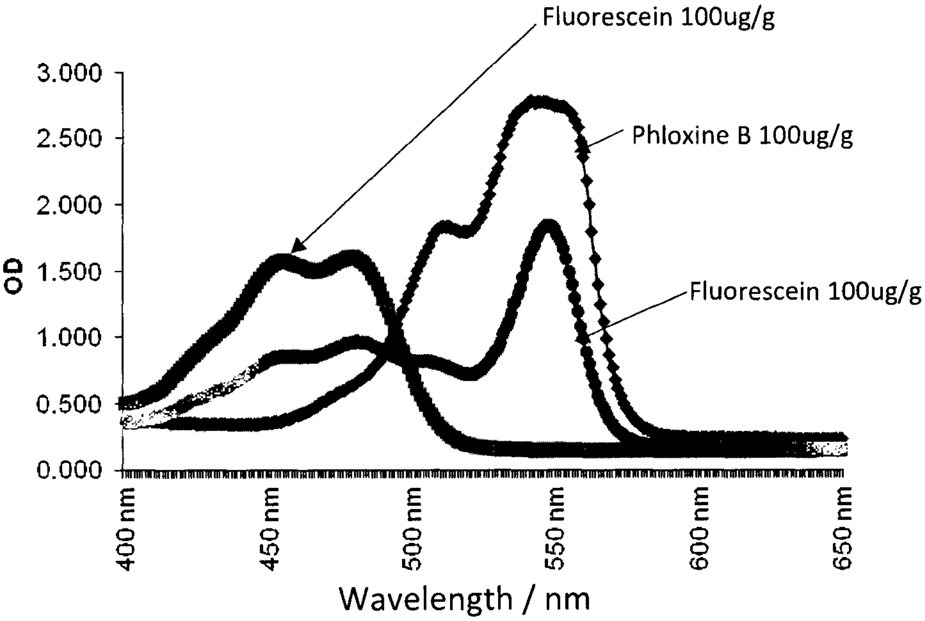

Figure 10A

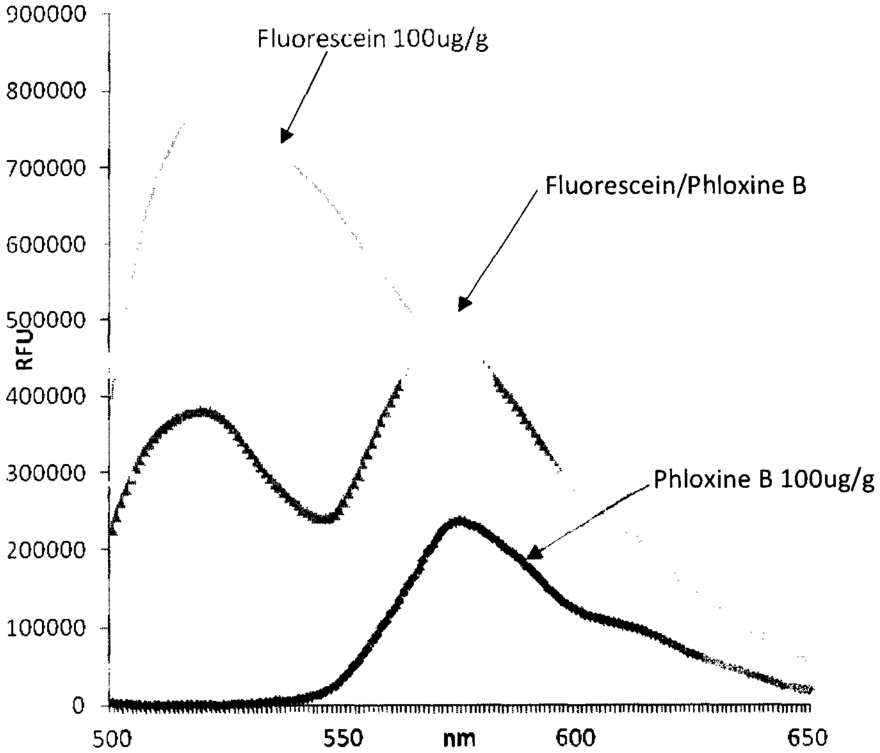

Figure 10B

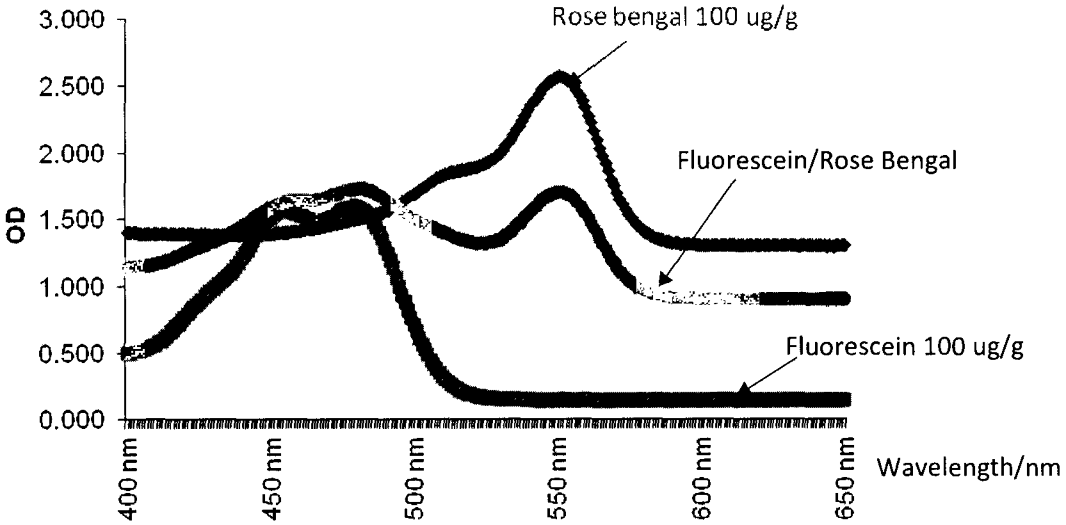

Figure 10A

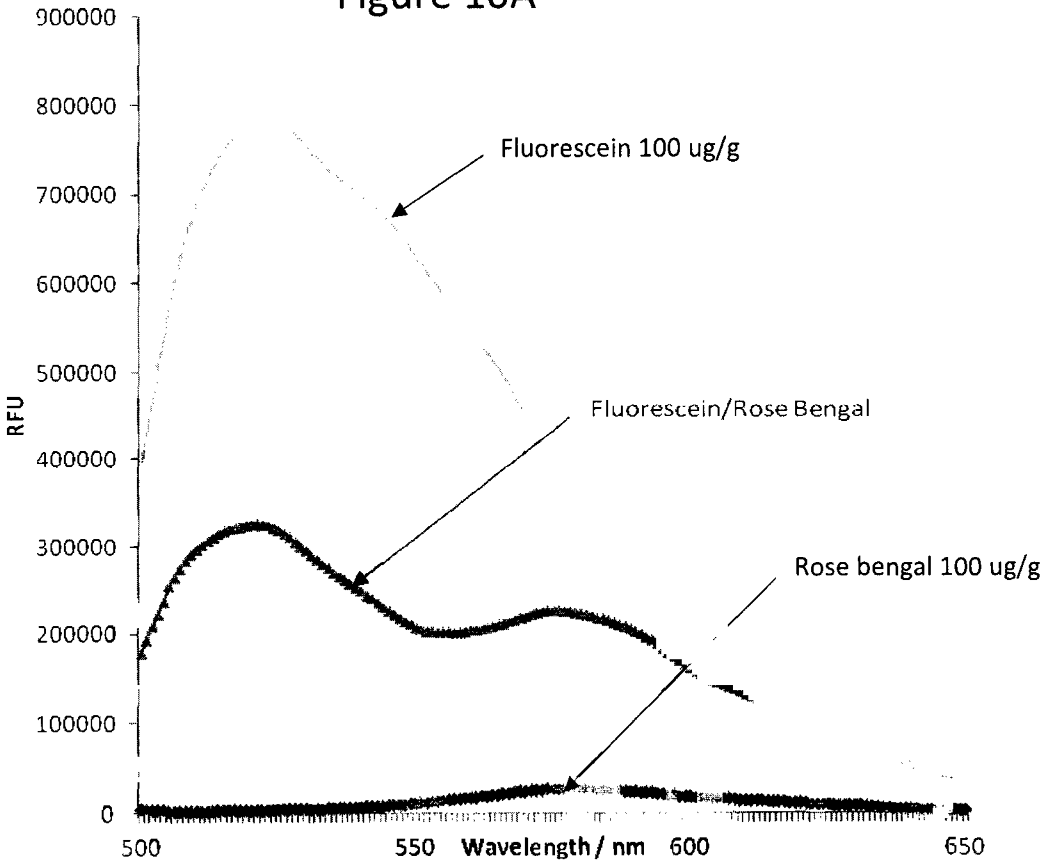

Figure 10B

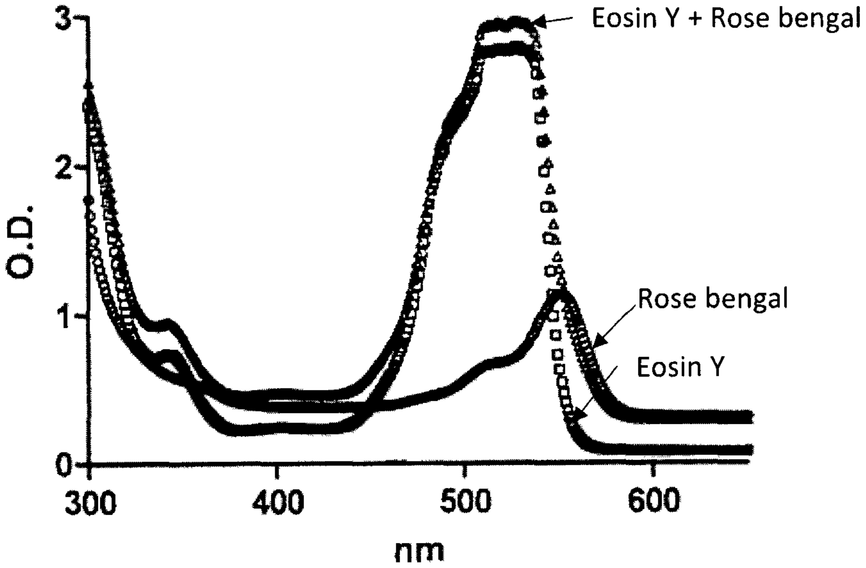

Figure 11A

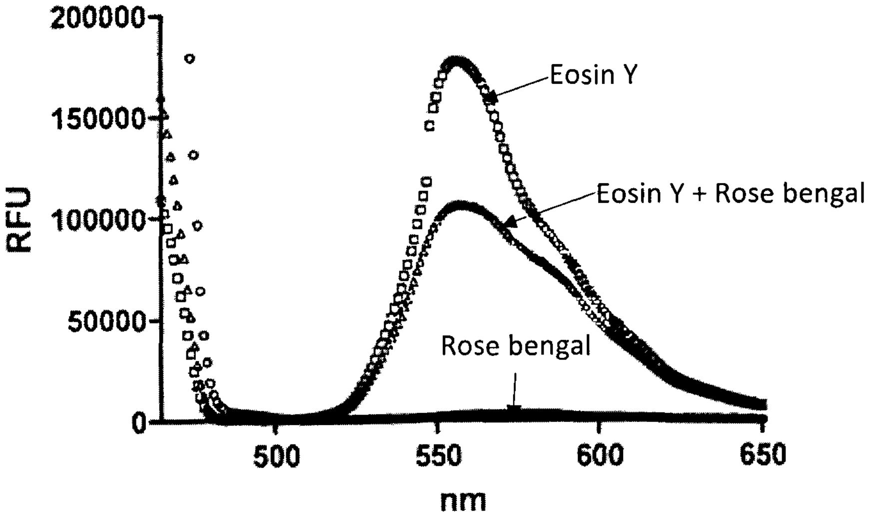

Figure 11B

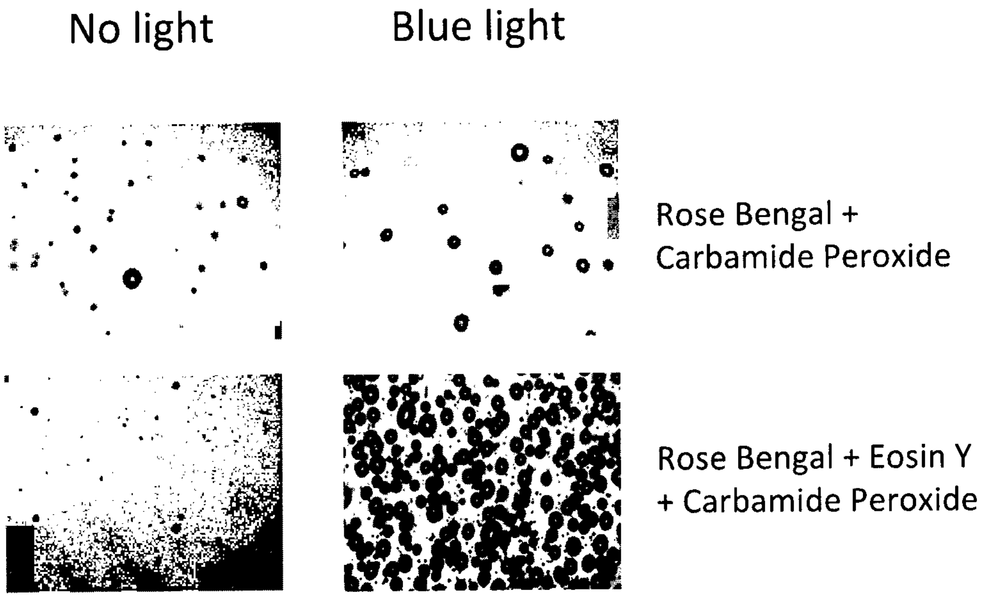
No light
Blue light
Rose Bengal + Carbamide Peroxide
Rose Bengal + Eosin Y + Carbamide Peroxide

Figure 12

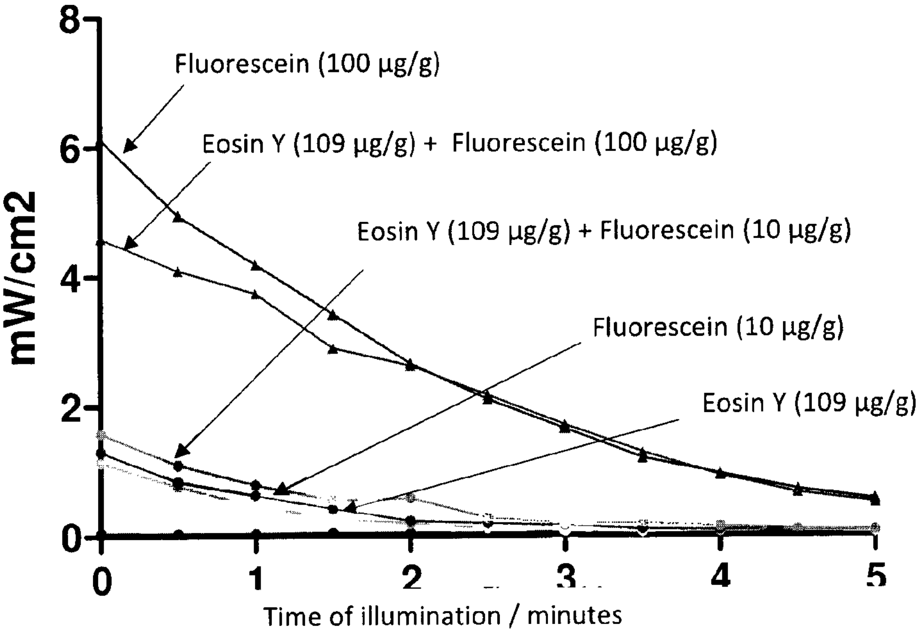

Figure 13A

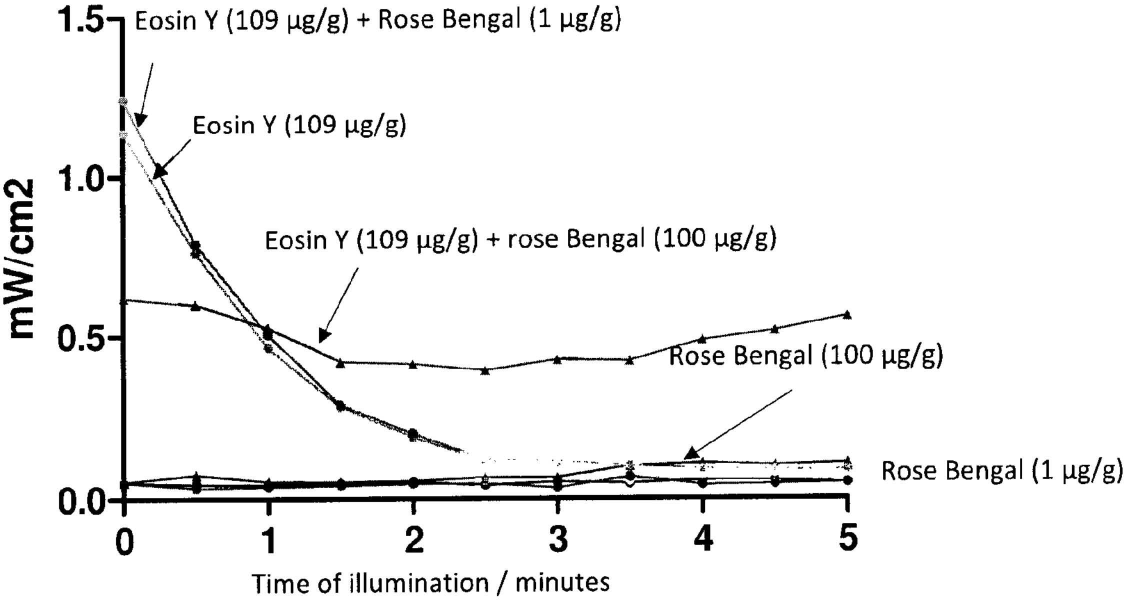

Figure 13B

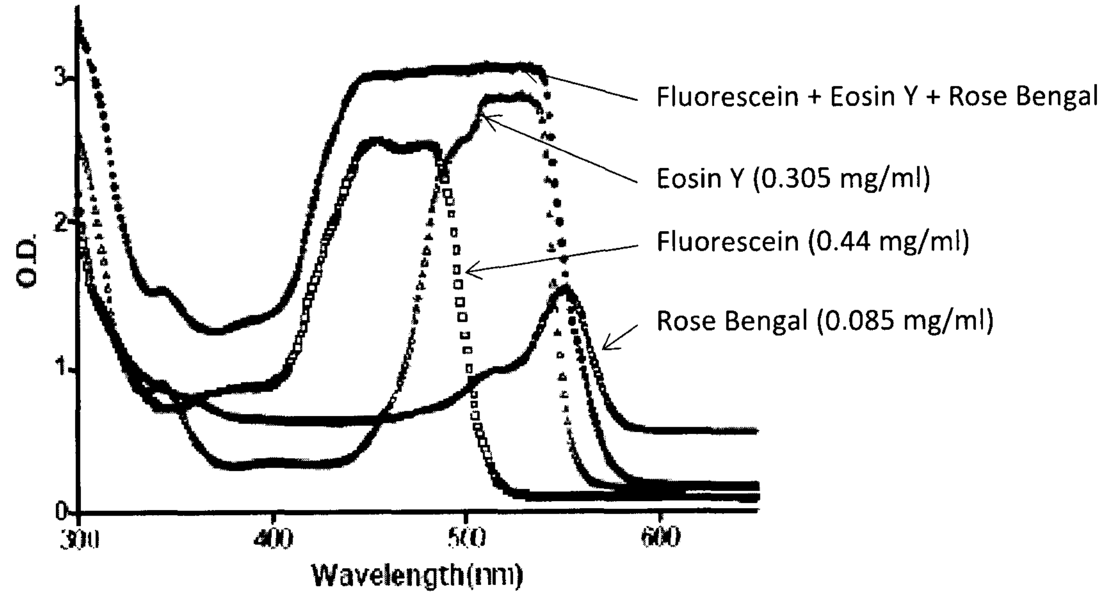

Figure 14A

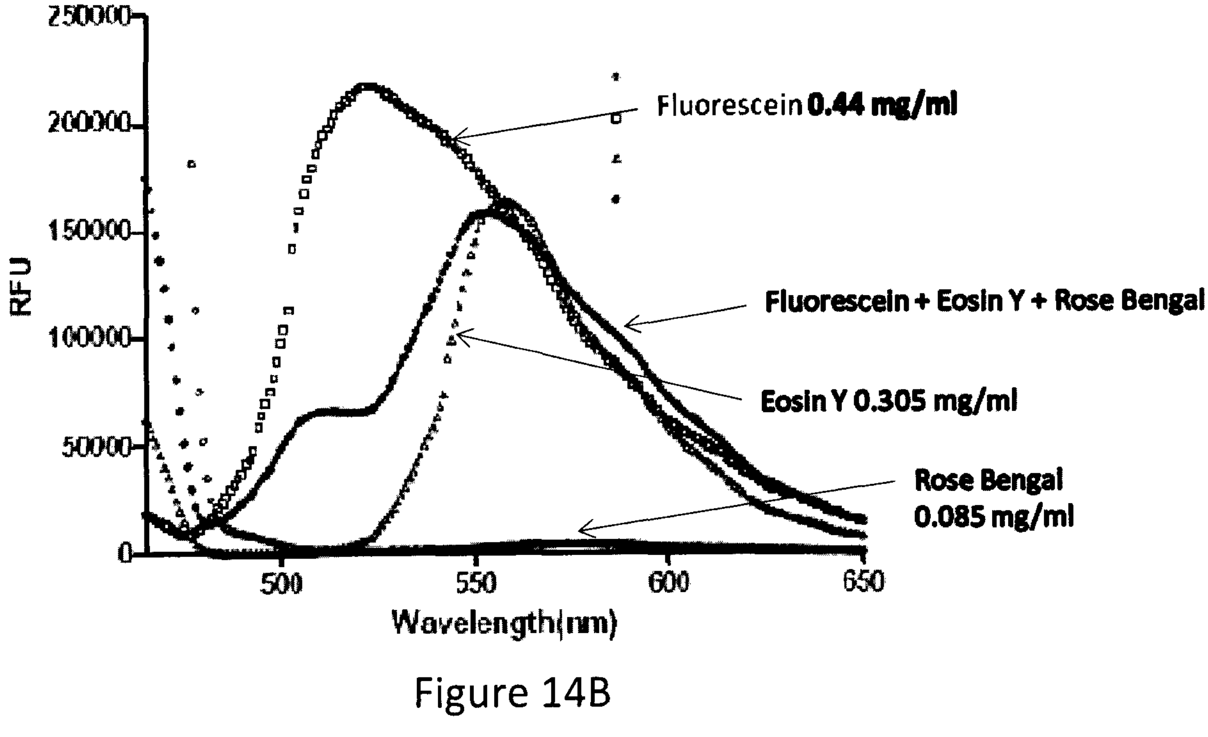

Figure 14B

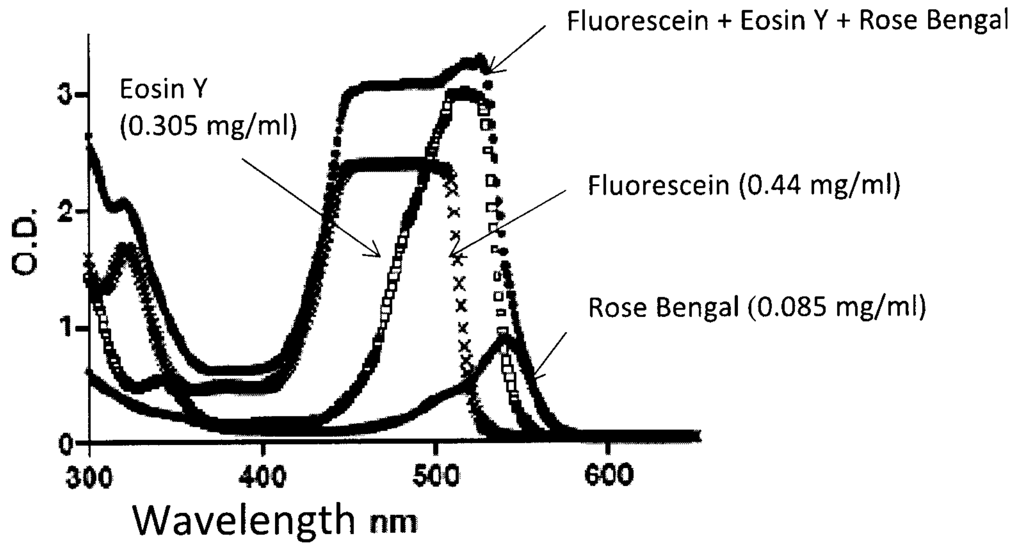

Figure 15A

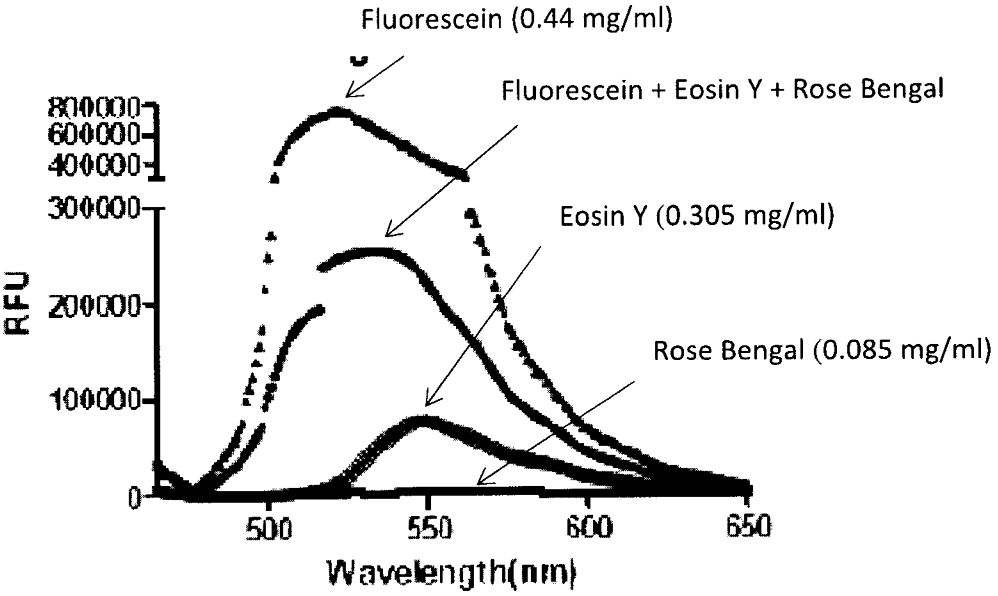

Figure 15B

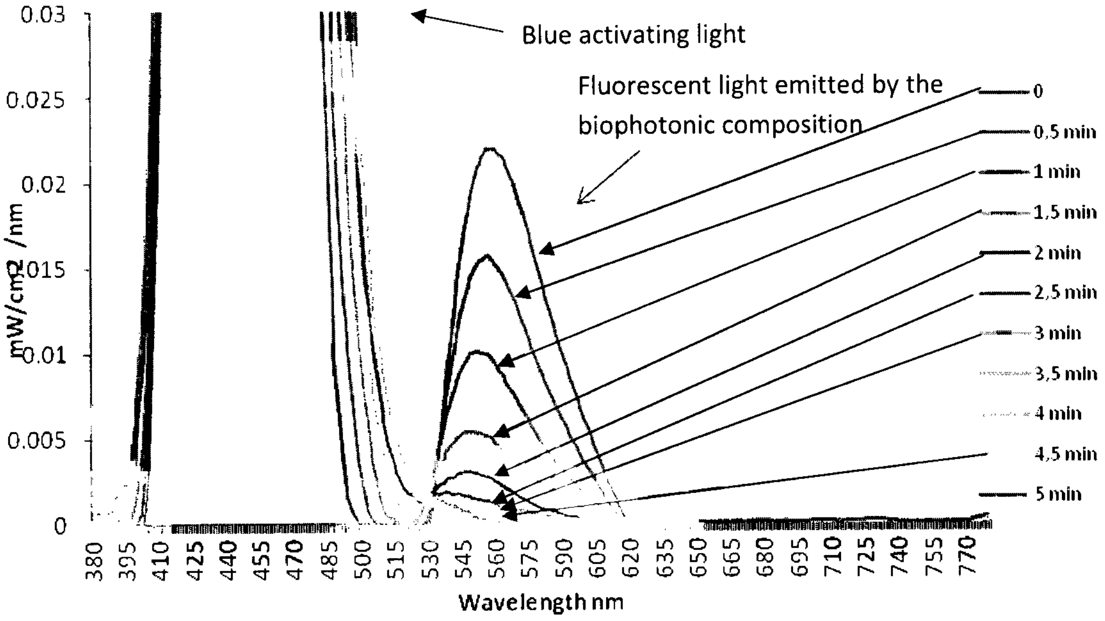
0.03
0.025
0.02
0.015
0.01
0.005
0
mW/cm2 /nm
380
395
410
425
440
455
470
485
500
515
530
545
560
575
590
605
620
635
650
665
680
695
710
725
740
755
770
Wavelength nm
Blue activating light
Fluorescent light emitted by the biophotonic composition
0
0,5 min
1 min
1,5 min
2 min
2,5 min
3 min
3,5 min
4 min
4,5 min
5 min

Figure 16

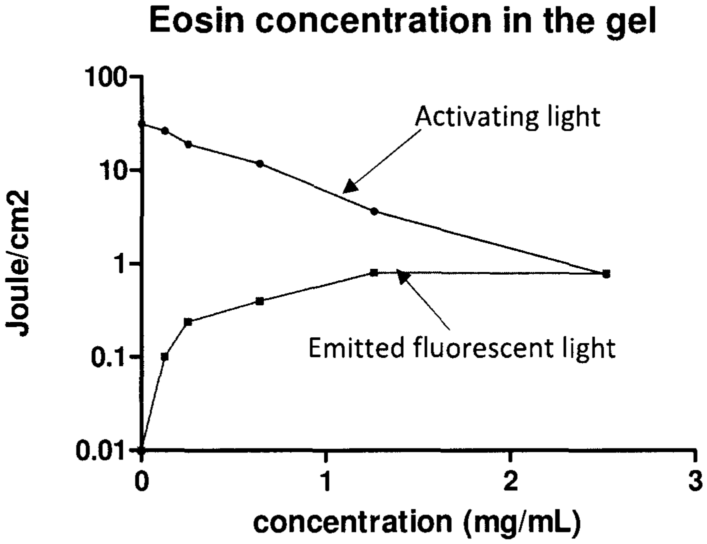

Figure 17A

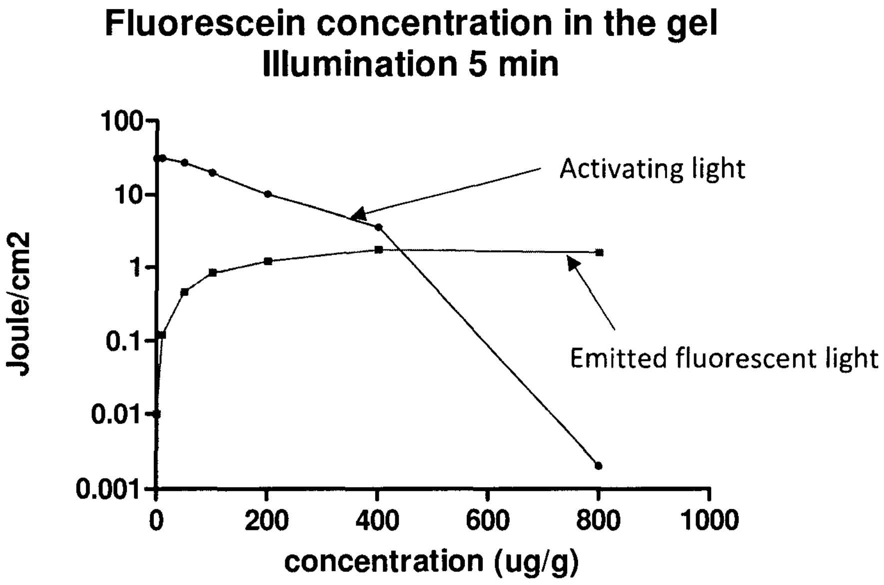

Figure 17B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2013/000786

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***A61K 41/00*** (2006.01) , ***A61P 17/00*** (2006.01) , ***A61P 17/02*** (2006.01) , *C09B 11/28* (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC: ***A61K 41/00*** (2006.01) , ***A61P 17/00*** (2006.01) , ***A61P 17/02*** (2006.01) , *C09B 11/**28*** (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent database, United States Patent database, EPOQUE (EPODOC), PubMed, Scopus, Google (Keywords: photodynamic, xanthene, Eosin Y, Fluorescein, Rose Bengal and related terms)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO2013155620 (LOUPIS et al.) 24 October 2013 (24-10-2013) (see claims 40-45, 59-64, 66-80 and 84)	1-138
A	WO0112181 (DEES et al.) 22 February 2001 (22-02-2001) (see entire document)	1-138
A	WO2010051636 (PIERGALLINI et al.) 14 May 2010 (14-05-2010) (see entire document)	1-138
A	US20050059731 (ALBRECHT et al.) 17 March 2005 (17-03-2005)	1-138

[ ] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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

Date of the actual completion of the international search 07 January 2014 (07-01-2014)	Date of mailing of the international search report 08 January 2014 (08-01-2014)
Name and mailing address of the ISA/CA Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Authorized officer Wesley Sharman (819) 934-2326

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

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

1. [X] Claim Nos. : 18-44, 57-72 and 138
because they relate to subject matter not required to be searched by this Authority, namely :

Although claims 18-44, 57-72 and 138 are directed to methods of medical treatment of the human or animal body (Rule 39.1(iv) of the PCT), a search has been carried out on the alleged effects obtained using a biophotonic composition comprising a first and second xanthene dye in photodynamic therapy.

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

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

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

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

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

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

Remark on Protest [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

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

[ ] No protest accompanied the payment of additional search fees.

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2013155620A1	24 October 2013 (24-10-2013)	GB201307157D0	29 May 2013 (29-05-2013)
		GB2499921A	04 September 2013 (04-09-2013)
		US2013281913A1	24 October 2013 (24-10-2013)
WO0112181A1	22 February 2001 (22-02-2001)	AR018674A1	28 November 2001 (28-11-2001)
		AR020337A1	08 May 2002 (08-05-2002)
		AR021061A1	12 June 2002 (12-06-2002)
		AR021750A1	07 August 2002 (07-08-2002)
		AR021967A1	04 September 2002 (04-09-2002)
		AR025223A1	13 November 2002 (13-11-2002)
		AR027630A1	02 April 2003 (02-04-2003)
		AR028285A1	30 April 2003 (30-04-2003)
		AR028321A1	07 May 2003 (07-05-2003)
		AR029721A1	10 July 2003 (10-07-2003)
		AT357912T	15 April 2007 (15-04-2007)
		AT384522T	15 February 2008 (15-02-2008)
		AT397408T	15 June 2008 (15-06-2008)
		AT406157T	15 September 2008 (15-09-2008)
		AT472325T	15 July 2010 (15-07-2010)
		AT529132T	15 November 2011 (15-11-2011)
		AU716504B2	24 February 2000 (24-02-2000)
		AU716507B2	24 February 2000 (24-02-2000)
		AU750633B2	25 July 2002 (25-07-2002)
		AU760418B2	15 May 2003 (15-05-2003)
		AU1452100A	22 May 2000 (22-05-2000)
		AU2368700A	12 July 2000 (12-07-2000)
		AU4346801A	17 September 2001 (17-09-2001)
		AU4410099A	30 December 1999 (30-12-1999)
		AU4761501A	08 October 2001 (08-10-2001)
		AU5152898A	22 May 1998 (22-05-1998)
		AU5312501A	23 October 2001 (23-10-2001)
		AU5391799A	28 February 2000 (28-02-2000)
		AU5425498A	22 May 1998 (22-05-1998)
		AU5460799A	28 February 2000 (28-02-2000)
		AU7057200A	13 March 2001 (13-03-2001)
		AU7686001A	30 January 2002 (30-01-2002)
		AU2003296371A1	30 June 2004 (30-06-2004)
		AU2008255204A1	25 June 2009 (25-06-2009)
		AU2008255204B2	11 July 2013 (11-07-2013)
		BR0013222A	09 July 2002 (09-07-2002)
		BR0109446A	24 June 2003 (24-06-2003)
		BR9712599A	21 December 1999 (21-12-1999)
		BR9713979A	02 May 2000 (02-05-2000)
		BR9911161A	24 April 2001 (24-04-2001)
		BR9912773A	16 October 2001 (16-10-2001)
		BR9912776A	08 May 2001 (08-05-2001)
		BR9914969A	10 July 2001 (10-07-2001)
		BR9916398A	11 September 2001 (11-09-2001)
		CA2252782 A1	07 May 1998 (07-05-1998)
		CA2252783A1	07 May 1998 (07-05-1998)
		CA2320795A1	11 November 1999 (11-11-1999)
		CA2333175A1	16 December 1999 (16-12-1999)
		CA2339252A1	17 February 2000 (17-02-2000)
		CA2339252C	12 February 2008 (12-02-2008)
		CA2339384A1	17 February 2000 (17-02-2000)
		CA2339384C	08 July 2008 (08-07-2008)
		CA2348094A1	11 May 2000 (11-05-2000)
		CA2352094A1	29 June 2000 (29-06-2000)
		CA2352094C	07 December 2010 (07-12-2010)
		CA2380805A1	22 February 2001 (22-02-2001)
		CA2380805C	13 April 2010 (13-04-2010)
		CA2402187A1	04 October 2001 (04-10-2001)
		CA2405554A1	18 October 2001 (18-10-2001)
		CA2415280A1	24 January 2002 (24-01-2002)
		CA2415280C	29 June 2010 (29-06-2010)
		CA2505975A1	24 June 2004 (24-06-2004)

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO0112181A1	22 February 2001 (22-02-2001)	CN1317952A	17 October 2001 (17-10-2001)
		CN1132557C	31 December 2003 (31-12-2003)
		CN1226147A	18 August 1999 (18-08-1999)
		CN1226148A	18 August 1999 (18-08-1999)
		CN1310596A	29 August 2001 (29-08-2001)
		CN1317951A	17 October 2001 (17-10-2001)
		CN1325284A	05 December 2001 (05-12-2001)
		CN100382846C	23 April 2008 (23-04-2008)
		CN1331797A	16 January 2002 (16-01-2002)
		CN1372467A	02 October 2002 (02-10-2002)
		CN1423557A	11 June 2003 (11-06-2003)
		CN101926789A	29 December 2010 (29-12-2010)
		DE60040079D1	09 October 2008 (09-10-2008)
		DE60127552D1	10 May 2007 (10-05-2007)
		DE60127552T2	13 December 2007 (13-12-2007)
		DE60132600D1	13 March 2008 (13-03-2008)
		DE60132600T2	29 January 2009 (29-01-2009)
		DE60142473D1	12 August 2010 (12-08-2010)
		DE69938870D1	17 July 2008 (17-07-2008)
		EP0977592A1	09 February 2000 (09-02-2000)
		EP0977592A4	10 January 2001 (10-01-2001)
		EP1032321A1	06 September 2000 (06-09-2000)
		EP1032321A4	06 September 2000 (06-09-2000)
		EP1076578A1	21 February 2001 (21-02-2001)
		EP1087717A1	04 April 2001 (04-04-2001)
		EP1087717A4	28 May 2003 (28-05-2003)
		EP1100394A1	23 May 2001 (23-05-2001)
		EP1100394A4	16 March 2005 (16-03-2005)
		EP1109506A1	27 June 2001 (27-06-2001)
		EP1109506A4	09 September 2009 (09-09-2009)
		EP1126782A1	29 August 2001 (29-08-2001)
		EP1126782A4	04 June 2003 (04-06-2003)
		EP1126782B1	04 June 2008 (04-06-2008)
		EP1192450A1	03 April 2002 (03-04-2002)
		EP1192450A4	21 May 2003 (21-05-2003)
		EP1210078A1	05 June 2002 (05-06-2002)
		EP1210078A4	09 June 2004 (09-06-2004)
		EP1210078B1	27 August 2008 (27-08-2008)
		EP1265546A1	18 December 2002 (18-12-2002)
		EP1265546A4	09 August 2006 (09-08-2006)
		EP1284727A1	26 February 2003 (26-02-2003)
		EP1284727A4	02 June 2004 (02-06-2004)
		EP1284727B1	30 June 2010 (30-06-2010)
		EP1292298A1	19 March 2003 (19-03-2003)
		EP1292298A4	25 June 2003 (25-06-2003)
		EP1292298B1	28 March 2007 (28-03-2007)
		EP1311261A1	21 May 2003 (21-05-2003)
		EP1311261A4	02 June 2004 (02-06-2004)
		EP1311261B1	23 January 2008 (23-01-2008)
		EP1578454A1	28 September 2005 (28-09-2005)
		EP2020242A2	04 February 2009 (04-02-2009)
		EP2020242A3	11 February 2009 (11-02-2009)
		EP2020242B1	19 October 2011 (19-10-2011)
		ES2283406T3	01 November 2007 (01-11-2007)
		ES2300344T3	16 June 2008 (16-06-2008)
		ES2307344T3	16 November 2008 (16-11-2008)
		ES2310521T3	16 January 2009 (16-01-2009)
		ES2346289T3	14 October 2010 (14-10-2010)
		ES2371948T3	11 January 2012 (11-01-2012)
		HK1039555A1	07 May 2004 (07-05-2004)
		HK1040899A1	24 December 2008 (24-12-2008)
		IL128355D0	31 January 2000 (31-01-2000)
		IL128355A	10 November 2002 (10-11-2002)
		IL128356D0	31 January 2000 (31-01-2000)
		IL128356A	25 November 2001 (25-11-2001)
		IL139915D0	10 February 2002 (10-02-2002)
		IL141243A	05 July 2006 (05-07-2006)
		IL141243D0	10 March 2002 (10-03-2002)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/CA2013/000786

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO0112181A1	22 February 2001 (22-02-2001)	IL141273D0	10 March 2002 (10-03-2002)
		IL142729D0	10 March 2002 (10-03-2002)
		IL143468D0	21 April 2002 (21-04-2002)
		IL148139D0	12 September 2002 (12-09-2002)
		IL151456D0	10 April 2003 (10-04-2003)
		IL151689D0	10 April 2003 (10-04-2003)
		IN185849A1	12 May 2001 (12-05-2001)
		IN186854A1	24 November 2001 (24-11-2001)
		IS5006A	19 March 1999 (19-03-1999)
		IS5007A	19 March 1999 (19-03-1999)
		JP2002533355A	08 October 2002 (08-10-2002)
		JP3735770B2	18 January 2006 (18-01-2006)
		JP2002522110A	23 July 2002 (23-07-2002)
		JP4662631B2	30 March 2011 (30-03-2011)
		JP2004503592A	05 February 2004 (05-02-2004)
		JP4989837B2	01 August 2012 (01-08-2012)
		JP2000511929A	12 September 2000 (12-09-2000)
		JP2001503748A	21 March 2001 (21-03-2001)
		JP2002513650A	14 May 2002 (14-05-2002)
		JP2002517419A	18 June 2002 (18-06-2002)
		JP2002522111A	23 July 2002 (23-07-2002)
		JP2002528472A	03 September 2002 (03-09-2002)
		JP2003506488A	18 February 2003 (18-02-2003)
		JP2003526091A	02 September 2003 (02-09-2003)
		JP2003528143A	24 September 2003 (24-09-2003)
		JP2003531834A	28 October 2003 (28-10-2003)
		JP2006510677A	30 March 2006 (30-03-2006)
		KR20000035893A	26 June 2000 (26-06-2000)
		KR20000035894A	26 June 2000 (26-06-2000)
		KR20030019329A	06 March 2003 (06-03-2003)
		MXPA01001336A	24 April 2002 (24-04-2002)
		MXPA02009329A	12 February 2003 (12-02-2003)
		MXPA02009881A	19 June 2003 (19-06-2003)
		MXPA03000353A	29 January 2004 (29-01-2004)
		MXPA05005460A	18 October 2005 (18-10-2005)
		NO991492D0	26 March 1999 (26-03-1999)
		NO991492A	31 May 1999 (31-05-1999)
		NO991493D0	26 March 1999 (26-03-1999)
		NO991493A	27 May 1999 (27-05-1999)
		NZ334190A	27 October 2000 (27-10-2000)
		NZ334191A	28 January 2000 (28-01-2000)
		TW396030B	01 July 2000 (01-07-2000)
		TW404845B	11 September 2000 (11-09-2000)
		TW515707B	01 January 2003 (01-01-2003)
		TWI231368B	21 April 2005 (21-04-2005)
		TWI271191B	21 January 2007 (21-01-2007)
		US5829448A	03 November 1998 (03-11-1998)
		US5832931A	10 November 1998 (10-11-1998)
		US5998597A	07 December 1999 (07-12-1999)
		US6042603A	28 March 2000 (28-03-2000)
		US6096036A	01 August 2000 (01-08-2000)
		US6270496B1	07 August 2001 (07-08-2001)
		US6331286B1	18 December 2001 (18-12-2001)
		US6493570B1	10 December 2002 (10-12-2002)
		US2002122236A1	05 September 2002 (05-09-2002)
		US6519076B2	11 February 2003 (11-02-2003)
		US2002033989A1	21 March 2002 (21-03-2002)
		US6525862B2	25 February 2003 (25-02-2003)
		US2001039413A1	08 November 2001 (08-11-2001)
		US6592581B2	15 July 2003 (15-07-2003)
		US2003167006A1	04 September 2003 (04-09-2003)
		US6986740B2	17 January 2006 (17-01-2006)
		US2003125376A1	03 July 2003 (03-07-2003)
		US6991776B2	31 January 2006 (31-01-2006)
		US7036516B1	02 May 2006 (02-05-2006)
		US7346387B1	18 March 2008 (18-03-2008)
		US7353829B1	08 April 2008 (08-04-2008)
		US7384623B1	10 June 2008 (10-06-2008)
		US2001022970A1	20 September 2001 (20-09-2001)

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO0112181A1	22 February 2001 (22-02-2001)	US7353829B1	08 April 2008 (08-04-2008)
		US7384623B1	10 June 2008 (10-06-2008)
		US2001022970A1	20 September 2001 (20-09-2001)
		US7390668B2	24 June 2008 (24-06-2008)
		US2005154049A1	14 July 2005 (14-07-2005)
		US7402299B2	22 July 2008 (22-07-2008)
		US2002161035A1	31 October 2002 (31-10-2002)
		US7648695B2	19 January 2010 (19-01-2010)
		US2008207746A1	28 August 2008 (28-08-2008)
		US7863047B2	04 January 2011 (04-01-2011)
		US2007208076A1	06 September 2007 (06-09-2007)
		US8470296B2	25 June 2013 (25-06-2013)
		US2008118567A1	22 May 2008 (22-05-2008)
		US8557298B2	15 October 2013 (15-10-2013)
		US2002001567A1	03 January 2002 (03-01-2002)
		US2003133940A1	17 July 2003 (17-07-2003)
		US2005019264A1	27 January 2005 (27-01-2005)
		US2005207976A1	22 September 2005 (22-09-2005)
		US2005282889A1	22 December 2005 (22-12-2005)
		US2006095097A1	04 May 2006 (04-05-2006)
		US2006095101A1	04 May 2006 (04-05-2006)
		US2006199859A1	07 September 2006 (07-09-2006)
		US2007010575A1	11 January 2007 (11-01-2007)
		US2007078076A1	05 April 2007 (05-04-2007)
		US2008118578A1	22 May 2008 (22-05-2008)
		US2009117199A1	07 May 2009 (07-05-2009)
		US2010076246A1	25 March 2010 (25-03-2010)
		US2011097280A1	28 April 2011 (28-04-2011)
		WO0007514A1	17 February 2000 (17-02-2000)
		WO0007514A9	19 April 2001 (19-04-2001)
		WO0007515A1	17 February 2000 (17-02-2000)
		WO0025665A1	11 May 2000 (11-05-2000)
		WO0037927A1	29 June 2000 (29-06-2000)
		WO0166030A1	13 September 2001 (13-09-2001)
		WO0172301A1	04 October 2001 (04-10-2001)
		WO0176595A1	18 October 2001 (18-10-2001)
		WO0205812A1	24 January 2002 (24-01-2002)
		WO9818398A1	07 May 1998 (07-05-1998)
		WO9818399A1	07 May 1998 (07-05-1998)
		WO9956810A1	11 November 1999 (11-11-1999)
		WO9963900A1	16 December 1999 (16-12-1999)
		WO2004052407A1	24 June 2004 (24-06-2004)
WO2010051636A1	14 May 2010 (14-05-2010)	AU2009311234A1	14 May 2010 (14-05-2010)
		AU2009311234B2	02 May 2013 (02-05-2013)
		AU2009311239A1	14 May 2010 (14-05-2010)
		AU2009311239B2	15 November 2012 (15-11-2012)
		CA2742942A1	14 May 2010 (14-05-2010)
		CA2742943A1	14 May 2010 (14-05-2010)
		CN102256591A	23 November 2011 (23-11-2011)
		CN102300587A	28 December 2011 (28-12-2011)
		EA201170650A1	30 December 2011 (30-12-2011)
		EA201170651A1	30 December 2011 (30-12-2011)
		EP2352488A1	10 August 2011 (10-08-2011)
		EP2352488A4	25 April 2012 (25-04-2012)
		EP2365829A1	21 September 2011 (21-09-2011)
		EP2365829A4	19 October 2011 (19-10-2011)
		IL212559D0	30 June 2011 (30-06-2011)
		IL212560D0	30 June 2011 (30-06-2011)
		JP2012508189A	05 April 2012 (05-04-2012)
		JP2012508190A	05 April 2012 (05-04-2012)
		KR20110090983A	10 August 2011 (10-08-2011)
		KR20110094017A	19 August 2011 (19-08-2011)
		MX2011004865A	17 October 2011 (17-10-2011)
		MX2011004868A	17 October 2011 (17-10-2011)
		NZ592650A	31 May 2013 (31-05-2013)
		NZ592651A	31 May 2013 (31-05-2013)
		US2011218482A1	08 September 2011 (08-09-2011)

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2010051636A1	14 May 2010 (14-05-2010)	NZ592651A	31 May 2013 (31-05-2013)
		US2011218482A1	08 September 2011 (08-09-2011)
		US2011224599A1	15 September 2011 (15-09-2011)
		US2013165845A1	27 June 2013 (27-06-2013)
		WO2010051641A1	14 May 2010 (14-05-2010)
US2005059731A1	17 March 2005 (17-03-2005)	BRPI0414331A	07 November 2006 (07-11-2006)
		CN101304766A	12 November 2008 (12-11-2008)
		EP1778294A2	02 May 2007 (02-05-2007)
		EP1778294A4	05 May 2010 (05-05-2010)
		JP2007509034A	12 April 2007 (12-04-2007)
		JP4943149B2	30 May 2012 (30-05-2012)
		MXPA06003033A	14 December 2006 (14-12-2006)
		RU2006112562A	10 August 2006 (10-08-2006)
		RU2368375C2	27 September 2009 (27-09-2009)
		WO2005032459A2	14 April 2005 (14-04-2005)
		WO2005032459A3	12 October 2006 (12-10-2006)

(19)中华人民共和国国家知识产权局

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PCT/CA2013/000395 2013.04.19 CA
1307157.6 2013.04.19 GB
61/701,513 2012.09.14 US
61/701,510 2012.09.14 US
61/701,502 2012.09.14 US
61/766,611 2013.02.19 US
13/830,488 2013.03.14 US
61/873,791 2013.09.04 US

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WO2014/040176 EN 2014.03.20

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(54)**发明名称**

生物光子用生色团组合

(57)**摘要**

本发明提供了可应用于光治疗的生物光子组合物和方法。尤其是，本发明的生物光子组合物包含至少两种呫吨染料。本发明的生物光子组合物和方法用于促进伤口愈合、皮肤更新及治疗痤疮和其它皮肤病。

1.一种局部施用于靶向组织的生物光子组合物，所述组合物包含第一呫吨染料和第二呫吨染料，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱重叠至少1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％、35-45％、50-60％、55-65％或60-70％，其中组合物中第一呫吨染料和第二呫吨染料的含量占所述组合物重量的大约0.001-0.5％。

2.一种局部施用于靶向组织的生物光子组合物，所述组合物包含曙红Y和荧光素。

3.一种局部施用于靶向组织的生物光子组合物，所述组合物包含曙红Y和玫瑰红。

4.一种局部施用于靶向组织的生物光子组合物，所述组合物包含荧光素和玫瑰红。

5.根据权利要求2所述的生物光子组合物，进一步包含第三呫吨染料玫瑰红。

6.根据权利要求1至5任一项所述的生物光子组合物，其中光活化导致第一呫吨染料和第二呫吨染料之间发生能量转移级联。

7.根据权利要求6所述的生物光子组合物，其中能量转移在使用时提供能穿入靶向皮肤组织位点处表皮与/或真皮内的光子。

8.根据权利要求6或7所述的生物光子组合物，其中所述能量转移并不同时伴随热量的产生。

9.根据权利要求6至8任一项所述的生物光子组合物，其中所述能量转移并不造成组织损伤。

10.根据权利要求1至9任一项所述的生物光子组合物，其中第一呫吨染料在可见光谱范围的波长处吸收。

11.根据权利要求1至10任一项所述的生物光子组合物，其中第二呫吨染料在可见光谱范围的波长处吸收。

12.根据权利要求1至11任一项所述的生物光子组合物，其中第一呫吨染料在波长400-500nm处吸收。

13.根据权利要求1至12任一项所述的生物光子组合物，其中第二呫吨染料在比第一呫吨染料相对长10-100nm的波长处吸收。

14.根据权利要求1至13任一项所述的生物光子组合物，进一步包含氧化剂。

15.根据权利要求14所述的生物光子组合物，其中至少第一或第二呫吨染料在接受光照时经历光漂白。

16.根据权利要求1至15任一项所述的生物光子组合物，其中组合物中第一呫吨染料和第二呫吨染料的含量占所述组合物重量的大约0.001-0.1％或0.001-0.01％。

17.根据权利要求1至16任一项所述的生物光子组合物，其中所述生物光子组合物促进皮肤病痊愈或伤口愈合。

18.一种用生物光子疗法治疗皮肤病的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是荧光素。

19.一种用生物光子疗法治疗皮肤病的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨

染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是玫瑰红。

20.一种用生物光子疗法治疗皮肤病的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是荧光素，第二呫吨染料是玫瑰红。

21.一种用生物光子疗法治疗伤口的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是荧光素。

22.一种用生物光子疗法治疗伤口的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是玫瑰红。

23.一种用生物光子疗法治疗伤口的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是荧光素，第二呫吨染料是玫瑰红。

24.一种促进伤口愈合的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是荧光素。

25.一种促进伤口愈合的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红Y，第二呫吨染料是玫瑰红。

26.一种促进伤口愈合的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；

-用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是荧光素，第二呫吨染料是玫瑰红。

27.一种用于促进皮肤更新的方法，包括：

－将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；及

－用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红 Y，第二呫吨染料是荧光素。

28. 一种用于促进皮肤更新的方法，包括：

－将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；及

－用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是曙红 Y，第二呫吨染料是玫瑰红。

29. 一种用于促进皮肤更新的方法，包括：

－将生物光子组合物局部施用于靶向组织，所述组合物包含第一呫吨染料和第二呫吨染料；及

－用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物，其中第一呫吨染料是荧光素，第二呫吨染料是玫瑰红。

30. 根据权利要求 26-28 任一项所述的方法，其中所述生物光子组合物促进胶原蛋白的合成。

31. 根据权利要求 18-20 任一项所述的方法，其中所述生物光子组合物促进皮肤病的痊愈。

32. 根据权利要求 18-20 任一项所述的方法，其中所述生物光子组合物能减缓炎症。

33. 根据权利要求 18-26 任一项所述的方法，其中所述生物光子组合物能减少疤痕组织的形成。

34. 根据权利要求 18、21、24 和 27 任一项所述的方法，进一步包含第三呫吨染料玫瑰红。

35. 根据权利要求 18-34 任一项所述的方法，其中所述生物光子组合物被照射至少 5 分钟。

36. 根据权利要求 18-35 任一项所述的方法，其中所述生物光子组合物被照射至少 3 分钟。

37. 根据权利要求 18-36 任一项所述的方法，其中所述生物光子组合物在经过光照之后从靶向组织清除。

38. 根据权利要求 18-37 任一项所述的方法，其中第一与/或第二呫吨染料在接受光照时经历光漂白。

39. 根据权利要求 18-38 任一项所述的方法，其中光照射导致生色团之间发生能量转移级联。

40. 根据权利要求 39 所述的方法，其中所述能量转移级联提供能穿入靶向组织位点处表皮与/或真皮内的光子。

41. 根据权利要求 39 或 40 所述的方法，其中所述能量转移级联并不同时伴随热量的产生。

42. 根据权利要求 39-41 所述的方法，其中所述能量转移级联并不导致组织损伤。

43. 根据权利要求 18-42 任一项所述的方法，其中第一呫吨染料在波长 200-600nm 处吸

收。

44. 根据权利要求18-43任一项所述的方法，其中第一和第二呫吨染料的含量占组合物的大约0.01-0.5％、大约0.001-0.1％或大约0.001-0.01％。

45. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，组合物的光吸收光谱的波长范围宽于单独的第一和第二生色团中的至少一种生色团。

46. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，组合物的光发射光谱的波长范围宽于单独的第一和第二生色团中的至少一种生色团。

47. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，组合物的光吸收峰的密度高于单独的第一和第二生色团中的至少一种生色团。

48. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，组合物的光发射峰的密度高于单独的第一和第二生色团中的至少一种生色团。

49. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，组合物产生的氧种类的含量高于单独的第一和第二生色团中的至少一种生色团。

50. 一种局部施用于靶向皮肤组织的生物光子组合物，所述组合物包含第一生色团和第二生色团，其中第一和第二生色团是第一和第二呫吨染料，当采用相同的活化光照射时，与单独的第一和第二生色团中的至少一种荧光团相比，组合物发射的荧光在照射时间内能得到实质上的维持。

51. 根据权利要求45所述的生物光子组合物，其中光发射光谱涵盖选自以下几种光的可见光谱的某些部分：绿色和黄色；黄色和橙色；绿色、黄色和橙色；黄色和红色；或橙色、黄色和红色。

52. 根据权利要求45-51任一项所述的生物光子组合物，其中第一和第二呫吨染料选自：荧光素和曙红Y；曙红Y和荧光桃红B；荧光素；曙红Y和玫瑰红；荧光素、曙红Y和玫瑰红。

53. 根据权利要求45-52任一项所述的生物光子组合物，其中所述活化光具有单一峰值发射波长。

54. 根据权利要求53任一项所述的生物光子组合物，其中所述活化光的峰值发射波长在蓝色与/或紫色光谱之内。

55. 根据权利要求45-52任一项所述的生物光子组合物，其中所述活化光是日光。

56. 根据权利要求45-55任一项所述的生物光子组合物，其中所述组合物进一步包含释氧剂。

57. 一种促进胶原蛋白形成的方法，包括：

-将生物光子组合物局部施用于靶向组织，所述组合物包含权利要求45-56任一项所述的生物光子组合物；及

-用波长与第一与/或第二呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

58. 一种促进皮肤病痊愈的方法，包括：

- 将生物光子组合物局部施用于靶向组织，所述组合物包含权利要求 45-56 任一项所述的生物光子组合物；及

- 用波长与第一与 / 或第二呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

59. 一种血管形成的方法，包括：

- 将生物光子组合物局部施用于靶向组织，所述组合物包含权利要求 45-56 任一项所述的生物光子组合物；及

- 用波长与第一与 / 或第二呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

60. 一种促进伤口愈合的方法，包括：

- 将生物光子组合物局部施用于靶向组织，所述组合物包含权利要求 45-56 任一项所述的生物光子组合物；及

- 用波长与第一与 / 或第二呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

61. 一种调节 MMP1、MMP3、MMP8、MMP10、MCP-2、IL-1R4/ST2、ENA78 和 TNFα 中任一项的表达而促进组织修复的方法，包括：

- 将生物光子组合物局部施用于靶向组织，所述组合物包含权利要求 45-56 任一项所述的生物光子组合物；及

- 用波长与第一与 / 或第二呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

62. 根据权利要求 57-61 任一项所述的方法，其中所述生物光子组合物被照射至少 5 分钟。

63. 根据权利要求 57-62 任一项所述的方法，其中所述生物光子组合物在光照射之后从靶向组织清除。

64. 根据权利要求 57-63 任一项所述的方法，其中第一与 / 或第二呫吨染料在光照射时经历至少部分光漂白。

65. 根据权利要求 57-64 任一项所述的方法，其中光照射导致生色团之间发生能量转移级联。

66. 根据权利要求 65 所述的方法，其中所述能量转移级联提供能穿入靶向组织位点内的光子。

67. 根据权利要求 65 或 66 所述的方法，其中所述能量转移级联并不同时伴随热量的产生。

68. 根据权利要求 65-67 所述的方法，其中所述能量转移级联并不导致组织损伤。

69. 根据权利要求 57-68 任一项所述的方法，其中第一呫吨染料在波长 400-600nm 处吸收。

70. 根据权利要求 57-68 任一项所述的方法，其中所述活化光具有单一峰值发射波长。

71. 根据权利要求 70 所述的方法，其中所述活化光的峰值发射波长在蓝色与 / 或紫色光谱之内。

72. 根据权利要求 57-69 任一项所述的方法，其中所述活化光是日光。

73. 一种试剂盒，包括：

第一成分，包含权利要求 1-17 或权利要求 45-56 任一项所述的生物光子组合物；及

任选第二成分，包含氧化剂。

74.一种试剂盒，包括：

第一成分，包含权利要求1-17或权利要求45-56任一项所述的生物光子组合物的第一呫吨染料；及

第二成分，包含权利要求1-17或权利要求45-56任一项所述的生物光子组合物的第二呫吨染料。

75.根据权利要求73或74所述的试剂盒，进一步包括向皮肤施用所述生物光子组合物的说明书。

76.根据权利要求75所述的试剂盒，其中所述说明书用于治疗皮肤病、皮肤更新或治疗伤口。

77.权利要求1-15或45-56任一项所述的组合物作为胶原蛋白合成的用途。

78.根据权利要求77所述的用途，其中所述胶原蛋白合成是在完好的皮肤内或在破损的皮肤内进行的。

79.权利要求1-17或45-56任一项所述的组合物作为调节MMP1、MMP3、MMP8、MMP10、MCP-2、IL-1R4/ST2、ENA78和TNFα中任一项的表达以促进组织修复的用途。

80.权利要求1-17或45-56任一项所述的组合物作为促进血管形成的用途。

81.一种组合物，包括：

氧化剂；

曙红Y和荧光素。

82.一种组合物，包括：

氧化剂；

曙红Y、荧光素和玫瑰红。

83.一种组合物，包括：

氧化剂；

曙红Y和玫瑰红。

84.一种组合物，包括：

氧化剂；

荧光素和玫瑰红。

85.一种组合物，包括：

氧化剂；

曙红Y、荧光素和赤藓红。

86.一种用于伤口愈合、皮肤更新或治疗皮肤病的组合物，所述组合物包括：

氧化剂；

曙红Y和荧光素。

87.一种用于伤口愈合、皮肤更新或治疗皮肤病的组合物，所述组合物包括：

氧化剂；

曙红Y、荧光素和玫瑰红。

88.一种用于伤口愈合、皮肤更新或治疗皮肤病的组合物，所述组合物包括：

氧化剂；

曙红Y和玫瑰红。

89. 一种用于伤口愈合、皮肤更新或治疗皮肤病的组合物，所述组合物包括：

氧化剂；

荧光素和玫瑰红。

90. 一种用于伤口愈合、皮肤更新或治疗皮肤病的组合物，所述组合物包括：

氧化剂；

曙红 Y、荧光素和赤藓红。

91. 根据权利要求 81-90 所述的组合物，进一步包括赤藓红。

92. 根据权利要求 81-91 任一项所述的组合物，其中氧化剂包括：

过氧化氢。

93. 根据权利要求 92 所述的组合物，其中组合物中过氧化氢的含量小于或等于其重量的 6％。

94. 根据权利要求 81-92 任一项所述的组合物，其中氧化剂包括：

过氧化脲。

95. 根据权利要求 94 所述的组合物，其中组合物中过氧化脲的含量小于或等于其重量的 22％。

96. 根据权利要求 81-95 任一项所述的组合物，其中氧化剂的总含量等于其重量的大约 6％。

97. 根据权利要求 81-96 任一项所述的组合物，进一步包含稳定剂。

98. 根据权利要求 81-97 任一项所述的组合物，进一步包含增稠剂。

99. 根据权利要求 98 所述的组合物，其中所述增稠剂是粒径小于 1 微米的二氧化硅与 / 或锻制氧化硅。

100. 根据权利要求 91-99 任一项所述的组合物，进一步包含亲水性胶凝剂。

101. 根据权利要求 100 所述的组合物，其中所述亲水性胶凝剂包括：

聚丙二醇、聚乙二醇、丙二醇、丙三醇或高分子量多元醇，或它们的任何组合。

102. 根据权利要求 91-101 任一项所述的组合物，进一步包含碱。

103. 根据权利要求 102 所述的组合物，其中所述碱是氢氧化钾。

104. 根据权利要求 91-103 任一项所述的组合物，其中所述组合物的 pH 是 2 至 10。

105. 根据权利要求 91-103 任一项所述的组合物，其中所述组合物的 pH 是 4 至 8，优选是 6 至 7，更优选是 6.5。

106. 根据权利要求 91-105 任一项所述的组合物，其中曙红 Y 的重量占组合物重量的 0.001％至 1％。

107. 根据权利要求 91-105 任一项所述的组合物，其中荧光素的重量占组合物重量的 0.001％至 1％。

108. 根据权利要求 91-105 任一项所述的组合物，其中玫瑰红的重量占组合物重量的 0.001％至 1％。

109. 根据权利要求 91-108 任一项所述的组合物，其中赤藓红的重量占组合物重量的 0.001％至 1％。

110. 一种试剂盒，包括：

包含氧化剂的第一成分；及

包含曙红 Y 和荧光素的第二成分。

111. 一种试剂盒，包括：

包含氧化剂的第一成分；及

包含曙红 Y、荧光素和玫瑰红的第二成分。

112. 一种试剂盒，包括：

包含氧化剂的第一成分；及

包含曙红 Y 和玫瑰红的第二成分。

113. 一种试剂盒，包括：

包含氧化剂的第一成分；及

包含荧光素和玫瑰红的第二成分。

114. 一种试剂盒，包括：

包含氧化剂的第一成分；及

包含曙红 Y、荧光素和赤藓红的第二成分。

115. 根据权利要求 110-113 的试剂盒，其中第二成分进一步包含赤藓红。

116. 根据权利要求 110-115 任一项所述的试剂盒，其中氧化剂包括过氧化氢或过氧化脲。

117. 根据权利要求 116 所述的试剂盒，其中所述氧化剂是过氧化脲。

118. 根据权利要求 110-117 任一项所述的试剂盒，其中第一和第二成分中的一种成分或两种成分进一步包含增稠剂。

119. 根据权利要求 118 所述的试剂盒，其中所述增稠剂是粒径小于 1 微米的二氧化硅与／或锻制氧化硅。

120. 根据权利要求 110-119 任一项所述的试剂盒，其中第一和第二成分中的一种成分或两种成分进一步包含亲水性胶凝剂。

121. 根据权利要求 110 至 120 任一项所述的试剂盒，进一步包括敷药器。

122. 根据权利要求 110 至 121 任一项所述的试剂盒，进一步包括试剂盒使用说明书、将第一成分和第二成分混合的设备、光源或评价组合物疗效的资料。

123. 根据权利要求 110 至 122 任一项所述的试剂盒，进一步包括向皮肤施用所述生物光子组合物的说明书。

124. 根据权利要求 123 所述的试剂盒，其中所述说明书用于治疗皮肤病、治疗伤口或皮肤更新。

125. 生物光子组合物用于治疗皮肤病的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红 Y，第二生色团是荧光素。

126. 生物光子组合物用于治疗皮肤病的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红 Y，第二生色团是玫瑰红。

127. 生物光子组合物用于治疗皮肤病的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是荧光素，第二生色团是玫瑰红。

128.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是荧光素。

129.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是玫瑰红。

130.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是荧光素，第二生色团是玫瑰红。

131.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是荧光素。

132.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是玫瑰红。

133.生物光子组合物用于生物光子治疗伤口的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是荧光素，第二生色团是玫瑰红。

134.生物光子组合物用于皮肤更新的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是荧光素。

135.生物光子组合物用于皮肤更新的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是曙红Y，第二生色团是玫瑰红。

136.生物光子组合物用于皮肤更新的用途，所述组合物包含第一生色团和第二生色团，其中所述生物光子组合物适合采用波长与第一生色团吸收光谱重叠的光照射，其中第一生色团是荧光素，第二生色团是玫瑰红。

137.权利要求1-17或45-56任一项所述的生物光子组合物，适用于治疗皮肤病、治疗伤口或皮肤更新的用途。

138.一种方法，该方法利用至少第一和第二荧光生色团之间的能量转移级联吸收与/或发射电磁谱可见光范围的光，用于治疗皮肤病、治疗伤口或皮肤更新。

生物光子用生色团组合

背景技术

[0001] 最近，光疗法被认为在医疗、美容及牙科领域具有广泛的应用，用于手术、治疗和检查。例如，已经开发用于下列方面的光疗法：治疗癌症和肿瘤、治疗皮肤病、作为抗菌处理对靶向部位进行消毒及促进伤口愈合。

[0002] 人们熟知的光疗技术包括光动力疗法，该方法包括全身性施用或摄入光敏剂或生色团到患病或受伤的组织内，然后针对该部位施用活化光。其它类型的光疗法包括利用发光二极管(LED)或荧光灯或激光器而仅仅向靶向组织施用特定波长的光。

[0003] 本发明的一个目的是提供用于光疗法的性能改善的新的组合物及方法。

发明内容

[0004] 本发明提供局部生物光子组合物及使用所述生物光子组合物对活组织进行生物光子治疗的方法。生物光子治疗包括皮肤更新；组织修复，包括伤口愈合、除疤及减少疤痕；治疗痤疮等各种皮肤病；及治疗牙周炎。

[0005] 本发明的生物光子组合物包括胶凝剂及至少二种呫吨类染料，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠5％、10％、20％、25％、30％、40％、50％、60％、70％。在一些实施例中，第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或60-70％。

[0006] 尤其有用的呫吨染料组合包括但不限于：荧光素+曙红Y；荧光素+曙红Y+玫瑰红；荧光素+曙红Y+荧光桃红B；曙红Y+玫瑰红；曙红Y+荧光桃红B；荧光素+赤藓红B+曙红Y；曙红Y+赤藓红；曙红Y+赤藓红B+玫瑰红；曙红Y+赤藓红B+荧光桃红B；荧光素+曙红Y+赤藓红B+玫瑰红；及荧光素+曙红Y+赤藓红B+荧光桃红B。

[0007] 胶凝剂可包括吸湿性物质。除此之外或者替代地，胶凝剂还可以是亲水性聚合物、水合聚合物或类脂。在一些实施例中，胶凝剂包括一种或多种丙三醇、乙二醇（如丙二醇）、聚丙烯酸聚合物、透明质酸、氨基葡萄糖硫酸盐或明胶。

[0008] 在一些实施例中，胶凝剂是粘度大约是20,000-80,000、20,000-100,000、25,000-90,000、30,000-80,000、30,000-70,000、30,000-60,000、25,000-40,000cP 的高分子量交联聚丙烯酸聚合物。在一些实施例中，所述交联聚丙烯聚合物是卡波姆，选自由Carbopol® 71G NF、971P NF、974P NF、980NF、981NF、5984EP,ETD 2020NF、Ultrez 10NF、934NF、934P NF、940NF、941NF或1342NF组成（但不限于由这些组成）的群组。

[0009] 在一些实施例中，所述生物光子组合物基本上是半透明与/或透明的。在一些实施例中，所述生物光子组合物在460nm时的半透明度是至少70％。在其它实施例中，所述组合物在460nm时的半透明度是至少20％、30％、40％、50％、60％、70％、75％、85％、90％、95％或100％。

[0010] 在一些实施例中，所述生物光子组合物是液体、凝胶、半固体、霜、泡沫、洗液、油、软膏、糊、悬浮液或气溶胶喷雾。

[0011] 在一些实施例中，所述生物光子组合物包裹在透明不透膜或者允许气体透过但不允许液体透过的透气膜内。所述膜可以包含类脂。

[0012] 在一些实施例中，所述生物光子组合物进一步包含产氧剂。在一些实施例中，所述产氧剂包括过氧化氢、过氧化脲、过氧化苯甲酰、分子氧或水。当释氧剂是过氧化物时，其含量可以低于 6％ H_2O_2,为 0.5-6wt％ H_2O_2（或相当）、0.5-5.5％、0.5-5.0％、0.5-4.5％、0.5-4.0％、0.5-3.5％、0.5-3.0％、0.5-2.5％、0.5-2％、0.5-1.5％或 0.5-1.0％。

[0013] 在一些实施例中，所述生物光子组合物在光照射之后并不产生大量的热。在一些实施例中，所述生物光子组合物散发的能量并不导致组织损伤。

[0014] 在一些实施例中，所述组合物中第一和第二呫吨染料的含量大约占组合物重量的 0.001-0.5％。

[0015] 在一些实施例中，所述生物光子组合物可以涂覆或浸渍到衬垫、敷料、织造布或无纺布等材料上。浸渍材料可以用作遮蔽物（如面罩）或敷料。

[0016] 在一些实施例中，所述生物光子组合物在组合物内或组合物附近进一步包含至少一种波导。所述波导可以是由传输与/或发射光的材料制造的颗粒、纤维或原纤网络。

[0017] 在一些实施例中，所述组合物不包含二氧化硅、鞣剂或非荧光染料。

[0018] 本发明还提供所述组合物的用途及生物光子处理活组织的方法。

[0019] 因此，在一些方面，本发明提供一种用生物光子疗法治疗伤口的方法，包括：向伤口施用一种包含至少第一呫吨染料和第二呫吨染料的生物光子组合物，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠 1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或 60-70％；采用波长与第一呫吨染料的吸收光谱重叠的光照射所述生物光子组合物。

[0020] 在一些提供用生物光子疗法治疗伤口的方法的实施例中，所述方法促进伤口的愈合。在一些方法实施例中，所述伤口包括，例如，慢性或急性伤口，如糖尿病足部溃疡、压迫性溃疡、静脉曲张性溃疡或截肢。在用生物光子疗法治疗伤口的一些实施例中，所述方法减少疤痕组织的形成。在一些实施例中，这种治疗可以每周一次、两次、三次、四次、五次或六次，每天或以任何其它频次施加到伤口内或伤口上。总的治疗时间可以是一周、两周、三周、四周、五周、六周、七周、八周、九周、十周、十一周、十二周或认为合适的任何其它时间长度。

[0021] 在其它方面，提供了一种用生物光子疗法治疗痤疮的方法，包括：向皮肤组织施用一种包含至少第一呫吨染料和第二呫吨染料的生物光子组合物，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠 1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或 60-70％；采用波长与第一呫吨染料的吸收光谱重叠的光照射生物光子组合物。在用生物光子疗法治疗痤疮的方法的一些实施例中，所述治疗可每周一次、两次、三次、四次、五次或六次，每天或以任何其它频次施加到皮肤组织，如面部。总的治疗时间可以是一周、两周、三周、四周、五周、六周、七周、八周、九周、十周、十一周、十二周或认为合适的任何其它时间长度。在一些实施例中，面部可以分为独立的区域（面颊、前额），每个区域分别治疗。例如，所述组合物可以局部施用到第一部分，并用光照射该部分，然后清除所述生物光子组合物。然后，将所述组合物施用到第二部分、对其进行照射和清除。最后，将所述组合物施用到第三部分，对其进行照射和清除。

[0022] 所述治疗痤疮或伤口的方法可以进一步包括，例如，在生物光子治疗之前、治疗期

间或治疗之后使用全身或局部药物。所述药物可以是抗生素、激素治疗或有助于治疗痤疮或伤口的任何其它药剂。全身治疗与局部生物光子治疗相结合可以缩短全身治疗的时间。

[0023] 在其它方面，提供了一种生物光子治疗皮肤病的方法，包括：向靶向皮肤组织施用一种包含至少第一呫吨染料和第二呫吨染料的生物光子组合物，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或60-70％；采用波长与第一呫吨染料的吸收光谱重叠的光照射生物光子组合物。

[0024] 在其它方面，提供了一种促进皮肤更新的方法，包括：向靶向皮肤组织施用一种包含至少第一呫吨染料和第二呫吨染料的生物光子组合物，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或60-70％；采用波长与第一呫吨染料的吸收光谱重叠的光照射生物光子组合物。

[0025] 在其它方面，提供了一种治疗牙周病的方法，包括：向牙周袋施用一种包含至少第一呫吨染料和第二呫吨染料的生物光子组合物，其中第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或60-70％；采用波长与第一呫吨染料的吸收光谱重叠的光照射生物光子组合物。

[0026] 在其它方面，提供了一种方法，该方法利用至少第一和第二荧光生色团之间的能量传递级联，吸收与/或发射电磁谱可见范围之内的光，用于治疗皮肤病和伤口、进行皮肤更新和治疗牙周炎。本发明所述方法和组合物还可用于治疗真菌感染和病毒感染。

[0027] 在本发明所述任一方法的某些实施例中，每次治疗时，所述生物光子组合物被照射任何时间，在治疗中生物光子组合物被活化例如1至30分钟。光源距生物光子组合物的距离可以是能够向生物光子组合物与/或皮肤组织传递适当光的功率密度的距离，例如，距离5、10、15或20cm。所述生物光子组合物局部施用任何合适的厚度。一般说来，所述生物光子组合物施用到皮肤或伤口上的厚度是至少大约2mm、大约2mm至大约10mm。

[0028] 在某些实施例中，本发明所述方法包括照射生物光子组合物至少30秒、2分钟、3分钟、5分钟、7分钟、10分钟、15分钟、20分钟、25分钟或30分钟的步骤。在一些实施例中，所述生物光子组合物被照射至少3分钟。

[0029] 在本发明所述方法的一些实施例中，在光照射之后，从治疗部位清除所述生物光子组合物。因此，在施用后至少30秒、2分钟、3分钟、5分钟、7分钟、10分钟、15分钟、20分钟、25分钟或30分钟内从治疗部位清除所述生物光子组合物。在一些实施例中，所述生物光子组合物被照射至少3分钟。在一些实施例中，所述生物光子组合物在施用到治疗部位至少3分钟后清除。

[0030] 在其它一些实施例中，所述生物光子组合物的保留时间高达一周、两周或三周，并以不同的间隔采用包括环境中的光进行照射。在这种情况下，所述组合物在两次暴露于光的间隔期间可以盖起来。例如，所述生物光子组合物可以浸在敷料中，并放在伤口上或伤口内，并让其保留较长的时间（例如，超过一天）。

附图说明

[0031] 图1描述了皮肤各层光的吸收情况(Samson et al.Evidence Report/Technology Assessment 2004,111,pages 1-97)。

[0032] 图2示出了斯托克斯频移。

[0033] 图3示出了供体和受体生色团的吸收和发射光谱。还示出了受体生色团的吸收光谱与供体生色团的发射光谱之间的光谱重叠情况。

[0034] 图4是雅布隆斯基图的示意图,示出了供体发射和受体吸收之间涉及的耦合跃迁。

[0035] 图5A和5B分别是脲凝胶中(i)浓度大约0.09mg/mL的荧光素钠盐,(ii)浓度大约0.305mg/mL的曙红Y,及(iii)浓度大约0.09mg/mL的荧光素钠盐和浓度大约0.305mg/mL的曙红Y的混合物(实例1)的吸收光谱和发射光谱。

[0036] 图6A和6B分别是水溶液中(i)终浓度大约0.18mg/mL的荧光素钠盐,(ii)浓度大约0.305mg/mL的曙红Y,及(iii)浓度大约0.18mg/mL的荧光素钠盐和浓度大约0.305mg/mL的曙红Y的混合物(实例2)的吸收光谱和发射光谱。

[0037] 图7A和图7B分别是12%脲凝胶中(i)终浓度0.25mg/mL的荧光桃红B,(ii)浓度大约0.05mg/mL的曙红Y及(iii)荧光桃红B(0.25mg/mL)和曙红Y(0.05mg/mL)的混合物(实例3)的吸收光谱和发射光谱。

[0038] 图8A和图8B分别是水溶液中(i)终浓度0.25mg/mL的荧光桃红B,(ii)浓度大约0.08mg/mL的曙红Y及(iii)荧光桃红B(0.25mg/mL)和曙红Y(0.08mg/mL)的混合物(实例4)的吸收光谱和发射光谱。

[0039] 图9A和9B分别是12%脲凝胶中(i)浓度100μg/g的荧光桃红B,(ii)浓度大约100μg/g的荧光素及(iii)荧光桃红B(100μg/g)和荧光素(100μg/g)的混合物(实例5)的吸收光谱和发射光谱。

[0040] 图10A和10B分别是12%脲凝胶中(i)浓度100μg/g的荧光桃红B,(ii)浓度大约100μg/g的荧光素及(iii)荧光桃红B(100μg/g)和荧光素(100μg/g)的混合物(实例6)的吸收光谱和发射光谱。

[0041] 图11A和图11B分别是12%脲凝胶中(i)终浓度0.305mg/mL的曙红Y,(ii)浓度大约0.085mg/mL的玫瑰红及(iii)曙红Y(0.305mg/mL)和玫瑰红(0.085mg/mL)的混合物(实例7)的吸收光谱和发射光谱。

[0042] 图12示出了曙红Y和玫瑰红以协同方式作用(实例8)。

[0043] 图13A和13B示出了包含(i)荧光素+曙红Y(图11A)及(ii)曙红Y+玫瑰红(图11B)的组合物随时间而变化的荧光发射(功率密度)情况(实例9)。

[0044] 图14A和14B分别是脲凝胶中(i)浓度大约0.085mg/mL的玫瑰红,(ii)终浓度大约0.44mg/mL的荧光素钠盐,(iii)浓度大约0.305mg/mL的曙红Y及(iv)上述(i)、(ii)和(iii)的混合物的吸收光谱和发射光谱(实例10)。

[0045] 图15A和15B分别是水溶液中(i)浓度大约0.085mg/mL的玫瑰红,(ii)终浓度大约0.44mg/mL的荧光素钠盐,(ii)浓度大约0.305mg/mL的曙红Y及(iii)上述(i)、(ii)和(iii)的混合物的吸收光谱和发射光谱(实例11)。

[0046] 图16是一个发射光谱,示出了实例12和实例13中的试验组合物发射的光的强度随时间变化的情况。

[0047] 图 17A 和 17B 表明，组合物中的曙红（上）和荧光素（下）发射的荧光的能量密度随生色团浓度的增加而迅速上升，但是，浓度进一步增加时，增速减慢，达到一个平台，而活化光随浓度增加而下降（实例 15）。

[0048] 发明详细说明

[0049] (1) 概述

[0050] 本发明提供了包含至少两个光活化生色团、能够将能量从一个生色团传递到另一个生色团的组合物，及提供了采用这些组合物治疗组织的方法，例如，促进组织修复（包括伤口愈合）、皮肤美容治疗（如皮肤更新，治疗皮肤病，如痤疮），及用于牙周病。

[0051] (2) 定义

[0052] 在继续更详细地描述本发明之前，应该理解的是，本发明公开并不限于具体的组合物或工艺步骤，因为这些都可能变化。必须指出的是，正如本说明书和所附权利要求书中使用的那样，除非上下文清楚显示，否则，单数形式“一”、“一个”及“该”包括复数指示对象。

[0053] 本发明中给出数值或范围时使用的词语“大约”指的是数值或范围与给出的数值或范围的差别在 20％以内，优选在 10％以内及更优选在 5％以内。

[0054] 本发明中使用的“与／或”指的是具体公开了两种规定特征或成分的每一种特征或成分，两者可以同时兼具或者单独具有。例如，“A 与／或 B”指的是公开了 (i)A、(ii)B 和 (iii)A 和 B 三种情形，每种情形都是独立的。

[0055] “生物光子性”指的是在生物相关背景中产生、操作、检测和应用光子。换句话说，生物光子组合物主要是由于光子的产生和操作而施加其生理效应。本发明所述“生物光子组合物”是可以通过光活化而产生光子的、用于生物相关应用的组合物。

[0056] “局部组合物”指的是施用于身体表面，如皮肤、粘膜、阴道、口腔、伤口等的组合物。局部组合物的形式包括但不限于霜、凝胶、油膏、洗液、细末、溶液、生物粘合剂、药膏、乳液等。局部组合物可以是浸渍材料，如衬垫、片材、织造布或纤维、敷料、喷雾、悬浮液、泡沫等。

[0057] 词语“生色团”、“光活化试剂”和“光活化剂”可以互换使用。生色团指的是光照射时能够吸收光的化合物，例如，呫吨染料。生色团能很容易地被光激发，然后，生色团会将其能量传递给其它分子或让其能量以光的形式散发出来。

[0058] “氧化剂”、“氧化试剂”或“释氧剂”在本发明中可以互换使用，指的是容易转移氧原子和氧化其它化合物的化合物。它包括分子氧以及含氧化合物，如水、过氧化物等。

[0059] “光漂白”指的是生色团的光化学破坏。

[0060] 词语“光化光”指的是特定光源（如灯、LED 或激光器）发出的且能够被物质（例如，上文定义的生色团或光活化剂）吸收的光能。在优选的实施例中，光化光是可见光。

[0061] “伤口”指的是任何组织的损伤，包括，例如，急性、亚急性、延缓愈合或难以愈合的伤口，以及慢性伤口。伤口的实例包括开放性伤口和闭合性伤口。伤口包括，例如，烧伤、切口、切除、损伤、撕裂伤、磨损、刺伤或贯通伤口、手术伤口、擦伤、血肿、压伤、溃疡（如压迫性、静脉、压迫性或糖尿病溃疡）、牙周炎造成的伤口（牙周发炎）及枪伤。

[0062] “伤口愈合”指的是促进或加速组织修复，包括伤口闭合、慢性伤口活化或减少疤痕形成。

[0063] “皮肤更新”指的是减少、消除、延缓或逆转皮肤老化的一个或多个症状的过程。例

如，皮肤老化的常见症状包括但不限于出现细纹或皱纹、皮肤变得薄而透明、皮下脂肪流失（造成脸颊下陷、眼窝深凹以及手部和颈部紧致性明显丧失）、骨质疏松（骨质疏松引起骨骼收缩，从而与皮肤分离，造成皮肤松驰）、皮肤干燥（可能会痒）、汗无法全部流出而冷却皮肤、面部长毛、雀斑、老年斑、蛛状静脉、皮肤粗糙和革质皮肤、拉开时会消失的细纹、皮肤松驰或长斑。本发明上文所述的皮肤老化的一种或多种症状可以通过本发明的组合物和方法减少、消除、延缓或甚至逆转。

[0064] (3) 生物光子组合物

[0065] 本发明提供生物光子组合物。从广义上来说，生物光子组合物是包含可由光活化且加速光能分散的生色团的组合物，从而使光对组合物本身与/或对组合物中包含的其它试剂具有光化学活化作用而发挥治疗作用（例如，当组合物中或治疗部位包含释氧剂时，释氧剂分解，形成氧自由基，如单线态氧）。本发明的生物光子组合物包含至少两种呫吨染料作为生色团。

[0066] 当生色团吸收某一波长的光子时，生色团被激发。这是一种不稳定的状态，分子将试图返回到基态，释放出多余的能量。对某些生色团来说，当其返回到基态时，最好是以光的形式放出多余的能量。该过程被称为发射荧光。由于转化过程失去能量，与吸收波长相比，发射荧光的峰值波长朝更长的波长移动。这种现象被称为斯托克斯频移，如图 2 所示。在合适的环境中（例如，在生物光子组合物中），许多能量被转移给组合物的其它成分或直接转移到治疗部位。

[0067] 人们认为，由于光活化生色团放射的荧光具有被生物细胞和组织认可的飞秒、皮秒或纳秒发射特性，导致有益的生物调节，因此，这种荧光具有治疗特性。但是，并不受限于这种理论。此外，发射的荧光的波长更长，因此，比活化光能更深地穿入组织内部。采用范围如此宽的波长（某些实施例中包括穿过组合物的活化光）照射组织，对细胞和组织具有不同的和互补的效果。此外，发明人观察到光活化生色团产生了氧（例如，单线态氧），在组合物内造成微泡，对其施用的组织存在物理影响，例如，使生物膜脱落和清除坏死组织或提供压力刺激。在采用本发明组合物进行治疗之前，生物膜还可以采用释氧剂进行预处理，从而弱化生物膜。

[0068] 此外，人们认为，与 LED 或激光器等光源相比，组合物中利用生色团来发射荧光能够在更大的程度上精细调节发射的光。例如，根据所需的治疗或处理，可以根据生色团发射的光的波长来选择生色团及选择适当的浓度，从而控制发射的光的功率密度。

[0069] 本发明的生物光子组合物基本上是透明/半透明的与/或具有较高的透光率，从而允许光散发到组合物内和穿过组合物。这样，组合物下面的组织区可以利用组合物发射的荧光治疗及利用光照射组合物使其活化。例如，利用 Perkin-Elmer Lambda 9500 系列 UV-可见分光光度计在波长 250nm 至 800nm 范围测量生物光子组合物的%透光率。在一些实施例中，本发明所述组合物的透光率在 460nm 处测定。

[0070] 由于透光率取决于厚度，因此，在将样品装到分光光度计中之前，采用卡规测量每个样品的厚度。根据下式，将透光率值归一化到厚度 100μm（或任何厚度）：

[0071]

$$F_{T-corr}(\lambda, t_2) = [e^{-\sigma_t(\lambda)t_1}]^{\frac{t_2}{t_1}} = [F_{T-corr}(\lambda, t_1)]^{\frac{t_2}{t_1}},$$

[0072] 式中，t_1＝试样实际厚度，t_2＝透光率测定归一化的厚度。

[0073] 在一些实施例中，生物光子组合物在 460nm 的透明度或半透明度超过 15％、20％、30％、40％、50％、55％、60％、65％、70％、75％、80％或 85％。在一些实施例中，生物光子组合物在 460nm 的透明度超过 70％、86％、87％、88％、89％、90％、91％、92％、93％、94％、95％、96％、97％、98％、99％。

[0074] 本发明的光子组合物适用于局部应用。这些组合物将根据构成组合物的成分进行说明。另外地或替代地，本发明的组合物具有功能和结构性质，这些性质可用于定义和描述组合物。下面将对本发明组合物的各个成分进行详细的说明。

[0075] (a) 生色团

[0076] 本发明的生物光子局部组合物包含至少两种呫吨染料作为生色团。呫吨染料结合可以通过结合的染料分子增加光吸收和增强吸收和光生物调节的选择性。这样创造了产生新的光敏性与／或选择性呫吨染料混合物的几种可能性。

[0077] 当这些多呫吨染料组合物采用合适波长的光照射而活化至少其中一种呫吨染料时，在呫吨染料之间会发生能量转移。这种被称为共振能量转移的过程是一种光物理过程，通过这一过程，被激发的“供体”呫吨染料（在本发明亦称为第一呫吨染料）将其激发能转移给“受体”呫吨染料（在本发明中亦称为第二呫吨染料）。共振能量转移的效率和导向性取决于供体和受体呫吨染料的光谱特征。特别是，呫吨染料之间的能量流动取决于反映吸收光谱和发射光谱相对位置和形状的光谱重叠。要发生能量转移，供体呫吨染料的发射光谱必须优选与受体呫吨染料的吸收光谱重叠（图 3）。

[0078] 通过供体发射的减少或猝灭和激发态寿命缩短，此外还有伴随受体发射强度的增加，证明了能量转移本身。图 4 是雅布隆斯基图，说明了供体发射和受体吸收之间涉及的耦合跃迁。

[0079] 为了提高能量转移效率，供体呫吨染料应具有良好的吸收光子和发射光子的能力。此外，人们认为，供体呫吨染料的发射光谱和受体呫吨染料的吸收光谱之间重叠越多，供体呫吨染料越能更好地向受体呫吨染料转移能量。

[0080] 在一些实施例中，第一呫吨染料的发射光谱与第二呫吨染料生色团的吸收光谱的至少大约 80％、50％、40％、30％、20％、10％重叠。在一个实施例中，第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱的至少大约 20％重叠。在一些实施例中，第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠 1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或 60-70％。

[0081] 本发明所述的％光谱重叠指的是在光谱四分之一最大全宽 (FWQM) 时测量的供体呫吨染料的发射波长范围与受体呫吨染料的吸收波长范围的重叠％。例如，图 3 表明了供体和受体呫吨染料的归一化吸收光谱和发射光谱。受体呫吨染料的吸收光谱的光谱 FWQM 是大约 60nm(515nm 至大约 575nm)。供体呫吨染料的光谱与受体呫吨染料的吸收光谱重叠大约 40nm（从 515nm 至大约 555nm)。因此，重叠％可以计算为 40nm/60nm x 100 ＝ 66.6％。

[0082] 在一些实施例中，第二呫吨染料在可见光谱范围的波长处吸收。在一些实施例中，在大约 50-250、25-150 或 10-100nm 内，第二呫吨染料的吸收波长比第一呫吨染料的相对更长。

[0083] 如上文所述，向本发明组合物照射光会在呫吨染料之间造成能量转移级联。在一

些实施例中，这种能量转移级联提供了光子，光子穿透靶向组织（包括，例如，伤口部位，或受痤疮或其它皮肤病折磨的组织）处的表皮、真皮与/或粘膜。在一些实施例中，这种能量转移级联并不伴随热量的产生。在一些实施例中，能量转移级联不会导致组织损伤。

[0084] 在一些实施例中，第一呫吨染料在可见光谱范围内的波长处吸收，如在波长大约380-800nm、380-700或380-600nm处吸收。在其它实施例中，第一呫吨染料在波长大约200-800nm、200-700nm、200-600nm或200-500nm处吸收。在一个实施例中，第一呫吨染料在波长大约200-600nm处吸收。在一些实施例中，第一呫吨染料在波长大约200-300nm、250-350nm、300-400nm、350-450nm、400-500nm、400-600nm、450-650nm、600-700nm、650-750nm或700-800nm处吸收。

[0085] 熟悉本领域的技术人员应该理解的是，具体呫吨染料的光学特性可能根据呫吨染料的周围介质而变化。因此，正如本发明所述，具体呫吨染料的吸收与/或发射波长（或光谱）与本发明生物光子组合物中测定的波长（或光谱）对应。

[0086] 示例性呫吨染料包括但不限于曙红B(4',5'-二溴-2',7'-二硝基-荧光素二价阴离子)；曙红Y；曙红Y(2',4',5',7'-四溴-荧光素二价阴离子)；曙红(2',4',5',7'-四溴-荧光素二价阴离子)；曙红(2',4',5',7'-四溴-荧光素二价阴离子)甲酯；曙红(2',4',5',7'-四溴-荧光素单价阴离子)p-异丙基苄酯；曙红衍生物(2',7'-二溴-荧光素二价阴离子)；曙红衍生物(4',5'-二溴-荧光素二价阴离子)；曙红衍生物(2',7'-二氯-荧光素二价阴离子)；曙红衍生物(4',5'-二氯-荧光素二价阴离子)；曙红衍生物(2',7'-二碘-荧光素二价阴离子)；曙红衍生物(4',5'-二碘-荧光素二价阴离子)；曙红衍生物（三溴荧光素二价阴离子）；曙红衍生物(2',4',5',7'-四氯-荧光素二价阴离子)；曙红；曙红联十六烷基吡啶氯离子对；赤藓红B(2',4',5',7'-四碘-荧光素二价阴离子)；赤藓红；赤藓红二价阴离子；赤藓红B；荧光素；荧光素二价阴离子；根皮红B(2',4',5',7'-四溴-3,4,5,6-四氯-荧光素二价阴离子)；根皮红B(四氯-四溴-荧光素)；根皮红B；玫瑰红(3,4,5,6-四氯-2',4',5',7'-四碘荧光素二价阴离子)；派若宁G，派若宁J，派若宁Y；罗丹明染料，如罗丹明包括4,5-二溴-罗丹明甲酯；4,5-二溴-罗丹明正丁酯；罗丹明101甲酯；罗丹明123；罗丹明6G；罗丹明6G己酯；四溴-罗丹明123；及四甲基-罗丹明乙酯。

[0087] 在一些实施例中，第一呫吨染料占组合物重量的大约0.01-40%，第二呫吨染料占组合物重量的大约0.001-40%。在一些实施例中，呫吨染料的总重量占组合物重量的大约0.01-40.001%。在一些实施例中，第一呫吨染料占组合物重量的大约0.01-1%、0.01-2%、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%或35-40%。在一些实施例中，第二呫吨染料占组合物重量的大约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%或35-40%。在一些实施例中，呫吨染料的总重量占组合物重量的大约0.5%以下、0.1%以下、0.001-0.1%、0.01-1%、0.01-2%、0.05-2%、0.001-0.5%、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％或 35-40.05％。所有含量都是以总浓度重量的重量百分数给出，及以相当的重量或体积含量形式给出。

[0088] 在一些实施例中，组合物中第一和第二呫吨染料的浓度比是 1:1 至 1:1000。在一些实施例中，曙红 Y：荧光素的相对浓度是曙红 Y 的含量比荧光素低，例如 1000:1 或 100:1 或 10:1 或 60-80％ :20-40％。在一些实施例中，曙红 Y 和玫瑰红的浓度比是 1:1 或 70-90％ :10-30％。在一些实施例中，荧光素和曙红 Y 和玫瑰红的浓度比是 20-40％ :30-60％ :10-20％。这一比例可以根据给定治疗或处理所要求的发射光谱进行定制。

[0089] 在一些实施例中，选择呫吨染料组合，从而光活化时其发射荧光是电磁谱中绿色、黄色、橙色、红色及红外部分内的一种或多种，例如，峰值波长在大约 490nm 至大约 800nm 范围之内。在某些实施例中，发射的荧光的功率密度是 0.005 至大约 10mW/cm^2、大约 0.5 至大约 5mW/cm^{2}或大约 0.05 至大约 2mW/cm^2。

[0090] 尤其有用的呫吨染料组合包括但不限于 ：荧光素 + 曙红 Y ；荧光素 + 曙红 Y+ 玫瑰红 ；荧光素 + 曙红 Y+ 荧光桃红 B ；曙红 Y+ 玫瑰红 ；曙红 Y+ 荧光桃红 B ；曙红 Y+ 赤藓红 ；荧光素 + 赤藓红 B+ 曙红 Y ；曙红 Y+ 赤藓红 B+ 玫瑰红 ；曙红 Y+ 赤藓红 B+ 荧光桃红 B ；荧光素 + 曙红 Y+ 赤藓红 B+ 玫瑰红 ；及荧光素 + 曙红 Y+ 赤藓红 B+ 荧光桃红 B。

[0091] 人们认为，这些组合中的至少部分组合在组合物内的某些浓度比上具有协同效应。例如，在某些浓度比上及采用合适的活化光活化时，曙红 Y 可以将能量转移给玫瑰红、赤藓红 B 或荧光桃红 B。然后，这种转移的能量以荧光放出与／或产生反应性的氧（如单线态氧）。

[0092] 当单个生色团和组合物采用同一活化光（发射光谱基本上相同的光）活化时，采用光吸收光谱的波长范围比组合物中单个生色团之一的单个光吸收光谱更宽的组合物，此协同效应非常明显。这一点使组合物能够采用波长范围更宽的活化光进行活化，例如，采用白光活化，从而不需要波长精确的活化光。

[0093] 当单个生色团和组合物采用同一活化光活化时，采用光发射光谱的波长范围比组合物中单个生色团之一的单个光吸收光谱更宽的组合物，此协同效应非常明显。这种吸收和再发射的光谱被认为在整个组合物中传输，还传输到治疗部位内。然后，这种发射的光谱将以不同的穿入深度照射靶向组织，（图 1），使靶向组织获得良好的治疗效果。例如，据报道，绿光具有伤口愈合性质。发射的波长范围越宽，可以实现的治疗效果范围越宽。这种发射的波长可以通过采用不同的生色团组合和浓度进行精细调节。

[0094] 当单个生色团和组合物采用同一活化光活化时，采用光吸收或发射峰值比组合物中单个生色团的单个光吸收／发射峰值更高的组合物，这种协同效应也非常明显。吸收和发射更高水平的光子的能力在某些应用中具有治疗效果。此外，为了实现某一功率密度，可能要求降低单个生色团的浓度。功率密度越高，治疗时间越短。

[0095] 当单个生色团和组合物采用同一活化光活化时，在释氧剂的存在下，采用比组合物中的单个生色团能产生更多氧种类的组合物，这种协同效应也非常明显。在某些情况下，不需延长治疗时间或提高活化光的功率密度就能产生更高水平的氧种类，这一点可能是非常有好处的。

[0096] 通过组合物中呫吨染料组合的协同效应，通常无法被活化光（如蓝光）活化的呫

吨染料，可以通过被活化光活化的呫吨染料的能量转移而得到活化。因此，可以根据所需的美容或治疗而利用和定制不同性质的光活化呫吨染料。

[0097] 例如，当玫瑰红在存在分子氧的条件下被光活化时，其单线态氧的收率较高，但玫瑰红的发射荧光量子产率较低。玫瑰红的峰值吸收在540nm附近，因此，通常用绿光活化。曙红Y的荧光量子产率高，可以采用蓝光活化。将玫瑰红和曙红Y相结合，得到的组合物被蓝光活化时，可以发射具有治疗效果的荧光和产生单线态氧。在这种情况下，人们认为，蓝光光活化曙红Y，曙红Y将其部分能量转移给玫瑰红并以荧光形式放出部分能量。

[0098] 在照射期间，这些生色团中的一个或多个生色团可以光漂白。这是“剂量”传递的可见确认。当生色团光漂白时，随着时间延长，它们发射的荧光减少。同时，随时间延长，它们吸收的活化光减少，因此，组织接收的活化光逐渐增加。因此，生色团对组织对于能提供某种保护效果的光的暴露进行调节。

[0099] (b)其它生色团

[0100] 除呫吨染料之外，本发明的生物光子局部组合物还包括但不限于下述染料：

[0101] 叶绿素染料

[0102] 示例性叶绿素染料包括但不限于叶绿素a；叶绿素b；油溶叶绿素；菌叶绿素a；菌叶绿素b；菌叶绿素c；菌叶绿素d；原叶绿素；原叶绿素a；两亲叶绿素衍生物1；两亲叶绿素衍生物2。

[0103] 亚甲基蓝染料

[0104] 示例性亚甲基蓝衍生物包括但不限于1-甲基亚甲基蓝；1,9-二甲基亚甲基蓝；亚甲基蓝；亚甲基蓝(16.mu.M)；亚甲基蓝(14.mu.M)；亚甲紫；溴亚甲基紫；4-碘亚甲基紫；1,9-二甲基-3-二甲基-氨基-7-二乙基-氨基-吩噻嗪；及1,9-二甲基-3-二乙基氨基-7-二丁基-氨基-吩噻嗪。

[0105] 偶氮染料

[0106] 示例性偶氮(或二偶氮-)染料包括但不限于甲基紫、中性红、对位红(颜料红1)、苋菜红(偶氮玉红S)、酸性红(偶氮玉红、食品红3、酸性红14)、诱惑红AC(FD&C 40)、酒石黄(FD&C黄5)、橙黄G(酸性橙10)、丽春红4R(食品红7)、甲基红(酸性红2)及紫脲酸铵-红紫酸铵。

[0107] 在本发明的某些方面，本发明生物光子组合物其它生色团可以独立地选自以下任一生色团：酸性黑1、酸性蓝22、酸性蓝93、酸性品红、酸性绿、酸性绿1、酸性绿5、酸性洋红、酸性橙10、酸性红26、酸性红29、酸性红44、酸性红51、酸性红66、酸性红87, 酸性红91, 酸性红92, 酸性红94, 酸性红101, 酸性红103, 酸性品红、酸性品红、酸性紫19、酸性黄1、酸性黄9、酸性黄23、酸性黄24、酸性黄36、酸性黄73、酸性黄S、吖啶橙、吖啶黄、阿尔新蓝、阿尔新黄、醇溶曙红、茜素、茜素蓝2RC、茜素卡红、茜素花青BBS、茜素花青R、茜素红S、茜素红紫、试铝灵、酰胺黑10B、氨基黑、苯胺蓝WS、蒽蓝SWR、金胺O、Azocannine B、偶氮卡红G、偶氮重氮(Azoic diazo)5、偶氮重氮48、天蓝A、天蓝B、天蓝C、碱性蓝8、碱性蓝9、碱性蓝12、碱性蓝15, 碱性蓝17、碱性蓝20、碱性蓝26、碱性棕1、碱性品红、碱性绿4、碱性橙14、碱性红2(Saffranin O)、碱性红5、碱性红9、碱性紫2、碱性紫3、碱性紫4、碱性紫10、碱性紫14、碱性黄1、碱性黄2、比布里希猩红、俾斯麦棕Y、亮结晶猩红6R、钙红、胭脂红、胭脂红酸(酸性4)、天青石蓝B、中国蓝、虫红、天青石蓝(Coelestine Blue)、铬紫CG、铬变素

2R、铬花青 R、刚果 corinth、刚果红、棉染蓝、棉红、Croceine 猩红、藏花素、结晶丽春红 6R、结晶紫、大丽紫、金刚绿 B、DiOC6、直接靛兰 14、直接蓝 58、直接红、直接红 10、直接红 28、直接红 80、直接黄 7、曙红 B、蓝色曙红、曙红、曙红 Y、黄色曙红、Eosinol、伊利石榴红 B、铬花青 R、赤藓红 B、乙基曙红、乙基绿、乙基紫、伊文思蓝、坚牢蓝 B、坚牢绿 FCF、坚牢红 B、坚牢黄、荧光黄、食品绿 3、焦酚酞、加拉明蓝、倍花青、龙胆紫、氧化苏木精、苏木精、苏木紫、日光坚牢品红 BBL、甲蓝、苏木因、苏木精、苏木紫、霍夫曼紫、皇家红、吲哚菁绿、阿利新蓝、阿利新蓝 1、阿利新黄 1、INT、洋红、胭脂酮酸、Kernechtrot、紫胶、紫胶酸、劳思氏紫、淡绿、丽丝胺绿 SF、Luxol 坚牢蓝、品红 0、品红 I、品红 II、品红 III、孔雀绿、曼彻斯特棕、马休黄、汞溴红、红汞、酸性间胺黄、亚甲基天蓝 A、亚甲基天蓝 B、亚甲基天蓝 C、亚甲基蓝、亚甲基绿、甲基紫、甲基紫 2B、甲基紫 10B、媒染蓝 3、媒染蓝 10、媒染蓝 14、媒染蓝 23、媒染蓝 32、媒染蓝 45、媒染红 3、媒染红 11、媒染紫 25、媒染紫 39、萘酚蓝黑、萘酚绿 B、萘酚黄 S、天然黑 1、天然红、天然红 3、天然红 4、天然红 8、天然红 16、天然红 25、天然红 28、天然黄 6、NBT、天然红、新品红、尼亚加拉蓝 3B、夜蓝、尼罗蓝、尼罗蓝 A、尼罗红、硫酸尼罗蓝、尼罗红、硝基 BT、硝基蓝四唑、核坚牢红、油红 0、橙 G、地衣红、副品红、荧光桃红 B、藻胆素、藻青素、藻红素。藻红蓝蛋白 (PEC)、酞菁、苦味酸、丽春红 2R、丽春红 6R、丽春红 B、二甲苯胺丽春红、丽春红 S、报春花、红紫素、派若宁 B、派若宁 G、派若宁 Y、罗丹明 B、玫瑰苯胺、玫瑰红、藏红、番红精 0、猩红 R、猩红、猩红 R、紫胶、天狼猩红 F3B、砂罗铬花青 R、可溶蓝、溶剂黑 3、溶剂蓝 38、溶剂红 23、溶剂红 24、溶剂红 27、溶剂红 45、溶剂黄 94、醇溶曙红、苏丹 III、苏丹 IV、苏丹黑 B、硫黄 S、瑞士蓝、酒石黄、硫黄素 S、硫黄素 T、劳氏紫、甲苯胺蓝、甲苯胺红、苯胺黄 G、吖啶黄、锥虫蓝、荧光素钠、维多利亚蓝 4R、维多利亚蓝 B、维多利亚绿 B、水溶蓝 I、水溶曙红、二甲苯胺丽春红或黄色曙红。

[0108]　在某些实施例中，除咕吨染料之外，本发明的组合物包括上文列出的其它生色团中的任何一个生色团，或它们的组合，从而在施用部位提供生物光子影响。这是这些试剂完全不同的应用，与生色团作为简单的染色剂或作为光聚合的催化剂的用途完全不同。

[0109]　例如，可以根据其发射波长性质（荧光团时）、根据其能量转移潜力、其产生反应性氧种类的能力或其抗菌效果，来选择生色团。这些需要因所需治疗的身体状况而不同。例如，叶绿素可能对面部的细菌具有抗菌效果。

[0110]　(c) 胶凝剂

[0111]　组合物任选可能包含一种胶凝剂。用于本发明的胶凝剂可包含适合用于本发明所述局部生物光子配方的任何成分。胶凝剂可以是能够形成交联（包括物理与／或化学交联）基质的试剂。胶凝剂优选是生物相容的，及生物可降解的。在一些实施例中，胶凝剂能够形成水凝胶或水胶体。合适的胶凝剂是能够形成粘稠液体或半固体的试剂。在优选的实施例中，胶凝剂与／或组合物具有适当的透光性。选择允许生色团生物光子活性的胶凝剂也是非常重要的。例如，要发射荧光，有些生色团需要水合环境。胶凝剂本身或其与其它组分（如水或另一种胶凝剂）结合能够形成凝胶，或在施用到治疗部位或用光照射时，能够形成凝胶。

[0112]　在一些实施例中，组合物是凝胶、霜、油膏、洗液、糊、喷雾或泡沫等形式。

[0113]　本发明各实施例中所述的胶凝剂可包括聚烷撑氧，尤其是聚乙二醇和聚（环氧乙烷）- 聚（环氧丙烷）共聚物，包括嵌段和无规共聚物；多元醇，如丙三醇、聚丙三醇（尤其

是高支化聚丙三醇），丙二醇和被一个或多个聚烷撑氧取代的丙二醇，如单 -、二 - 和三 - 聚氧乙烯丙三醇、单 - 和双 - 聚氧乙烯丙二醇，及单 - 和双 - 聚氧乙烯丙二醇；聚氧乙烯山梨醇、聚氧乙烯葡萄糖；丙烯酸聚合物及其类似物和共聚物，如聚丙烯酸本身、聚甲基丙烯酸、聚（羟基乙基甲基丙烯酸酯）、聚（羟基乙基丙烯酸酯）、聚（甲基烷基亚砜甲基丙烯酸酯）、聚（甲基烷基亚砜丙烯酸酯）及它们的共聚物，与 / 或与其它丙烯酸酯的共聚物，如氨基乙基丙烯酸酯和单 -2-（丙烯醛基氧基）- 乙基琥珀酸酯；聚马来酸；聚（丙烯酰胺），如聚丙烯酰胺本身、聚（甲基丙烯酰胺）、聚（二甲基丙烯酰胺），及聚（N- 异丙基 - 丙烯酰胺）；聚（烯醇），如聚（乙烯醇）；聚（N- 乙烯基内酰胺），如聚（乙烯基吡咯烷酮）、聚（N- 乙烯基己内酰胺），及它们的共聚物，聚恶唑啉，包括聚（甲基恶唑啉）和聚（乙基恶唑啉）；及聚乙烯胺。

[0114] 本发明各实施例中所述的胶凝剂可包括聚合物，选自任何合成的或半合成的聚合物材料、聚丙烯酸酯共聚物、纤维素衍生物和聚甲基乙烯基醚 / 马来酸酐共聚物。在一些实施例中，亲水性聚合物包括高分子量聚合物（即摩尔质量大于大约 5000，在某些情况下，大于大约 10,000 或 100,000 或 1,000,000）与 / 或交联聚丙烯酸聚合物。在一些实施例中，聚合物是聚丙烯酸聚合物，粘度是大约 15,000-100,000、15,000-90,000、15,000-80,000、20,000-80,000、20,000-70,000、20,000-40,000cP。在一些实施例中，聚合物是高分子量与 / 或交联聚丙烯酸聚合物，其中聚丙烯酸聚合物的粘度是大约 15,000-80,000cP。

[0115] 可以使用卡波姆。卡波姆是与烯丙基蔗糖或季戊四醇的烯丙基醚交联的合成高分子量丙烯酸聚合物，分子量是大约 3×10^6。胶凝机理取决于羧酸单元的形成可溶盐的中和作用。聚合物是亲水的，中和时形成光洁透明的凝胶。卡波姆凝胶具有良好的热稳定性，其凝胶粘度和产率基本上不受温度的影响。作为局部产品，卡波姆具有最佳的流变性能。这种固有的假塑性流动允许其在剪切终止时立即恢复粘度，其高收率和迅速破裂使其易于分配。由于存在游离的羧酸基，Carbopol® 的水溶液本质上呈酸性。这种溶液的中和作用使聚合物交联和胶质化，形成具有所需粘度的粘稠的整体结构。

[0116] 卡波姆可以是分散在水中而形成低粘度酸性胶态悬浮液的白色细粉末（1% 分散液的 pH 大约是 3）。悬浮液采用碱，例如，氢氧化钠、氢氧化钾或氢氧化铵、低分子量的胺和烷醇胺中和，形成半透明的凝胶。烟碱盐，如烟碱氯化物在 pH 大约 3.5 时与卡波姆形成稳定的水溶络合物，在最佳 pH 大约 5.6 时非常稳定。

[0117] 在本发明的某些实施例中，卡波姆是 Carbopol。此类聚合物可从 B.F.Goodrich 或 Lubrizol 获得，商品名称是 Carbopol® 71G NF、420、430、475、488、493、910、934、934P、940、971PNF、974P NF、980NF、981NF 等。正如 Brock(Pharmacotherapy,14:430-7(1994)) 和 Durrani(Pharmaceutical Res.(Supp.)8:S-135(1991)) 所述，Carbopol 是多用途的控释聚合物，属于与聚烯基聚醚交联的合成高分子量非线性丙烯酸聚合物卡波姆系列。在一些实施例中，卡波姆是 Carbopol® 974P NF、980NF、5984EP、ETD 2020NF、Ultrez 10NF、934NF、934P NF 或 940NF。在某些实施例中，卡波姆是 Carbopol® 980NF、ETD 2020NF、Ultrez 10NF、Ultrez 21 或 1382 聚合物、1342NF、940NF。

[0118] 在某些实施例中，胶凝剂包括吸湿性材料。吸湿性材料指的是能够吸收水的物质，例如，甚至在室温（如 25℃）、相对湿度低达 50% 条件下，能够吸收或吸附水的物质。吸湿

性材料包括但不限于葡糖胺、糖胺聚糖、聚（乙烯醇）、聚（2-羟基乙基甲基丙烯酸酯）、聚环氧乙烷、胶原蛋白、脱乙酰壳多糖、藻酸盐、聚（丙烯腈）-类水凝胶、聚（乙二醇）/聚（丙烯酸）互穿聚合物网络水凝胶、聚环氧乙烷-聚对苯二甲酸丁二醇酯、透明质酸、高分子量聚丙烯酸、聚（羟基乙基甲基丙烯酸酯）、聚（乙二醇）、四甘醇二丙烯酸酯、聚乙二醇甲基丙烯酸酯和聚（丙烯酸甲酯-共-丙烯酸羟乙酯）。

[0119] 本发明的生物光子组合物可以进一步包裹在例如膜中。所述膜可以是透明的与/或基本上或完全不渗透的。所述膜可以是液体不能透过但气体如空气可以透过的。在某些实施例中，组合物可以形成包裹生物光子局部组合物的生色团的膜，其中所述膜基本上不渗透液体与/或气体。

[0120] 所述组合物可以包括任何其它载体。

[0121] (d) 释氧剂

[0122] 根据一些实施例，本发明的组合物可以任选进一步包含释氧剂，例如，作为氧的来源。

[0123] 当本发明包含释氧剂的生物光子组合物被光照射时，呫吨染料被激发到更高的能态。当呫吨染料的电子返回到更低的能态时，它们放出能级更低的光子，从而发射出波长更长的光（斯托克斯频移）。在合适的环境中，某些释放的能量被转移给氧或反应性过氧化氢，导致形成氧自由基，如单线态氧。人们认为，生物光子组合物活化产生的单线态氧及其它反应性氧种类是以刺激效应方式操作的。也就是说，通过刺激和调节靶向组织细胞中的应激反应路径，在较少暴露于通常有毒的刺激（如反应性氧）的情况下可以实现有益的健康效果。对外源产生的自由基（反应性氧）的内源性响应得到调节，使其对抗外源性自由基的防御能力增加，从而诱导愈合和再生过程的加速。此外，组合物的活化还产生抗菌效果。细菌对暴露于自由基极为敏感，使本发明的组合物实际上是一种杀菌组合物。

[0124] 如上文所述，某些实施例中组合物引起的氧种类的产生伴随微泡的产生，有利于施用部位生物膜的脱落或去除。这可以改善治疗部位活化光与/或荧光的穿透情况，例如，使细菌菌落失活，使其数量减少。

[0125] 组合物中可以包含的合适的释氧剂包括但不限于过氧化物，如过氧化氢、过氧化脲和过氧化苯甲酰。过氧化物是含有过氧基(R-O-O-R)的释氧剂，具有链状结构，包含两个氧原子，每个氧原子与另一个氧原子连接并与基团或某些元素连接。

[0126] 过氧化氢(H_2O_2)是制备有机过氧化物的初始材料。H_2O_2是强大的释氧剂，过氧化氢具有独特性质，它分解为水和氧，不会形成任何持久性的有毒残余化合物。组合物中使用的过氧化氢可以在凝胶中使用，例如含6%过氧化氢。本发明组合物中可以使用的合适的过氧化氢的浓度是大约0.1%至大约6%。

[0127] 过氧化脲（亦称为尿素过氧化物、脲过氧化氢、过碳酰胺）可溶于水，并包含大约35%过氧化氢。组合物中使用的过氧化脲可作为凝胶使用，例如，16%过氧化脲，包含5.6%过氧化氢，或12%过氧化脲。本发明组合物中可以使用的合适的过氧化脲的浓度是大约0.3%至大约16%。过氧化脲以缓-释方式分解为脲和过氧化氢，该过程可通过加热或光化学反应加速。释放的脲[脲，$(NH_2)CO_2$)]易溶于水，是强大的蛋白质变性剂。它会提高某些蛋白质的溶解度和增强皮肤与/或粘膜的再水化。

[0128] 过氧化苯甲酰由两个与过氧化基团连接的苯甲酰基组成（苯甲酸中的羧酸H被脱

除）。人们发现，在用于痤疮的治疗中，过氧化苯甲酰的浓度是 2.5%至 10%。释放的过氧化基团在灭菌方面非常有效。过氧化苯甲酰还可以促进皮肤更新和毛孔清除，进一步减少细菌数量和减少痤疮。过氧化苯甲酰与皮肤接触时分解为苯甲酸和氧，这两者都没毒。本发明组合物中可以使用的合适的过氧化苯甲酰的浓度是大约 2.5%至大约 5%。

[0129] 其它释氧剂包括分子氧、水、过硼酸盐和碳酸盐。释氧剂可以是组合物内的粉末、液体或凝胶形式。组合物可以包含一定含量的释氧剂，通过将释氧剂单独施用于治疗部位而增强效果。

[0130] 或者，释氧剂也可以与组合物分开施用于组织。

[0131] (e) 愈合因子

[0132] 本发明的组合物可以包含愈合因子。愈合因子包含在组合物施用部位上的、能促进或增强组织愈合或再生过程的化合物。在本发明组合物光活化期间，治疗部位处皮肤、伤口或粘膜的分子吸收增加。一段时间内，观察到治疗部位血液流动量增加。加入愈合因子，因自由基级联的动态交互作用所引起的淋巴引流增加及可能的渗透平衡变化，都会得到改善或甚至被强化。合适的愈合因子包括但不限于：

[0133] 透明质酸（透明质酸盐）：是一种非硫酸化的糖胺聚糖，广泛分布在整个结缔组织、上皮组织及神经组织中。它是细胞外基质的主要成分之一，显著影响细胞增殖和细胞迁移。透明质酸是皮肤的主要成分，参与组织修复。虽然透明质酸在细胞外基质中大量存在，但是，它对组织流体力学、细胞运动和增殖都有影响，并参与大量细胞表面受体相互作用，特别是那些包含主受体 CD44 的相互作用。透明质酸酶降解透明质酸。人体内有至少七种类型的透明质酸酶类酶，其中几种酶是肿瘤抑制剂。透明质酸的降解产物、低聚糖及分子量非常低的透明质酸具有促血管生成的性质。此外，最近的研究表明，透明质酸片段，而不是透明质酸的天然高分子量，可以在组织损伤中的巨噬细胞和树突细胞中诱导炎症反应。透明质酸非常适合针对皮肤的生物应用。由于具有较高的生物相容性，透明质酸被用于刺激组织再生。研究已经表明，在愈合的初期出现透明质酸，实际上为介导免疫响应的白细胞创建了空间。它用于合成生物支架，用于伤口愈合应用及皱纹处理。本发明组合物中可以使用的透明质酸的合适浓度是大约 0.001%至大约 3%。

[0134] 葡糖胺：是人体组织内含量最多的单糖之一和生物合成糖基化蛋白质和类脂的前体。它通常用于治疗骨关节炎。葡糖胺的常用形式是其硫酸盐。葡糖胺具有多种作用，包括消炎活性、刺激蛋白聚糖的合成及蛋白水解酶的合成。本发明组合物中可以使用的葡糖胺的合适浓度是大约 0.01%至大约 3%。

[0135] 尿囊素：是乙醛酸的二酰脲。它具有促角质分离的作用，提高细胞外基质的含水量，增强死亡（凋亡）皮肤细胞上层的脱落，及促进皮肤增殖和伤口愈合。

[0136] (f) 杀菌剂

[0137] 本发明的组合物可以包含杀菌剂。杀菌剂杀灭微生物或抑制它们的生长或累积。示例性杀菌剂（或抗菌剂）在美国专利申请公开 20040009227 和 20110081530 中引用。本发明方法中使用的合适的杀菌剂包括但不限于酚类和氯化酚类化合物、间苯二酚及其衍生物、双酚化合物、苯甲酸酯（对羟基苯甲酸酯）、卤代碳酰苯胺、聚合物类杀菌剂、噻唑啉、三氯甲基硫代酰亚胺、天然杀菌剂（亦称为“天然精油”）、金属盐和广谱抗生素。

[0138] 本发明中可以使用的具体的酚类和氯代酚类杀菌剂包括但不限于：苯酚；2- 甲基

苯酚;3-甲基苯酚;4-甲基苯酚;4-乙基苯酚;2,4-二甲基苯酚;2,5-二甲基苯酚;3,4-二甲基苯酚;2,6-二甲基苯酚;4-正-丙基苯酚;4-正-丁基苯酚;4-正-戊基苯酚;4-叔戊基苯酚;4-正-己基苯酚;4-正-正庚基苯酚;单-和多-烷基和芳基卤代酚;对-氯苯基;甲基对-氯酚;乙基对-氯酚;正-丙基对-氯酚;正-丁基对-氯酚;正戊基-对-氯酚;仲-戊基对-氯酚;正-己基对-氯酚;环己基对-氯酚;正-庚基对-氯酚;正-辛基对-氯酚;邻-氯酚;甲基邻-氯酚;乙基邻-氯酚;正-丙基邻-氯酚;正-丁基邻-氯酚;正-戊基邻-氯酚;叔-戊基邻-氯酚;正-己基邻-氯酚;正-庚基邻-氯酚;邻-苯甲基对-氯酚;邻-苯甲基-间-甲基对-氯酚;邻-苯甲基-m,m-二甲基对-氯酚;邻-苯基乙基对-氯酚;邻-苯基乙基-间-甲基对-氯酚;3-甲基对-氯酚;3,5-二甲基对-氯酚;6-乙基-3-甲基对-氯酚、6-正-丙基-3-甲基对-氯酚;6-异-丙基-3-甲基对-氯酚;2-乙基-3,5-二甲基对-氯酚;6-仲-丁基-3-甲基对-氯酚;2-异-丙基-3,5-二甲基对-氯酚;6-二乙基甲基-3-甲基对-氯酚;6-异-丙基-2-乙基-3-甲基对-氯酚;2-仲-戊基-3,5-二甲基对-氯酚;2-二乙基甲基-3,5-二甲基对-氯酚;6-仲-辛基-3-甲基对-氯酚;对-氯-间-甲酚对-溴苯酚;甲基对-溴苯酚;乙基对-溴苯酚;正-丙基对-溴苯酚;正-丁基对-溴苯酚;正-戊基对-溴苯酚;仲-戊基对-溴苯酚;正-己基对-溴苯酚;环己基对-溴苯酚;邻-溴苯酚;叔-戊基邻-溴苯酚;正-己基邻-溴苯酚;正-丙基-m,m-二甲基邻-溴苯酚;2-苯基苯酚;4-氯-2-甲基苯酚;4-氯-3-甲基苯酚;4-氯-3,5-二甲基苯酚;2,4-二氯-3,5-二甲基苯酚;3,4,5,6-四溴-2-甲基苯酚;5-甲基-2-戊基苯酚;4-异丙基-3-甲基苯酚;对-氯-间二甲苯酚(PCMX);氯百里酚;苯氧乙醇;苯氧异丙醇;及5-氯-2-羟基二苯甲烷。

[0139] 间苯二酚及其衍生物也可用作杀菌剂。具体的间苯二酚衍生物包括但不限于:甲基间苯二酚;乙基间苯二酚;正-丙基间苯二酚;正-丁基间苯二酚;正-戊基间苯二酚;正-己基间苯二酚;正-庚基间苯二酚;正-辛基间苯二酚;正-壬基间苯二酚;苯基间苯二酚;苯甲基间苯二酚;苯乙基间苯二酚;苯丙基间苯二酚;对-氯苯甲基间苯二酚;5-氯-2,4-二羟基二苯甲烷;4'-氯-2,4-二羟基二苯甲烷;5-溴-2,4-二羟基二苯甲烷;及4'-溴-2,4-二羟基二苯甲烷。

[0140] 本发明中可以使用的具体的双酚抗菌剂包括但不限于:2,2'-亚甲基双-(4-氯苯酚);2,4,4'-三氯-2'-羟基-二苯醚,Ciba Geigy(地址:美国新泽西州 Florham Park)以商品名 Triclosan®销售的产品;2,2'-亚甲基双-(3,4,6-三氯苯酚);2,2'-亚甲基双-(4-氯-6-溴苯酚);双-(2-羟基-3,5-二氯对-己基)硫化物;及双-(2-羟基-5-氯苯甲基)硫化物。

[0141] 本发明中可以使用的具体的苯甲酸酯(对羟基苯甲酸酯)包括但不限于:对羟基苯甲酸甲酯;对羟基苯甲酸丙酯;对羟基苯甲酸丁酯;对羟基苯甲酸乙酯;对羟基苯甲酸异丙酯;对羟基苯甲酸异丁酯;对羟基苯甲酸苄酯;对羟基苯甲酸甲酯钠;及对羟基苯甲酸丙酯钠。

[0142] 本发明中可以使用的具体的卤代碳酰苯胺包括但不限于:3,4,4'-三氯碳酰苯胺,如3-(4-氯苯基)-1-(3,4-二氯苯基)脲,Ciba-Geigy(地址:美国新泽西州 Florham Park)以商品名 Triclocarban®销售的产品;3-三氟甲基-4,4'-二氯碳酰苯胺;及3,3',4-三氯碳酰苯胺。

[0143] 本发明中可以使用的具体的聚合物抗菌剂包括但不限于：聚六亚甲基双胍盐酸盐；及聚（亚胺基酰亚胺羰基亚胺基酰亚胺羰基亚胺基六亚甲基盐酸盐），以商品名 Vantocil® IB 销售。

[0144] 本发明中可以使用的具体的噻唑啉包括但不限于：以商品名 Micro-Check® 销售的产品；及 2- 正 - 辛基 -4- 异噻唑啉 -3- 酮，以商品名 Vinyzene® IT-3000DIDP 销售。

[0145] 本发明中可以使用的具体的三氯甲基硫代酰亚胺包括但不限于：N-（三氯甲基硫代）邻苯二甲酰亚胺，以商品名 Fungitrol® 销售；及 N- 三氯甲基硫代 -4- 环己烯 -1，2- 二甲酰亚胺，以商品名 Vancide® 销售。

[0146] 本发明中可以使用的具体的天然抗菌剂包括但不限于下述物质的油：大茴香；柠檬；柑桔；迷迭香；鹿蹄草；百里香；熏衣草；丁香；酒花；茶树；枫茅；小麦；大麦；芸香草；雪松叶；杉木；肉桂；旋复花草（fleagrass）；老鹳草；檀香；紫罗兰；越橘；桉树；马鞭草；薄荷；安息香胶；罗勒；茴香；枞树；凤仙花；薄荷醇；牛至（ocmea origanuin）；黄连碱；角叉菜（carradensis）；小檗科（Berberidaceac daceae）；拉坦尼根（Ratanhiae longa）；及姜黄。归入天然抗菌剂一类的还包括人们发现的植物油中能够提供抗菌好处的关键化学成分。这些化学物质包括但不限于：茴香脑；儿茶酚；莰烯；百里酚；丁香酚；桉叶醇；阿魏酸；法尼醇；扁柏酚；草酚酮；柠檬烃；薄荷醇；水杨酸甲酯；香芹酚；松油醇；马鞭草烯酮；小檗碱；拉坦尼根（ratanhiae）精华；丁香烯氧化物；香茅酸；姜黄；橙花叔醇；及香叶醇。

[0147] 本发明中可以使用的具体的金属盐包括但不限于元素周期表 3a-5a、3b-7b 和 8 族金属的盐。金属盐的具体实例包括但不限于以下金属的盐：铝；锆；锌；银；金；铜；镧；锡；汞；铋；硒；锶；钪；钇；铈；镨；钕；钷；钐；铕；钆；铽；镝；钬；铒；铊；镱；镏；及它们的混合物。金属离子类抗菌剂的一个实例是 HealthShield Technology（地址：美国马萨诸塞州 Wakefield）生产、以商品名 HealthShield® 销售的一种抗菌剂。[此处给出其它实例，如施乐辉（smith and nephew）]

[0148] 本发明可以使用的具体的广谱抗菌剂包括但不限于本发明其它抗菌剂类别中引用的那些抗菌剂。

[0149] 本发明方法中可以使用的其它抗菌剂包括但不限于：吡硫翁，特别是吡硫翁 - 包括锌络合物，如以商品名 Octopirox® 销售的产品；二甲基羟甲基乙内酰脲，以商品名 Glydant® 销售的产品；甲基氯异噻唑啉酮 / 甲基异噻唑啉酮，以商品名 Kathon CG® 销售的产品；亚硫酸钠；亚硫酸氢钠；咪唑烷基脲，以商品名 Germall 115® 销售的产品；二唑烷基脲，以商品名 Germall 11® 销售的产品；苯甲醇 v2- 溴 -2- 硝基丙烷 -1，3- 二醇，以商品名 Bronopol® 销售的产品；福尔马林或甲醛；碘代丙烯基丁氨基甲酸酯，以商品名 Polyphase P100® 销售；氯乙酰氨；六亚甲基四胺；甲基二溴腈戊二腈（1，2- 二溴 -2，4- 二氰基丁烷），以商品名 Tektamer® 销售；戊二醛；5- 溴 -5- 硝基 -1，3- 二恶烷，以商品名 Bronidox® 销售；苯乙醇；邻 - 苯基苯酚 / 钠邻 - 苯基苯酚钠羟甲基甘氨酸盐，以商品名 Suttocide A® 销售；聚甲氧基双环恶唑烷，以商品名 Nuosept C® 销售；硫柳汞；二氯苯

甲醇；克菌丹；chlorphenenesin；双氯酚；氯丁醇；月桂酸甘油酯；卤代二苯醚；2,4,4'－三氯－2'－羟基－二苯醚，以商品名Triclosan®销售，并且可从Ciba-Geigy（地址：美国新泽西州Florham Park）获得；及2,2'－二羟基－5,5'－二溴－二苯醚。

[0150] 本发明方法中可以使用的其它抗菌剂包括美国专利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；和5,919,554，及美国专利申请公开Nos.20040009227和20110081530中公开的那些抗菌剂。

[0151] (g) 胶原蛋白和促进胶原蛋白合成的试剂

[0152] 本发明的组合物可以包含胶原蛋白及促进胶原蛋白合成的试剂。胶原蛋白是真皮成纤维细胞产生的纤维状蛋白质，构成皮肤的70％。胶原蛋白使皮肤光滑、紧致。因此，当胶原蛋白合成减少时，将出现皮肤老化，皮肤紧致度和光滑度迅速降低。因此，皮肤将失去光泽和出现皱纹。另一方面，当胶原蛋白代谢被皮肤中胶原蛋白合成刺激活化时，真皮基质的成分将增加，引起皱纹改善、紧致度改善及皮肤强度增加等效果。因此，胶原蛋白和促进胶原蛋白合成的试剂在本发明中也有用。促进胶原蛋白合成的试剂（即原胶原蛋白合成试剂）包括氨基酸、缩氨酸、蛋白质、类脂；小化学分子、天然产品及天然产品提取物。

[0153] 例如，人们发现，摄入维生素C、铁和胶原蛋白可有效提高皮肤或骨骼中胶原蛋白的数量。参见，例如，美国专利申请公开20090069217。维生素C的实例包括抗坏血酸的衍生物，如L-抗坏血酸或L-抗坏血酸钠，利用乳化剂等涂敷抗坏血酸得到的抗坏血酸制剂，及含任意比例的两种或多种维生素C的混合物。此外，也可以使用含维生素C的天然产品，如金虎尾和柠檬。铁制剂的实例包括：无机铁，如硫酸亚铁、柠檬酸亚铁钠，或焦磷酸铁；有机铁，如血红素铁、铁蛋白铁，或乳铁蛋白铁；含任意比例的两种或多种这些铁制剂的混合物。此外，还可以使用含铁天然产品，如菠菜或动物肝脏。此外，胶原蛋白的实例包括：用酸或碱处理哺乳动物如牛或猪的骨头、皮肤等获得的提取物；用蛋白酶如胃蛋白酶、胰蛋白酶或糜蛋白酶水解提取物获得的缩氨酸；及含任意比例的两种或多种这些胶原蛋白的混合物。还可以使用从植物提取的胶原蛋白。

[0154] 例如，在美国专利7598291、7722904、6203805、5529769等及美国专利申请公开20060247313、20080108681、20110130459、20090325885、20110086060等中对其它原胶原蛋白合成剂进行了描述。

[0155] (4) 使用方法

[0156] 本发明的光子组合物具有多种用途。本发明的生物光子组合物可以促进伤口愈合或组织修复，但并不受限于这一理论。本发明的生物光子组合物还可用于治疗皮肤病。本发明的生物光子组合物还可用于治疗痤疮。本发明的生物光子组合物还可用于皮肤更新。本发明的生物光子组合物还可用于治疗急性炎症。因此，本发明的目的是提供一种可以用生物光子疗法治疗伤口的方法，其中所述方法促进伤口愈合。本发明的另一个目的是提供一种用生物光子疗法治疗患痤疮的皮肤组织的方法，其中所述方法用于治疗痤疮。本发明还有一个目的是提供一种用生物光子疗法治疗患皮肤病的皮肤组织的方法，其中所述方法用于治疗皮肤病。本发明还有一个目的是提供一种用生物光子疗法治疗皮肤组织的方法，其中所述方法用于促进皮肤更新。

[0157] 在一些实施例中，本发明提供一种用生物光子疗法治疗伤口的方法，包括：将本发

明生物光子组合物施用（例如，局部施用）到伤口部位，及用波长与生物光子组合物中第一呫吨染料（例如，供体呫吨染料）的吸收光谱重叠的光照射该生物光子组合物。

[0158] 在另一方面，本发明提供一种促进皮肤更新的方法。在一些实施例中，本发明提供一种促进皮肤更新的方法，包括：将本发明生物光子组合物施用（例如，局部施用）到皮肤上，及用波长与生物光子组合物中第一呫吨染料（例如，供体呫吨染料）的吸收光谱重叠的光照射该生物光子组合物。

[0159] 在另一方面，本发明提供一种用生物光子疗法治疗患有皮肤病的靶向皮肤组织的方法。在一些实施例中，本发明提供一种用生物光子疗法治疗靶向皮肤组织的方法，包括：将本发明生物光子组合物施用（例如，局部施用）到靶向皮肤组织，及用波长与生物光子组合物中第一呫吨染料（例如，供体呫吨染料）的吸收光谱重叠的光照射该生物光子组合物。

[0160] 在另一方面，本发明提供一种用生物光子疗法治疗患有痤疮的靶向皮肤组织的方法。在一些实施例中，本发明提供一种用生物光子疗法治疗患有痤疮的靶向皮肤组织的方法，包括：将本发明生物光子组合物施用（例如，局部施用）到靶向皮肤组织，及用波长与生物光子组合物中第一呫吨染料（例如，供体呫吨染料）的吸收光谱重叠的光照射该生物光子组合物。

[0161] 在一些实施例中，本发明提供一种治疗急性炎症的方法，包括：将本发明生物光子组合物局部施用到患有急性炎症的靶向皮肤组织上，及用波长与生物光子组合物中第一呫吨染料（例如，供体呫吨染料）的吸收光谱重叠的光照射该生物光子组合物。

[0162] 适合本发明方法用途的生物光子组合物选自上述生物光子组合物的任一实施例。例如，用于本发明方法的生物光子组合物可以包含光照射时发生至少部分光漂白的第一呫吨染料。第一呫吨染料可以在波长大约200-800nm、200-700nm、200-600nm或200-500nm处吸收。在一个实施例中，第一呫吨染料在波长大约200-600nm处吸收。在某些实施例中，第一呫吨染料在波长大约200-300nm、250-350nm、300-400nm、350-450nm、400-500nm、450-650nm、600-700nm、650-750nm或700-800nm吸收。第二呫吨染料的吸收光谱与第一呫吨染料的发射光谱应至少重叠大约80％、50％、40％、30％或20％。在一些实施例中，第一呫吨染料的发射光谱与第二呫吨染料的吸收光谱至少重叠1-10％、5-15％、10-20％、15-25％、20-30％、25-35％、30-40％，35-45％、50-60％、55-65％或60-70％。

[0163] 利用光照射生物光子组合物会导致能量从第一呫吨染料转移到第二呫吨染料。然后，第二呫吨染料以荧光与/或产生反应性氧种类的形式放出能量。在本发明的一些实施例中，光照射导致的能量转移并不伴随热量的产生，或者并不导致组织损伤。

[0164] 用于本发明的生物光子组合物可以采用任何载体配制。在一些实施例中，载体是一种胶凝剂。胶凝剂包括但不限于：类脂（如丙三醇）、乙二醇（如丙二醇）、透明质酸、硫酸盐葡萄糖胺、纤维素衍生物（羟丙基甲基纤维素、羟乙基纤维素、羟丙基纤维素、甲基纤维素等）、非纤维素多糖（半乳糖甘露聚糖、瓜尔豆胶、角豆树胶、阿拉伯胶、苹婆胶、琼脂、藻朊酸盐等）及丙烯酸聚合物。

[0165] 在本发明方法中，可以使用任何光化光光源。任何类型的卤素灯、LED或等离子弧灯或激光器都是合适的。合适的光化光光源的主要特点是其发射一个（或多个）波长适合活化组合物中一个或多个生色团的光。在一个实施例中，使用氩激光器。在另一个实施例中，使用磷酸钛氧钾(KTP)激光器（如GreenLight^{TM}激光器）。在另一个实施例中，可以

采用日光。在另一个实施例中，光化光光源是 LED 光固化设备。在另一个实施例中，光化光光源是波长大约 200 至 800nm 的光源。在另一个实施例中，光化光光源是波长大约 400 至 600nm 的可见光光源。此外，光化光光源应具有合适的功率密度。非准直光源（LED、卤素灯或等离子灯）合适的功率密度是大约 $1mW/cm^2$至大约 $200mW/cm^2$。激光器光源合适的功率密度是大约 $0.5mW/cm^2$至大约 $0.8mW/cm^2$。

[0166] 在本发明方法的一些实施例中，光源在受试者皮肤、伤口或粘膜表面处的能量是大约 $1mW/cm^2$至大约 $500mW/cm^2$、$1\text{-}300mW/cm^2$或 $1\text{-}200mW/cm^2$，其中施用的能量至少取决于治疗条件、光的波长、受试者皮肤距光源的距离及生物光子组合物的厚度。在一些实施例中，受试者皮肤处的光是大约 $1\text{-}40mW/cm^2$，或 $20\text{-}60mW/cm^2$，或 $40\text{-}80mW/cm^2$，或 $60\text{-}100mW/cm^2$，或 $80\text{-}120mW/cm^2$，或 $100\text{-}140mW/cm^2$，或 $120\text{-}160mW/cm^2$，或 $140\text{-}180mW/cm^2$，或 $160\text{-}200mW/cm^2$，或 $110\text{-}240mW/cm^2$，或 $110\text{-}150mW/cm^2$，或 $190\text{-}240mW/cm^2$。

[0167] 在一些实施例中，可采用移动设备活化本发明实施例中的生物光子组合物，其中所述移动设备可以发射与生物光子组合物中供体呫吨染料的吸收光谱相重叠的发射光谱。所述移动设备有一个显示屏，光发射穿过该显示屏与 / 或移动设备可以从闪光灯发射光，发射的光可以光活化生物光子组合物。

[0168] 在一些实施例中，可以采用电视机或计算机上的显示屏活化生物光子组合物，其中显示屏可以发射与生物光子组合物中供体呫吨染料的吸收光谱相重叠的发射光谱。

[0169] 在一些实施例中，第一与 / 或第二呫吨染料可以通过来源于太阳或其它光源的环境光进行光活化。环境光可视为普通照射，从无可见的光源的房间内的所有方向照过来。在一些实施例中，第一与 / 或第二呫吨染料可以通过电磁谱可见范围内的光进行光活化。照射环境光的时间可以比照射直射光的时间更长。

[0170] 在一些实施例中，可以采用不同的光源活化生物光子组合物，如结合环境光和直射 LED 光。

[0171] 所需的暴露于光化光的时间长短，将取决于治疗区的表面；治疗损伤、创伤或伤害的类型；光源的功率密度、波长和带宽；生物光子组合物的厚度及治疗时距光源的距离。荧光照射治疗区可在数秒内发生或甚至在不到一秒内发生，但是，为了利用吸收、反射和重新发射的光对本发明组合物的协调效应及其与治疗组织的相互作用，延长照射时间是有益的。在一个实施例中，施用生物光子组合物的组织、皮肤或伤口照射光化光的时间是 1 分钟至 5 分钟。在另一个实施例中，施用生物光子组合物的组织、皮肤或伤口照射光化光的时间是 1 分钟至 5 分钟。在一些实施例中，生物光子组合物照射 1 分钟至 3 分钟。在一些实施例中，光照射的时间是 1-30 秒、15-45 秒、30-60 秒、0.75-1.5 分钟、1-2 分钟、1.5-2.5 分钟、2-3 分钟、2.5-3.5 分钟、3-4 分钟、3.5-4.5 分钟、4-5 分钟、5-10 分钟、10-15 分钟、15-20 分钟、20-25 分钟或 20-30 分钟。在另一个实施例中，光化光的光源在治疗区上方连续移动，照射合适的时间。在另一个实施例中，多次施用生物光子组合物和光化光。在一些实施例中，组织、皮肤或伤口至少照射两次、三次、四次、五次或六次光化光。在一些实施例中，在照射光化光之前先新施用生物光子组合物。

[0172] 在本发明的方法中，可任选在光照射后将生物光子组合物从治疗部位清除。在一些实施例中，生物光子组合物留在治疗部位 30 分钟以上、1 小时以上、2 小时以上、3 小时以上。可以采用环境光照射。为了防止干燥，组合物可以采用照射前可以取下的透明或半透

明遮盖物（例如聚合物膜）或不透明遮盖物进行遮盖。

[0173]　(5) 伤口和伤口愈合

[0174]　本发明的生物光子组合物和方法可用于治疗伤口和促进伤口愈合。本发明的生物光子组合物和方法可以治疗的伤口包括，例如，不同方式引发的皮肤和皮下组织的伤口（如卧床时间太长引起的压迫性溃疡、外伤引起的伤口、牙周炎等症状引起的伤口）及具有不同特点的伤口。在一些实施例中，本发明提供治疗例如，灼伤、切口、切除术、撕裂、磨损、刺伤或贯通伤口、手术伤口、挫伤、血肿、压伤、枪伤、疮和溃疡与／或促进这些伤口的愈合的生物光子组合物和方法。

[0175]　本发明的生物光子组合物和方法可用于治疗慢性皮肤溃疡或伤口与／或促进这些伤口的愈合，这些伤口无法通过一系列有序、及时的事件而实现持久的结构性、功能性及美观性闭合。绝大多数慢性伤口可以根据其病原学分为三类：压迫性溃疡、神经病变性（糖尿病足部）溃疡及血管性（静脉或动脉）溃疡。

[0176]　在其它一些实施例中，本发明提供了用于治疗 I-IV 级溃疡与／或促进 I-IV 级溃疡愈合的生物光子组合物和方法。在一些实施例中，本申请提供尤其适合 II 级溃疡的组合物。根据伤口的深度，溃疡可以分为四级：i) I 级：伤口限于上皮组织；ii) II 级：伤口延伸到真皮；iii) III 级：伤口延伸到皮下组织，及 iv) IV 级（或全层伤口）：骨头露出来的伤口（例如，位于例如较大的转节或骶骨等骨压点的伤口）。

[0177]　例如，本发明提供用于治疗糖尿病溃疡与／或促进糖尿病溃疡愈合的生物光子组合物和方法。由于神经和血管并发症，糖尿病患者容易患足部溃疡和其它溃疡。周围神经病变会导致足部与／或腿部感觉改变或完全丧失。晚期神经病变的糖尿病患者完全丧失了辨别剧痛的能力。患神经病变的患者足部出现任何伤口或创伤时，他们可能数天或数周都完全没注意到。晚期神经病变患者丧失了感觉持续压力刺激的能力，结果，可能出现组织缺血和坏疽，导致，例如，足底溃疡。微血管疾病是糖尿病显著的并发症之一，也会导致溃疡。在一些实施例中，本发明提供了治疗慢性伤口的组合物和方法，其中慢性伤口的特征在于因糖尿病神经病变与／或血管并发症而引起的糖尿病足部溃疡与／或溃疡。

[0178]　在其它实例中，本发明提供用于治疗压迫性溃疡与／或促进压迫性溃疡愈合的生物光子组合物和方法。压迫性溃疡包括褥疮、褥疮性溃疡和坐骨结节溃疡，这些溃疡会给患者带来巨大的痛苦和不适。压迫性溃疡是由长期施加到皮肤上的压力引起的。因此，由于个人的重量或质量，压力可以施加到患者的皮肤上。当皮肤一个区域供血阻塞或中断两小时或三小时以上时，会形成压迫性溃疡。受影响的皮肤区域会变红、疼痛及坏死。如果不进行治疗，皮肤会露出来，受到感染。因此，疮疡是因长期卧床、坐轮椅与／或戴石膏绷带所造成的压力下皮肤某一区域发生的皮肤溃疡。当一个人卧床不起、失去意识、无法感觉疼痛或不能动时，会发生压迫性溃疡。压迫性溃疡通常发生在身体的骨头突出部分，如臀部区（骶骨或髂嵴）或足跟。

[0179]　在其它实例中，本发明提供用于治疗急性伤口与／或促进急性伤口愈合的生物光子组合物和方法。

[0180]　本发明的生物光子组合物和方法可以治疗的其它类型的伤口包括美国专利申请公开 No.20090220450 中披露的那些伤口，该专利通过引用而并入本发明中。

[0181]　成年人组织中伤口的愈合是一个复杂的修复过程。例如，皮肤的愈合过程涉及伤

口部位各种专门细胞的补充、细胞外基质和基底膜沉积、血管形成、选择性的蛋白酶活性及表皮再植。

[0182] 伤口愈合过程有三个明显的阶段。首先，在炎症阶段，通常从伤口出现到两天至五天，血小板聚集从而沉积肉牙，促进纤维蛋白的沉积及刺激生长因子的释放。白血球迁移到伤口部位，开始消解伤口处的碎片和将碎片运走。在此炎症阶段，单核细胞还转化为巨噬细胞，后者释放出生长因子，刺激血管的形成和成纤维细胞的产生。

[0183] 其次，在增生阶段，通常发生在两天至三周，肉牙组织形成，开始上皮形成和收缩。成纤维细胞是该阶段的关键细胞类型，它们通过增生和合成胶原蛋白来填充伤口，提供强大的基质供上皮细胞生长。当成纤维细胞产生胶原蛋白时，从附近血管延伸形成血管，导致形成肉牙组织。肉牙组织通常从伤口底部生长。上皮形成涉及上皮细胞从伤口表面迁移，从而封住伤口。上皮细胞被接触类似细胞的需求推动，并受到充当网格作用的纤维蛋白链网络的引导，这些细胞在网格上迁移。在伤口处出现称为肌成纤维细胞的收缩细胞，帮助伤口闭合。这些细胞显示出胶原蛋白合成和收缩性，并且在肉牙性伤口中比较常见。

[0184] 再次，重塑阶段，即伤口愈合的最后阶段，从三周到几年，伤疤中的胶原蛋白经历反复降解和重新合成。在此阶段，新形成的皮肤的拉伸强度提高。

[0185] 但是，当伤口愈合速度增加时，通常伤疤形成相应增加。结疤是大多数成年动物和人类组织愈合过程的结果。疤痕组织与其代替的组织不同，其功能质量通常更差。疤痕的类型包括但不限于萎缩性疤痕、增生性疤痕和瘢痕瘤（keloidal scar），以及瘢痕挛缩。萎缩性疤痕呈扁平，其表面低于周围皮肤下，形成谷或洞。增生性疤痕是留在原损伤边界内的隆起疤痕，通常含有以异常方式布置的过多的胶原蛋白。瘢痕瘤（keloidal scar）是在原伤口边缘外扩散的隆起疤痕，以位点特异性方式侵入到正常皮肤附近，通常含有以异常方式布置的胶原蛋白螺旋。

[0186] 与此相反的是，正常皮肤由以网织篮式方式布置的胶原蛋白纤维，有助于真皮的强度和弹性。因此，为了使伤口愈合过程更顺利，需要一种方法，不仅刺激胶原蛋白的产生，而且还以减少疤痕形成的方式刺激胶原蛋白的产生。

[0187] 本发明的生物光子组合物和方法通过促进基本上均匀的上皮形成、促进胶原蛋白合成、促进控制收缩，与/或减少疤痕组织形成而促进伤口愈合。在一些实施例中，本发明的生物光子组合物和方法可以通过促进基本上均匀的上皮形成而促进伤口愈合。在一些实施例中，本发明的生物光子组合物和方法促进胶原蛋白合成。在一些实施例中，本发明的生物光子组合物和方法促进控制性收缩。在一些实施例中，本发明的生物光子组合物和方法，例如，通过减少疤痕组织的形成或通过加速伤口闭合过程而促进伤口愈合。在一些实施例中，本发明的生物光子组合物和方法，例如，通过减少炎症而促进伤口愈合。在一些实施例中，生物光子组合物可在伤口闭合后使用，用于优化疤痕修复。在这种情况下，可以定期施用生物光子组合物，例如，一周一次，或医生认为合适的间隔。

[0188] 生物光子组合物可以浸到织物或无纺材料中或海绵中，以伤口敷料的形式施用。可在伤口敷料或组合物内部或附近提供光源，如LED或波导来照射组合物。波导可以是不仅从其两端，而且从其纤体传输光的光纤。例如，由聚碳酸酯或聚甲基丙烯酸甲酯制造的光纤。

[0189] 可以使用局部或全身的辅助治疗，如抗生素治疗。也可使用负压辅助伤口闭合来

帮助伤口闭合与/或清除组合物。

[0190] (6) 痤疮和痤疮疤痕

[0191] 本发明的生物光子组合物和方法还可用于治疗痤疮。本发明所述“痤疮”指的是皮肤腺或毛囊炎症引起的皮肤病。本发明的生物光子组合物和方法可用于治疗痤疮的早期萌前阶段，也可用于在其病灶已可见的后期阶段治疗痤疮。本发明的生物光子组合物和方法实施例可用于治疗轻度、中度和重度痤疮。痤疮早期萌前阶段通常开始于皮脂分泌过度，或由位于毛皮脂内的皮脂腺所分泌的皮肤油。皮脂通过毛囊管到达皮肤表面。管道中和皮肤上存在过多皮脂会阻塞或淤塞毛囊管的正常的皮脂流动，从而使皮脂增厚和固化，形成称为粉刺的固体塞。在痤疮形成的正常顺序中，刺激毛囊孔形成过度角质化，从而完全堵住管道。通常的结果是丘疹、脓疱或囊肿，通常被细菌污染，导致继发性感染。痤疮的特征尤其在于存在粉刺、炎性丘疹或囊肿。痤疮的外观可能是轻微的皮肤刺激性至坑点，甚至可能发展成影响容貌的疤。因此，本发明的光子组合物和方法可用于治疗一种或多种与痤疮有关的皮肤刺激、坑点、疤痕形成、粉刺、炎性丘疹、囊肿、角质化及皮脂增厚和硬化。

[0192] 组合物可以浸到或涂到织物或无纺材料中或海绵中，以遮盖物的形式施用到身体各部位，如面部、身体、手臂、腿部等。可在遮盖物或组合物内部或附近提供光源(如LED或波导)，用以照射组合物。波导可以是不仅从其两端，而且从其纤体传输光的光纤。例如，由聚碳酸酯或聚甲基丙烯酸甲酯制造的光纤。

[0193] 本发明的生物光子组合物和方法可用于治疗各种类型的痤疮。例如，痤疮的一些类型，包括寻常痤疮、囊性痤疮、萎缩性痤疮、溴痤疮、氯痤疮、聚合性痤疮、美容性痤疮、去污剂痤疮、流行痤疮、表皮痤疮、夏季痤疮、暴发性痤疮、卤素痤疮、硬结性痤疮、碘痤疮、瘢痕瘤性痤疮、机械性痤疮、丘疹性痤疮、发蜡痤疮、月经前座疮、脓疱性痤疮、坏血病性痤疮、结核性痤疮、荨麻疹性痤疮、痘样痤疮、中毒性痤疮、丙酸痤疮、人工痤疮、革兰氏阴性痤疮、类固醇痤疮及结节囊性痤疮。

[0194] (7) 皮肤老化和更新

[0195] 真皮是皮肤的第二层，含有皮肤的结构性元素结缔组织。真皮内有具有不同功能的各种类型的结缔组织。弹性纤维赋予皮肤弹性，胶原蛋白赋予皮肤强度。

[0196] 真皮和表皮之间的接合处是一个非常重要的结构。真皮-表皮接合处连结形成与手指类似的表皮嵴。表皮细胞从真皮内的血管接收其营养物质。表皮嵴增加表皮暴露于这些血管和所需营养物质的表面积。

[0197] 皮肤的老化伴随皮肤的显著生理学变化。新皮细胞的产生减缓，真皮-表皮接合处的表皮嵴变平。虽然弹性纤维的数量增加，但是，其结构和凝聚性下降。此外，胶原蛋白的数量和真皮的厚度随着皮肤老化而降低。

[0198] 胶原蛋白是皮肤细胞外基质的主要成分，提供结构性框架。在老化过程期间，胶原蛋白合成下降及胶原蛋白纤维不溶解，导致真皮变薄，丧失皮肤的生物力学性质。

[0199] 皮肤的生理学变化导致出现显著的老化症状，通常被称为时序老化、内在老化和光老化。皮肤变得更干燥、粗糙和脱屑增加，外表变得更暗淡无光，出现明显的细纹和皱纹。皮肤老化的其它症状包括但不限于：皮肤变薄和透明、底层脂肪流失(导致双颊凹陷和眼睛深陷，以及手部和颈部明显失去紧致度)、骨质疏松(由于骨质疏松，骨骼与皮肤分离，导致皮肤松驰)、皮肤干燥(可能发痒)、无法排汗充分冷却皮肤、面部长毛、雀斑、老年斑、蛛

状静脉、皮肤粗糙和革质皮肤、拉开时会消失的细纹、皮肤松弛或长斑。

[0200]　真皮－表皮接合处是基底膜，将表皮中的角化细胞与位于表皮下面的细胞外基质分离。该基底膜由两层组成：与角化细胞接触的基底层及与细胞外基质接触的下层网状层。基底层富含 IV 型胶原蛋白和层粘连蛋白，这些分子在为细胞连接而提供结构性网络和生物粘合特征方面起到作用。

[0201]　层粘连蛋白是糖蛋白，仅存在于基底膜内。它由三种多肽链（α 、β 和 γ）组成，以不对称交叉形状布置，通过二硫键保持在一起。这三种链以不同的亚型存在，导致层粘连蛋白有十二种不同的同分异构体，包括层粘连蛋白 -1 和层粘连蛋白 -5。

[0202]　真皮通过 VII 型胶原蛋白纤维被锚定在基底膜角化细胞的半桥粒处，半桥粒是位于角化细胞上的特异性接合点，由 α－整联蛋白和其它蛋白组成。层粘连蛋白，特别是层粘连蛋白 -5，在基底角化细胞中的半桥粒跨膜蛋白及 VII 型胶原蛋白之间构成实际的锚定点。

[0203]　已经证明，在老化皮肤中，层粘连蛋白 -5 合成和 VII 型胶原蛋白表达减少。这导致真皮和表皮之间的接触丧失，导致皮肤失去弹性和松弛。

[0204]　最近，通常称为表情纹的另一种类型的皱纹得到了普遍认可。这些皱纹要求弹性丧失，尤其是在真皮中，因此，当产生面部表情的面部肌肉施加应力到皮肤上时，皮肤不再能够恢复其原来状态，导致表情纹。

[0205]　本发明的组合物和方法促进皮肤更新。在一些实施例中，本发明的生物光子组合物和方法促进胶原蛋白的合成。在一些实施例中，本发明的组合物和方法可以减少、消除、延缓或甚至逆转一种或多种皮肤老化的症状，包括但不限于出现细纹或皱纹、皮肤变薄和透明、底层脂肪流失（导致双颊凹陷和眼睛深陷，以及手部和颈部明显失去紧致度）、骨质疏松（由于骨质疏松，骨骼与皮肤分离，导致皮肤松弛）、皮肤干燥（可能发痒）、无法排汗充分冷却皮肤、面部长毛、雀斑、老年斑、蛛状静脉、皮肤粗糙和革质皮肤、拉开时会消失的细纹、皮肤松弛或长斑。在一些实施例中，本发明的组合物和方法可以诱导毛孔缩小、增强皮肤各部分的紧致性，与／或增强皮肤半透明性。

[0206]　(8) 皮肤病

[0207]　本发明的生物光子组合物和方法可用于治疗皮肤病，包括但不限于红斑、毛细管扩张、光化毛细血管扩张、牛皮癣、皮肤癌、天疱疮、晒斑、皮炎、湿疹、疹子、脓疱病、单纯慢性苔癣、肥大性酒渣鼻、口周皮炎、须部假性毛囊炎、药疹、多形红斑、结节性红斑、环形肉芽肿、光化性角化病、紫癜、斑秃、口疮性口炎、药物皮炎、干性皮肤、皲裂、干燥病、寻常性鱼鳞癣、真菌感染、寄生虫感染、病毒感染、单纯疱疹、擦烂、瘢瘤、角化症、粟丘疹、触染性软疣、玫瑰糠疹、瘙痒症、荨麻疹和血管瘤和各种变形。皮炎包括接触性皮炎、特异反应性皮炎、脂溢性皮炎、钱币状皮炎、全身剥落性皮炎及静态性皮炎。皮肤癌包括黑色素瘤、基底细胞癌和鳞状细胞癌。

[0208]　某些皮肤病具有不同的症状，包括皮肤发红、潮红、灼痛、脱屑、疙瘩、丘疹、脓疱、粉刺、斑点、节结、囊、水泡、毛细管扩张、蛛状静脉、疮、表面刺激或疼痛、痒、炎症、红、紫或蓝色斑片或褪色、痣与／或肿瘤。因此，本发明的生物光子组合物和方法可用于治疗皮肤发红、潮红、灼痛、脱屑、疙瘩、丘疹、脓疱、粉刺、斑点、节结、囊、水泡、毛细管扩张、蛛状静脉、疮、表面刺激或疼痛、痒、炎症、红、紫或蓝色斑片或褪色、痣与／或肿瘤。急性炎症本身呈现

疼痛、发热、发红、肿胀和丧失功能。它包括虫咬等引起的变态反应的那些症状，如蚊子、蜜蜂、黄蜂叮咬、毒常春藤中毒、后消融治疗等。

[0209] 组合物可以浸到或涂到机织或无纺布材料上或海绵上，并以遮盖物形式施用到身体部位，用于治疗皮肤病。可在遮盖物或组合物内部或附近提供光源（如LED或波导），用以照射组合物。波导可以是不仅从其两端，而且从其纤体传输光的光纤。例如，由聚碳酸酯或聚甲基丙烯酸甲酯制造的光纤。

[0210] (9) 试剂盒

[0211] 本发明还提供制备与／或施用本发明任一组合物的试剂盒。所述试剂盒包含本发明的生物光子局部组合物。所述组合物可以包含占组合物重量大约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％或30％-40％的释氧剂。第一呫吨染料的含量占组合物重量的大约0.01-40％，第二呫吨染料的含量占组合物重量的大约0.01-40％。在一些实施例中，第一呫吨染料占组合物重量的大约0.001-1％、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％或35-40％。在一些实施例中，第二呫吨染料占组合物重量的大约0.001-1％、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％或35-40％。在一些实施例中，呫吨染料占组合物重量的大约0.05-40.05％。在一些实施例中，呫吨染料占组合物重量的大约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％或35-40.05％。

[0212] 在一些实施例中，所述试剂盒包含不止一种组合物，例如，包含第一组合物和第二组合物。第一组合物可以包含释氧剂，第二组合物可以包含液体或粉末形式的呫吨染料。在一些实施例中，所述试剂盒包括含本发明组合物的容器。

[0213] 所述组合物可装在容器内。所述容器可以是不透光的、不透气的与／或防泄漏的。示例性容器包括但不限于注射器、小瓶或小袋。例如，容器可以是双室注射器，在将组合物从室内往外推送时，室内的物质发生混合。在另一个实例中，小袋可以包括被一个易碎的膜所分隔的两个室。在另一个实例中，一个组分可以装在注射器中，并可注射到包含第二组分的容器内。容器可以是可以加压或不能加压的喷雾罐。组合物可以是液体与／或气体形式。

[0214] 生物光子组合物还可以装在容器内，容器包括用于容纳生物光子组合物的一种或多种成分的一个或多个室，及一个与一个或多个室相连接的、用于从容器内排出生物光子组合物的出口。

[0215] 在其它实施例中，试剂盒包括用于增强组合物治疗效果的全身或局部药物。例如，试剂盒包括用于治疗痤疮或伤口愈合的全身性或局部性抗生素或激素治疗药物。

[0216] 试剂盒中可以包含关于如何按照本发明使用生物光子组合物的书面说明书，或者书面说明书可以包括在包含本发明组合物的容器上，或与该容器放在一起。

[0217] 在一些实施例中，试剂盒可以进一步包含敷料。所述敷料可以是多孔或半多孔结构，用于接收生物光子组合物。敷料可以包含机织或无纺纤维材料。

[0218] 在试剂盒的一些实施例中，试剂盒可进一步包含光源，如波长适合用于活化生物光子组合物中的生色团的便携式灯。便携式灯可以用电池或可以充电。

[0219] 在一些实施例中，试剂盒进一步包含一种或多种波导。

[0220] 根据本发明，识别相当的组合物、方法和试剂盒是本领域技术人员熟悉的，除常规试验外不再需要进行其它实验。根据下述实例，将更全面地了解本发明，这些实例仅作解释用，无论如何都不应视为限制本发明。

[0221] 实例

[0222] 下面的实例用于解释本发明各实施例的实施。它们并不旨在限制或定义本发明的全部范围。

[0223] 实例 1- 凝胶中的荧光素和曙红 Y 的吸收 / 发射光谱

[0224] 对凝胶（含大约 12％过氧化脲）中 (i) 浓度大约 0.09mg/mL 的荧光素钠盐，(ii) 浓度大约 0.305mg/mL 的曙红 Y，及 (iii) 浓度大约 0.09mg/mL 的荧光素钠盐和浓度大约 0.305mg/mL 的曙红 Y 的混合物的光动学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0225] 吸收光谱和发射光谱示于图 5A 和 5B 中，图中表明，组合中生色团之间发生了能量转移。特别是，与采用单个生色团相比，采用曙红 Y 和生色团组合，吸收光谱和发射光谱都更宽。这意味着采用带宽更宽的光，可以活化多生色团组合物，在照射之后，多生色团光可以发射带宽更宽的光。换句话说，与单个生色团相比，多生色团组合物的发射的波长范围更宽。在此实例中，组合物发射可见光谱中的绿色、黄色和橙色波长的光。在照射期间，观察到曙红 Y 存在光漂白。此外，结果（未显示）表明，凝胶中过氧化物的存在并不影响吸收光谱和发射光谱。在本发明组合物和方法中，过氧化物是任选存在的。

[0226] 实例 2- 水溶液中荧光素和曙红 Y 的吸收 / 发射光谱

[0227] 对水溶液中 (i) 终浓度 0.18mg/mL 的荧光素，(ii) 浓度大约 0.305mg/mL 的曙红 Y，及 (iii) 浓度大约 0.18mg/mL 的荧光素和浓度大约 0.305mg/mL 的曙红 Y 的混合物的光动力学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0228] 吸收光谱和发射光谱示于图 6A 和 6B 中，图中表明，组合中生色团之间发生了能量转移。此外，与图 5A 和 5B 一样，与采用单个生色团相比，采用曙红 Y 和生色团组合，发射光谱更宽。组合物发射可见光谱中的绿色、黄色和橙色波长的光。实例 1 和实例 2 之间的吸收光谱和发射光谱的区别可以用介质（实例 1 是凝胶，实例 2 是水溶液）的光学区别进行解释，也可能用荧光浓度翻倍的影响进行解释。从中可以看出，向曙红 Y 中加入荧光素，加宽了曙红 Y 的吸收峰和发射峰的带宽。这一点使多生色团组合能够吸收用于光活化的范围更宽的波长，同时也能发射范围更宽的波长，这就可能产生不同的治疗作用。在照射期间，观察到曙红 Y 存在光漂白。

[0229] 实例 3- 凝胶中荧光桃红 B 和曙红 Y 的吸收 / 发射光谱

[0230] 对 12％脲凝胶中 (i) 终浓度 0.25mg/mL 的荧光桃红 B，(ii) 浓度大约 0.05mg/mL 的曙红 Y，及 (iii) 荧光桃红 B(0.25mg/mL) 和曙红 Y(0.05mg/mL) 的混合物的光动力学性质

进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0231] 吸收光谱和发射光谱示于图 7A 和 7B 中，图中表明，组合中生色团之间发生了能量转移。与前面两个实例一样，与采用单个生色团相比，采用荧光桃红 B 和曙红 Y 生色团组合，吸收光谱和发射光谱更宽。组合物发射可见光谱中的绿色、黄色、橙色和红色波长的光。

[0232] 实例 4- 水溶液中荧光桃红 B 和曙红 Y 的吸收 / 发射光谱

[0233] 对水溶液中 (i) 终浓度 0.25mg/mL 的荧光桃红 B，(ii) 浓度大约 0.08mg/mL 的曙红 Y，及 (iii) 荧光桃红 B(0.25mg/mL) 和曙红 Y(0.08mg/mL) 的混合物的光动力学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0234] 吸收光谱和发射光谱示于图 8A 和 8B 中，图中表明，组合中生色团之间发生了能量转移。与采用单个生色团相比，采用荧光桃红 B 和曙红 Y 生色团组合，吸收光谱和发射光谱更宽。组合物发射可见光谱中的绿色、黄色、橙色和红色波长的光。

[0235] 实例 5- 凝胶中荧光桃红 B 和荧光素的吸收 / 发射光谱

[0236] 对 12％脲凝胶中 (i) 终浓度大约 100μg/g 的荧光素，(ii) 浓度大约 100μg/g 的荧光桃红 B，及 (iii) 荧光素 (100μg/g) 和荧光桃红 B(100μg/g) 的光动学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0237] 吸收光谱和发射光谱示于图 9A 和 9B 中，图中表明，组合中生色团之间发生了能量转移。对于这种特定的生色团组合及在此浓度条件下，生色团组合中观察到与荧光素和荧光桃红 B 发射所对应的两个峰，在大约 577nm 吸收处的峰较高 (与单个生色团相比)。

[0238] 实例 6- 凝胶中的荧光素和玫瑰红的吸收 / 发射光谱

[0239] 对 12％脲凝胶中 (i) 终浓度大约 100μg/g 的荧光素，(ii) 浓度大约 100μg/g 的玫瑰红，及 (iii) 荧光素 (100μg/g) 和荧光桃红 B(100μg/g) 的光动学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0240] 吸收光谱和发射光谱示于图 10A 和 10B 中，图中表明，组合中生色团之间发生了能量转移。对于这种特定的生色团组合及在此浓度条件下，组合生色团组合物中观察到两个峰，在大约 580nm 吸收处的峰较高 (与单个生色团相比)。

[0241] 实例 7- 凝胶中玫瑰红和曙红 Y 的吸收 / 发射光谱

[0242] 对 12％脲凝胶中 (i) 终浓度 0.305mg/mL 的曙红 Y，(ii) 浓度大约 0.085mg/mL 的玫瑰红，(iii) 曙红 Y(0.305mg/mL) 和玫瑰红 (0.085mg/mL) 的混合物的光动力学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0243] 吸收光谱和发射光谱示于图 11A 和 11B 中，图中表明，组合中生色团之间发生了能量转移。对于这种特定的生色团组合及在此浓度条件下，与单个生色团相比，生色团组合实现了更高的吸收。这种特定组合的发射光谱的功率密度比单独的曙红 Y 低。在照射期间或照射之后，组合物中并未出现温度上升，这种能量的明显损失可能是由于产生反应性氧种类所引起的（参见下面的实例 8）。

[0244] 实例 8- 曙红和玫瑰红产生氧种类

[0245] 制备下述组合，对本发明各实施例中两个生色团之间的协同效应进行了研究：

[0246] 1 - 12％脲凝胶中的曙红 Y(0.035％)+ 玫瑰红 (0.085％)。

[0247] 2 - 12％脲凝胶中的玫瑰红 (0.085％)。

[0248] 众所周知的是，在存在释氧剂的条件下，当用绿光进行光活化时，玫瑰红的单线态氧量子产率高（水中单线态氧的量子产率是大约 75％ [Murasecco-Suardi et al,Helvetica Chimica Acta,Vol.70,pp.1760-73,1987]）。众所周知，在被光活化时，曙红 Y 的发射荧光量子产率高；当它处于凝胶中时，它可以被蓝光至少部分地活化。在释氧剂的存在下，光活化的曙红 Y 的单线态氧量子产率低得多（当完全活化时，单线态氧的量子产率是大约 4％）[Gandin et al,Photochemistry and Photobiology,Vol.37,pp.271-8,1983]）。

[0249] 当曙红 Y 和玫瑰红结合时，似乎这两个生色团都被同样的蓝光活化，如图 12 所证明。

[0250] 图 12，左边，示出了光学显微镜下看到的 (x250) 组合物在暴露于活化蓝光之前的照片。可以看到，这两个组合物中的气泡都非常少。采用蓝光照射后，可以看到，包含曙红 Y 和玫瑰红组合的组合物的气泡急剧增多，而仅含玫瑰红或仅含曙红 Y 的组合物的气泡并未增多（未显示）。这表明能量从曙红 Y 转移到玫瑰红，导致氧种类的形成。脲凝胶中的曙红 Y 单独存在时具有与玫瑰红类似的性质。荧光素和玫瑰红也观察到类似的效果。

[0251] 实例 9- 改变生色团的浓度比

[0252] 对于改变本发明各实施例中的多生色团组合物中各个单独的生色团的浓度的影响进行了研究。包含 (i) 荧光素 - 曙红 Y，及 (ii) 曙红 Y - 玫瑰红的组合物的荧光发射随时间的变化分别示于图 13A 和 13B 中。

[0253] 如图 13A 所示，对下述组合的发射性质进行了研究：(i)109μg/g 曙红 Y+10μg/g 荧光素，(ii)109μg/g 曙红 Y+100μg/g 荧光素，(iii)109μg/g 曙红 Y，(iv)10μg/g 荧光素，(v)100μg/g 荧光素，所有这些组合均在过氧化脲凝胶中。采用 SP-100 分光光度计测量用蓝光照射（波长大约 440-480nm，功率密度小于 150mW/cm^2，照射大约 5 分钟）时，从不同组合物中检测到的光信号的功率密度谱图 (mW/cm^2，相对于波长）。荧光被作为 519-700nm 范围内的光进行测量。

[0254] 从中可以看出，在所有浓度条件下，发射的荧光均随时间而衰退。这种衰退通常伴随组合物中的一种或多种生色团的光漂白。多生色团组合物中的荧光浓度越高，初始发射的荧光就越高，持续时间也就越长，即寿命越长。对于曙红 Y(109μg/g) 和荧光素 (100μg/g) 组合物来说，初始发射荧光略低于仅含 100μg/g 荧光素的组合物。这可能是由于利用能量形成氧种类的原因（如上面实例 6 所述）。因此，可以改变多生色团组合物中各生色团的相对浓度，对得到的荧光和氧种类的性质进行定制。

[0255] 在图 13B 中，对过氧化脲凝胶中的下述组合物进行了评价：(i)109μg/g 曙红 Y+1μg/g 玫瑰红（两者之比是大约 10:1），(ii)109μg/g 螺红 Y+100μg/g 玫瑰红（两者之比是大约 1:1），(iii)109μg/g 曙红 Y，(iv)1μg/g 玫瑰红，(v)100μg/g 玫瑰红。图 13A 单独的曙红 Y、曙红 Y-1μg/g 玫瑰红，以及曙红 Y-10μg/g 玫瑰红均观察到具有相同的衰退趋势（未显示）。还观察到，当采用蓝光活化时，单独的玫瑰红的两种浓度均呈现非常低的荧光水平。令人吃惊的是，观察到 109μg/g 曙红 Y+100μg/g 玫瑰红的荧光水平比单独的曙红 Y 及曙红 Y+1μg/g 玫瑰红要低，但其荧光持久。在此组合物中，曙红 Y 未观察到任何光漂白。人们认为，曙红 Y 没有光漂白是因为在此曙红 Y/ 玫瑰红比例上，曙红 Y 能够将其吸收的所有能量转移给玫瑰红，后者然后将能量发射出来，从而防止了曙红 Y 分子的光降解，但是，并不受限于这一理论。109μg/g 曙红 Y+100μg/g 玫瑰红组合物的峰值发射波长与玫瑰红的峰值发射波长更接近，而不是与曙红 Y 更接近。

[0256] 在包含相对浓度之比大约是 1:10:10 的荧光素、曙红 Y 和玫瑰红的组合物中，也观察到类似的持续荧光效应（未显示）。

[0257] 实例 10- 凝胶中荧光素、曙红 Y 和玫瑰红的吸收 / 发射光谱

[0258] 对本发明的一个实施例中的含大约 12％过氧化脲的凝胶中的 (i) 浓度大约 0.085mg/mL 的玫瑰红，(ii) 终浓度大约 0.44mg/mL 的荧光素钠盐，(ii) 浓度大约 0.305mg/mL 的曙红 Y，及 (iii) 本发明 (i)、(ii) 和 (iii) 的混合物的光动力学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0259] 吸收光谱和发射光谱示于图 14A 和 14B 中，图中表明，生色团组合中的各生色团之间发生了能量转移。从图 14B 中可以明显看出，荧光素、曙红 Y 和玫瑰红组合的带宽比单独使用曙红 Y 时宽。

[0260] 实例 11- 水溶液中荧光素、曙红 Y 和玫瑰红的吸收 / 发射光谱

[0261] 对本发明的一个实施例的水溶液中 (i) 浓度大约 0.085mg/mL 的玫瑰红，(ii) 终浓度大约 0.44mg/mL 的荧光素钠盐，(ii) 浓度大约 0.305mg/mL 的曙红 Y，及 (iii) 上述 (i)、(ii) 和 (iii) 的混合物的光动力学性质进行了评价。采用 flexstation 384II 分光光度计测量发射的荧光，测量参数如下：模式：荧光，激发：460nm，发射光谱：465-750nm。采用协同 HT 酶标仪读取吸光度：模式：吸光度；光谱：300-650nm。

[0262] 吸收光谱和发射光谱示于图 15A 和 15B 中，图中表明在没有过氧化物，但存在其它释氧剂（如水）的条件下，生色团组合中的各生色团之间存在能量转移。

[0263] 参考本发明各组合物在过氧化脲凝胶中的吸收光谱和发射光谱，同一生色团的光谱与凝胶中没有过氧化物时是一样的。

[0264] 实例 12- 本发明组合物的血管生成潜力

[0265] 为了对本发明组合物的血管生成潜力进行评价，建立了人体皮肤模型。简单地说，将包含曙红 Y 和赤藓红的组合物放置在含成纤维细胞和角化细胞的人体皮肤模型上面。利用孔径 20 微米的尼龙网将皮肤模型和组合物分开。然后，用蓝光（“活化光”）照射组合物 5 分钟，组合物距离光源 5cm。活化光由 LED 灯发射的光组成，光的平均峰值波长大约 400-470nm，10cm 处测量的功率密度是 7.7J/cm^{2}至 11.5J/cm^2。采用活化光照射时，组合物发射出荧光。由于组合物与细胞的接触受限，成纤维细胞与角化细胞主要暴露于活化光和

组合物发射的荧光。然后，向之前平板接种在Matrigel®中的人体主动脉内皮细胞上涂覆来自经过处理的人体3D皮肤模型的条件介质。通过显微镜观察和监测内皮细胞管的形成情况，24小时后开展图像分析。来自经过光照射处理的3D皮肤模型的条件介质诱导了体外内皮管的形成，表明光处理（蓝光和荧光）可通过成纤维细胞和角化细胞而产生因子，从而对血管形成产生间接影响。采用来自未经处理的皮肤样品的普通介质及条件介质作为对照，它们都没有诱导内皮管的形成。

[0266] 图16是一个发射光谱，它表明了生物光子组合物发射的光的强度与时间的关系（实例9，采用分光光度计测量）。可以合理推断的是，其它具有相当发射光谱的生色团组合也将诱导血管形成。从图16可以看出，发射荧光的波长是大约520-620nm，峰值波长是大约560nm。采用曙红Y和荧光素（图5B）；曙红Y和荧光桃红B（图7B，图8B）；曙红Y和玫瑰红（图11B）；荧光素、曙红Y和玫瑰红（图14B，图15B），观察到类似的发射光谱。也可以采用其它具有类似发射光谱的生色团组合物，有理由预期它们也具有血管生成特性。

[0267] 实例13-蛋白质分泌和基因表达谱

[0268] 采用受伤的和未受伤的3D人体皮肤模型(EpiDermFT，MatTek Corporation)用于评价本发明组合物引发明显的蛋白质分泌和基因表达谱的潜力。简单地说，将包含曙红和赤藓红的组合物放不同条件下（生长因子(1X)、50％生长因子(0.5X)和没有生长因子(0X)）培养的受伤和未受伤3D人体皮肤模型的上面。不同的条件分别模拟未受损的愈合、半饥饿状态和饥饿状态。利用孔径20微米的尼龙网将皮肤模型和组合物分开。然后，用蓝光（“活化光”）照射每种皮肤模型-组合物组合5分钟，组合物距离光源5cm。活化光由LED发射的光组成，光的平均峰值波长是大约440-470nm，5cm处的功率密度是60-150mW/cm^2，5分钟后的总能量密度是大约18-39J/cm^2。对照样由未采用光照射的3D皮肤模型组成。

[0269] 光照射24小时后测量基因表达谱和蛋白质分泌谱。通过抗体阵列(RayBio Human Cytokine抗体阵列）分析细胞因子的分泌，通过PCR阵列(PAHS-013A，SABioscience)分析基因表达，并通过GAPDH和LDH释放测定细胞毒性。结果（表1和表2)表明，在受伤皮肤插入片段上、在非饥饿状态中，光处理能提高伤口愈合初期炎症阶段的蛋白质分泌和基因表达水平。在模拟慢性伤口的饥饿状态下，与对照样相比，分泌的炎性蛋白水平并未增加。令人感兴趣的是，在细胞水平上，光处理对未受伤皮肤模型的影响远低于其对受伤皮肤插入片段的影响，表明光处理具有细胞水平上的影响。这似乎加速了伤口愈合过程的炎症阶段。由于缺少其它细胞类型，例如3D皮肤模型中的巨噬细胞，没有出现抗炎反馈，这似乎可以用于解释伤口闭合延迟的原因。在光处理中未观察到细胞毒性。曙红Y和赤藓红B组合物具有同样的发射特性，如图16所示。如上文所述，可以合理推断的是，其它具有相当发射光谱的生色团组合也将诱导本实例中看到的蛋白质分泌或基因表达。

[0270] 表1-在第3天时，经处理样品和未经处理对照样品之间的分泌比具有统计学显著差异的蛋白质的清单。双箭头表示分泌比超过2倍。

[0271]

	介质 1X	**介质 0.5X**	**介质 0X**
增加		**ENA78 p=0.04 ↑↑** **Il-1R4/ST2 p=0.02 ↑↑** **MMP3 p=0.01 ↑↑** **MCP-2 p=0.04 ↑↑**	血管生成素 p=0.03 ↑ CXCL16 p=0.04 ↑
减少	BMP6 p=0.01 ↓ TNFα p=0.005 ↓	BMP6 p=0.02 ↓	

[0272] 表 2 - 第一个 24 小时期间经处理样品和未经处理对照样品的表达比具有统计学显著差异的基因的清单。双箭头表示分泌比超过 2 倍。

[0273]

	介质 1X	**介质 0.5X**	**介质 0X**
增加	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 ↑
递减	HAS1 p=0.009 ↓↓ NCAM1 p=0.05 ↓↓ VCAM1 p=0.03 ↓↓ COL7A1 p=0.04 ↓ CTNNA1 p=0.03 ↓	**NCAM1 p=0.02 ↓↓** VCAN p=0.02 ↓ LAMC1 p=0.002 ↓ COL6A1 p=0.007 ↓ MMP7 p=0.003 ↓	

[0274] 实例 14- 曙红 Y 和荧光素诱导胶原蛋白的形成

[0275] 对本发明的一个实施例中的、包含位于载体基质（1.8％卡波姆凝胶）中的0.01％曙红Y和0.01％荧光素的组合物的诱导胶原蛋白形成的潜力进行了评价。将真皮人成纤维细胞平板接种在带孔玻底培养皿（MatTek®）内。每孔内有大约 4000 个细胞。48 小时后，将玻底培养皿倒过来，通过玻璃底 (i) 无光照射（对照），(ii) 在中午利用日光照射大约 13 分钟（对照），(iii) 将组合物施用到细胞另一侧的玻璃孔底部（无光照射），(iv) 将组合物施用到细胞另一侧玻璃孔底部（中午日光照射大约 13 分钟），及 (v) 将组合物施用到细胞另一侧玻璃孔底部（蓝光照射大约 5 分钟）对细胞进行处理。在 (iii)、(iv) 和 (v) 的情况下，细胞和组合物之间不存在直接接触。在 (iv) 和 (v) 的情况下，当分别照射日光和蓝光时，细胞暴露于来自和穿过曙红 Y 和荧光素组合物的光。在 (iv) 和 (v) 中观察到至少部分光漂白。在处理后，洗涤细胞，并在普通介质中培养 48 小时。然后，采用苦味酸 - 天狼星红方法，对上层清液开展胶原蛋白分析。这包括将天狼星红染料的苦味酸溶液加到上清液中，轻轻振荡培养 30 分钟，然后离心分离，形成颗粒。首先用 0.1N HCl，然后用 0.5N NaOH 洗涤颗粒，脱除游离染料。离心分离后，对 I 型胶原蛋白来说，读取悬浮液在 540nm 处的读

数。结果见表 1。

[0276] 表 1 - 将暴露于下述条件的真皮人成纤维细胞上清液中 I 型胶原蛋白的浓度进行了定性对比 :(i) 无光照射 (对照),(ii) 中午日光照射大约 13 分钟 (对照),(iii) 任何曙红 Y 和荧光素组合物发射的穿过玻璃隔离物的光 (无光照射),(iv) 任何曙红 Y 和荧光素组合物发射的穿过玻璃隔离物的光 (中午日光照射大约 13 分钟),及 (v) 组合物施用到细胞另一侧的玻璃孔底部 (蓝光照射大约 5 分钟)。++ 表示胶原蛋白含量是 + 的两倍,+++ 表示胶原蛋白含量是 + 的三倍。

[0277]

	无光照射(对照)	日光(对照)	曙红 Y + 荧光素 - 无光	曙红和荧光素 - 日光	曙红和荧光素 - 蓝光
胶原蛋白的浓度	+	+	++	+++	+++

[0278] 照射日光和蓝光的曙红 Y 和荧光素组合物诱导的胶原蛋白含量与无光和仅日光的对照样品相比存在统计学上的显著差别。

[0279] 胶原蛋白的产生指示了组织修复的潜力,包括肉芽组织的稳定及伤口尺寸的变小。还与细纹减少、毛孔收缩、完好皮肤的质地的改善及拉伸强度的改善相关联。本实例中的曙红 Y 和荧光素组合物的发射光谱具有单峰发射,波长是大约 480-620nm。在采用日光照射后,峰的功率密度减小,表明 13 分钟内出现了至少部分光漂白,通过组合物颜色的变化也可观察到这一点。与采用蓝光活化的曙红 Y 和荧光素组合物 (如实例 5 和 6 中的组合物) 相比,采用日光 (白光) 照射时,荧光发射 / 光漂白速率减慢。

[0280] 实例 15- 选择生物光子组合物中生色团的浓度

[0281] 采用分光光度计和活化蓝光 (如实例 9),对生色团浓度不同的组合物的荧光光谱进行了研究。曙红 Y 和荧光素的示例性荧光光谱在图 17A 和 17B 中给出。从图中发现,生色团发射的荧光随浓度增加而迅速增加,但是,浓度进一步增加时,荧光增加速度减慢,达到一个平台。由于生色团浓度增加时更多的光被生色团吸收,因此,穿过组合物的活化光随浓度增加而下降。因此,根据本实例,可以根据处理组织所要求的活化光和荧光素的比例和水平,选择本发明之组合物中的生色团的浓度。在一些实施例中,对曙红 Y 来说,浓度应选择在迅速增加的区域之后,即 0.5 至 1mg/mL(图 17A)。

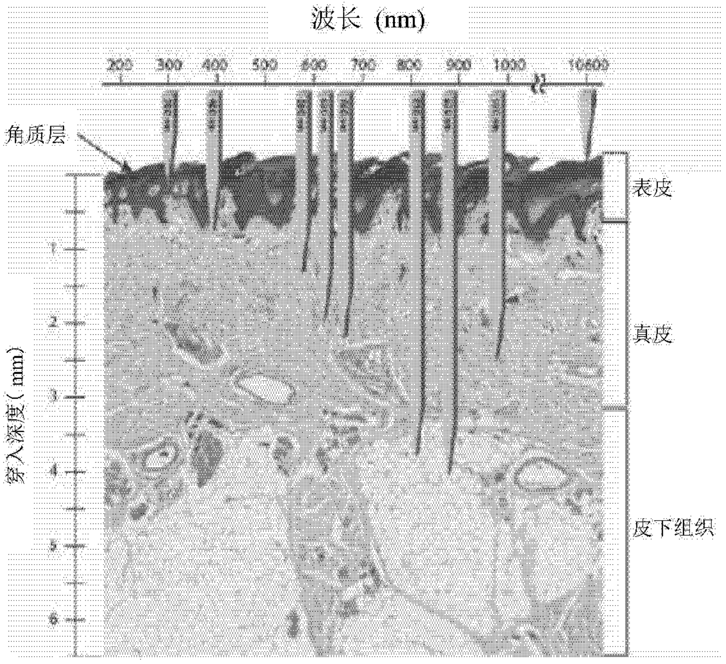
[0282] 因此,可以根据所需的活化光和荧光来选择浓度。在一些实施例中,对曙红 Y 来说,浓度应选择在迅速增加的区域之后,即 0.5 至 1mg/mL(图 17A)。

[0283] 含玫瑰红的组合物的表现与此略有不同,随着浓度增加,可能由于形成气泡的原因,组合物变得更不透明。

[0284] 同样,对组织接收的光的功率密度与照射时间之间的关系进行了研究。我们发现,活化光的功率密度一开始较低,但会随着时间的进展而增加。这种现象与吸收光的生色团的光漂白呈相关性,也与穿过组合物到达组织的活化光的数量更多呈相关性。与此同时,由于一个或多个生色团被光漂白,组合物发射的荧光随时间的进展而下降。总之,用于处理组织的光的总功率密度随照射时间的进展而逐渐下降。

[0285] 应该理解的是,本发明并不限于此处描述和阐明的具体实施例,而是包括所附权

利要求书中定义的本发明范围之内的所有变动和更改。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表皮
真皮
皮下组织
穿入深度 (mm)
1
2
3
4
5
6

图 1

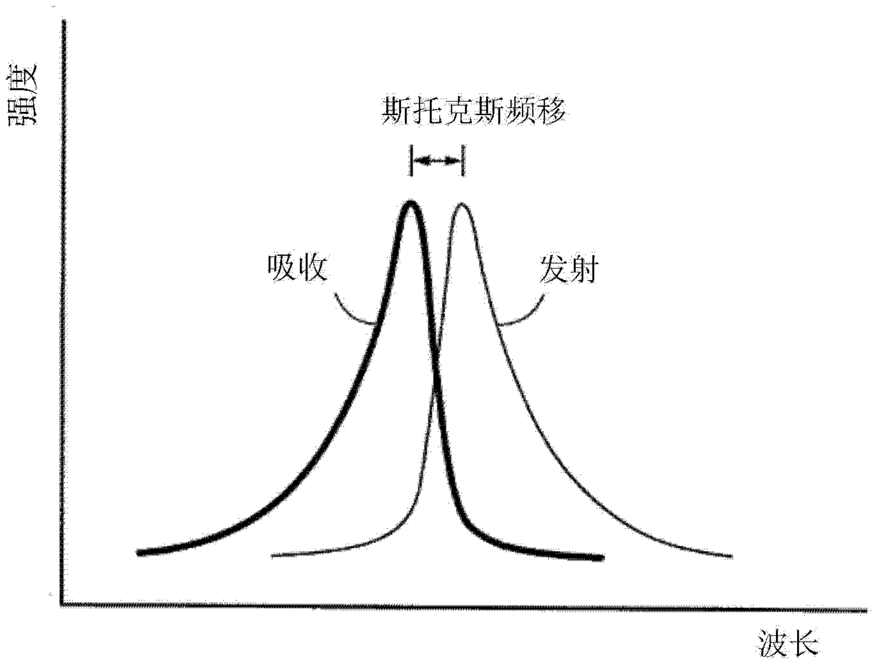
强度
斯托克斯频移
吸收
发射
波长

图 2

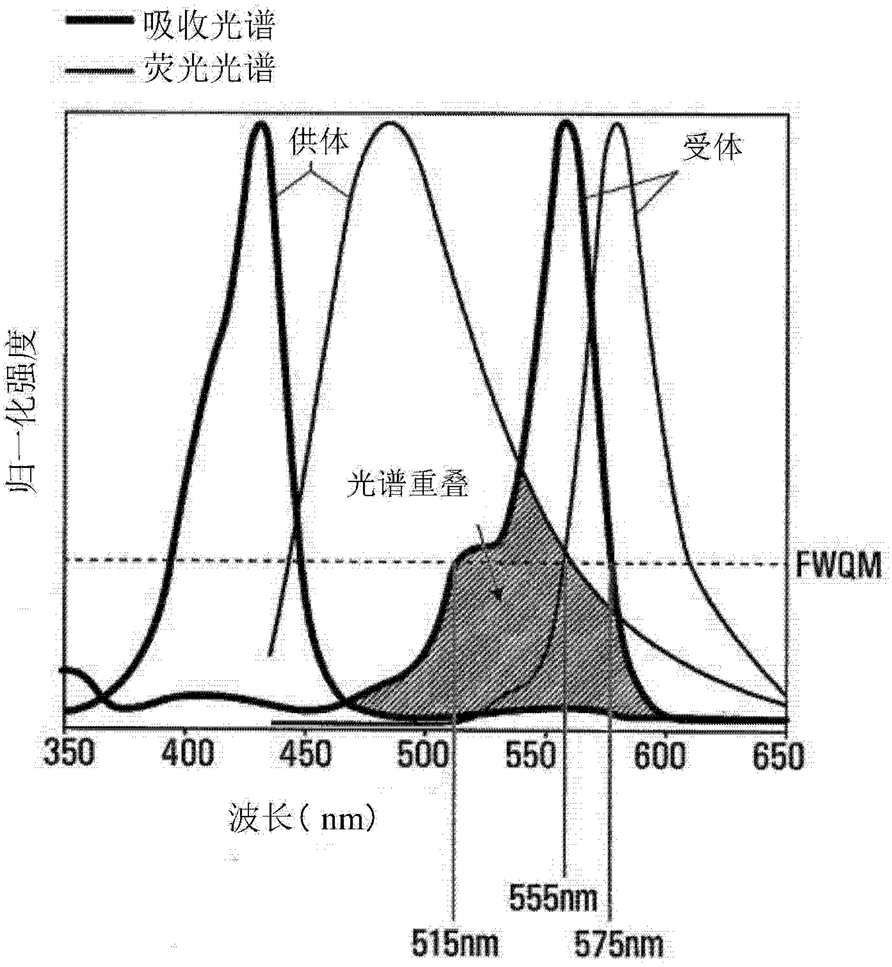
吸收光谱
荧光光谱
供体
受体
归一化强度
光谱重叠
FWQM
350
400
450
500
550
600
650
波长(nm)
555nm
515nm
575nm

图 3

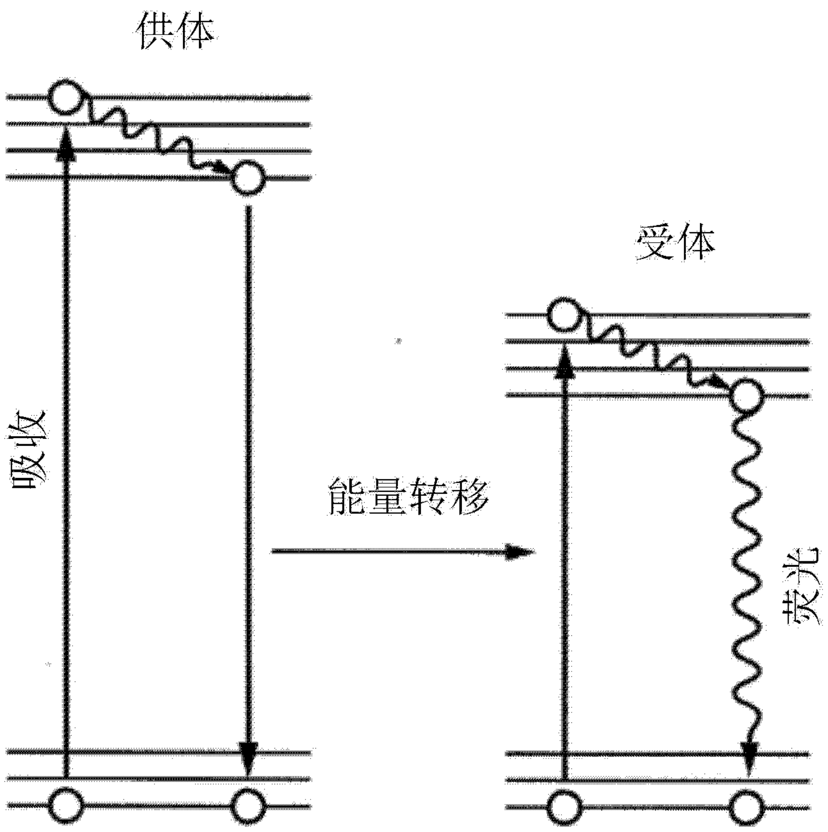

图 4

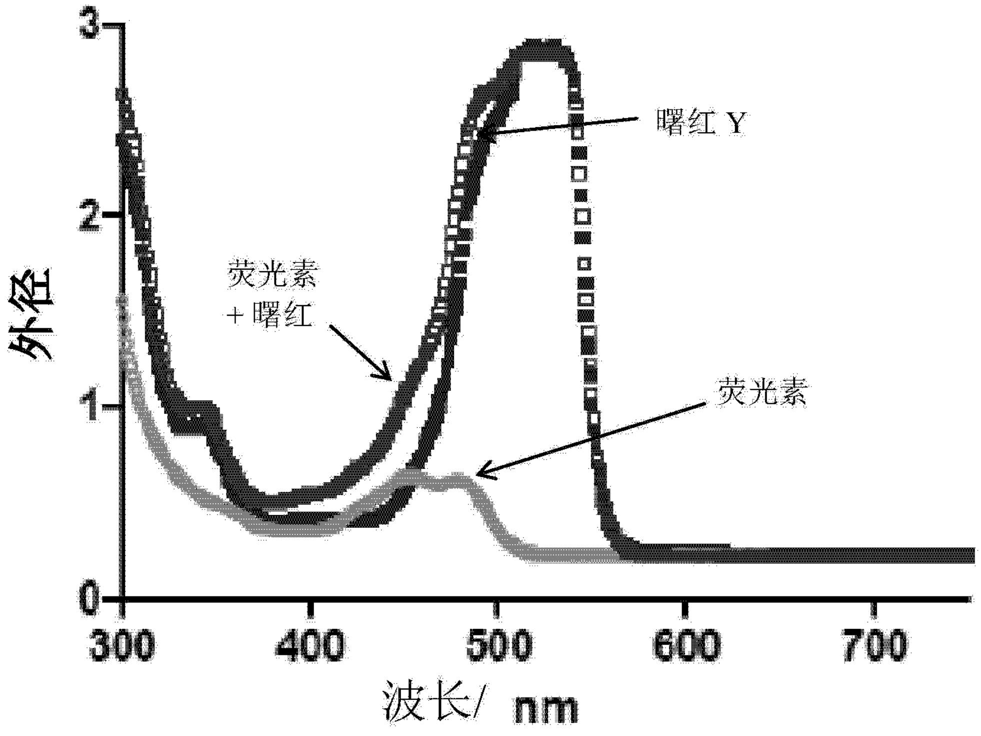

图 5A

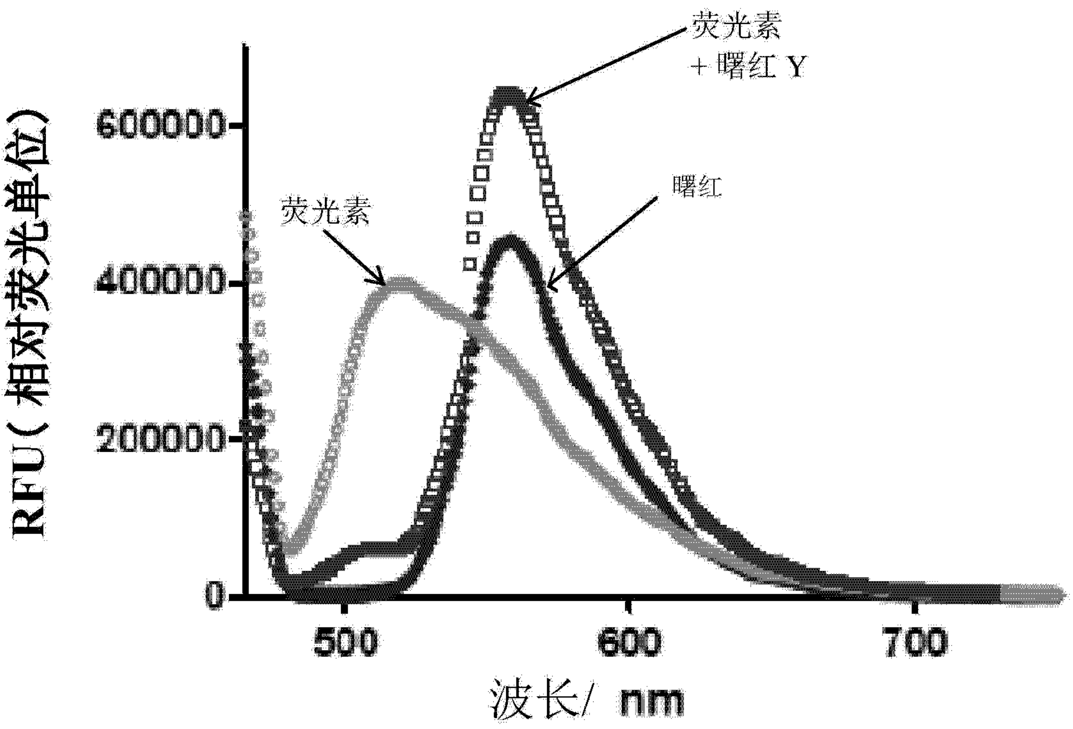
荧光素
+曙红 Y
曙红
荧光素
RFU（相对荧光单位）
600000
400000
200000
0
500
600
700
波长/ nm

图 5B

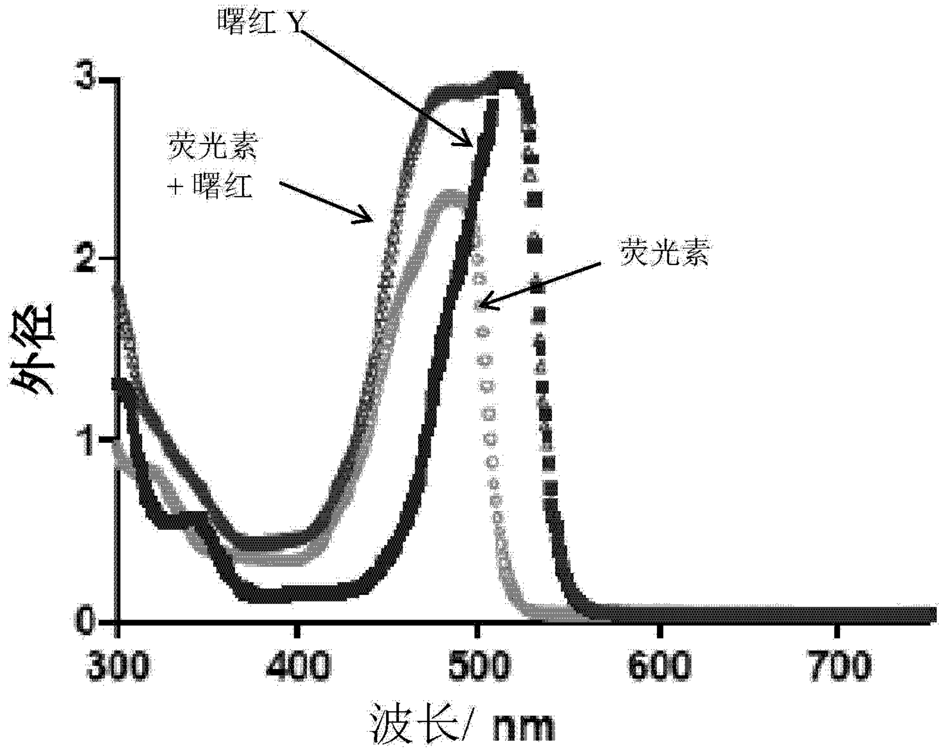
曙红 Y
荧光素
+曙红
荧光素
外径
3
2
1
0
300
400
500
600
700
波长/ nm

图 6A

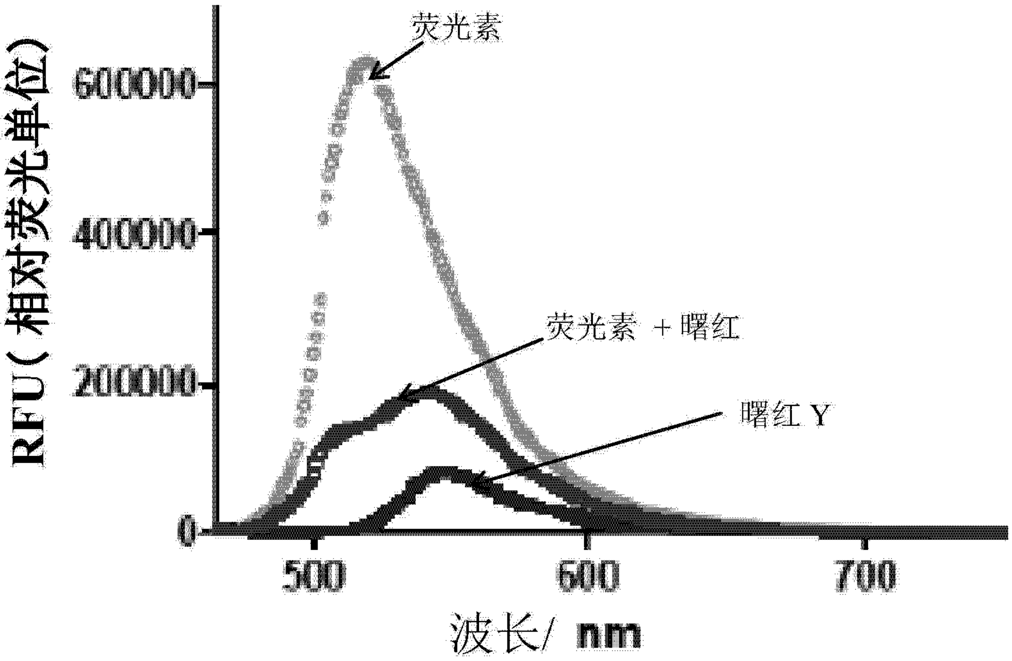
荧光素
荧光素 ＋曙红
曙红 Y
RFU（相对荧光单位）
600000
400000
200000
0
500
600
700
波长/ nm

图 6b

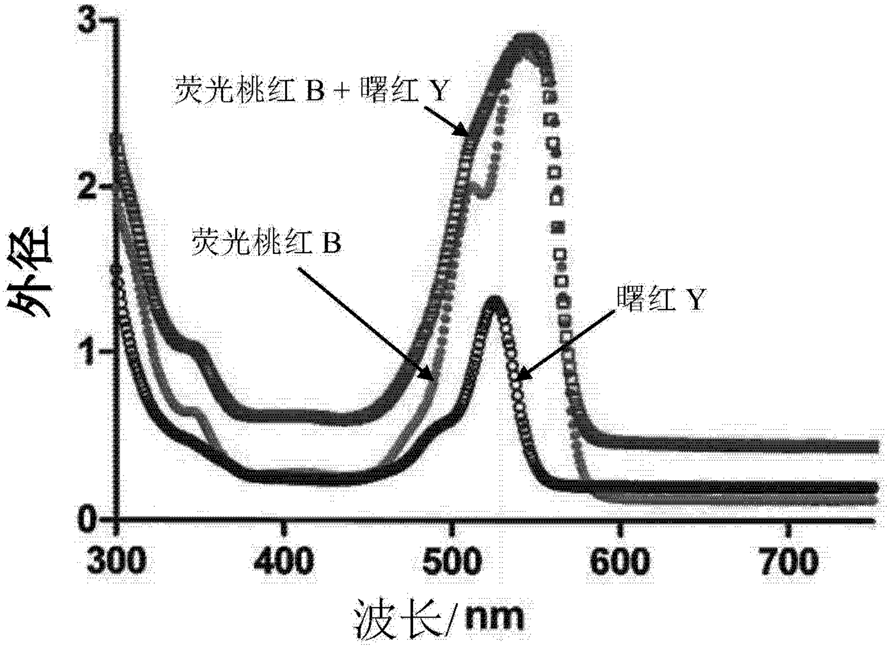
荧光桃红 B＋曙红 Y
荧光桃红 B
曙红 Y
外径
3
2
1
0
300
400
500
600
700
波长/nm

图 7A

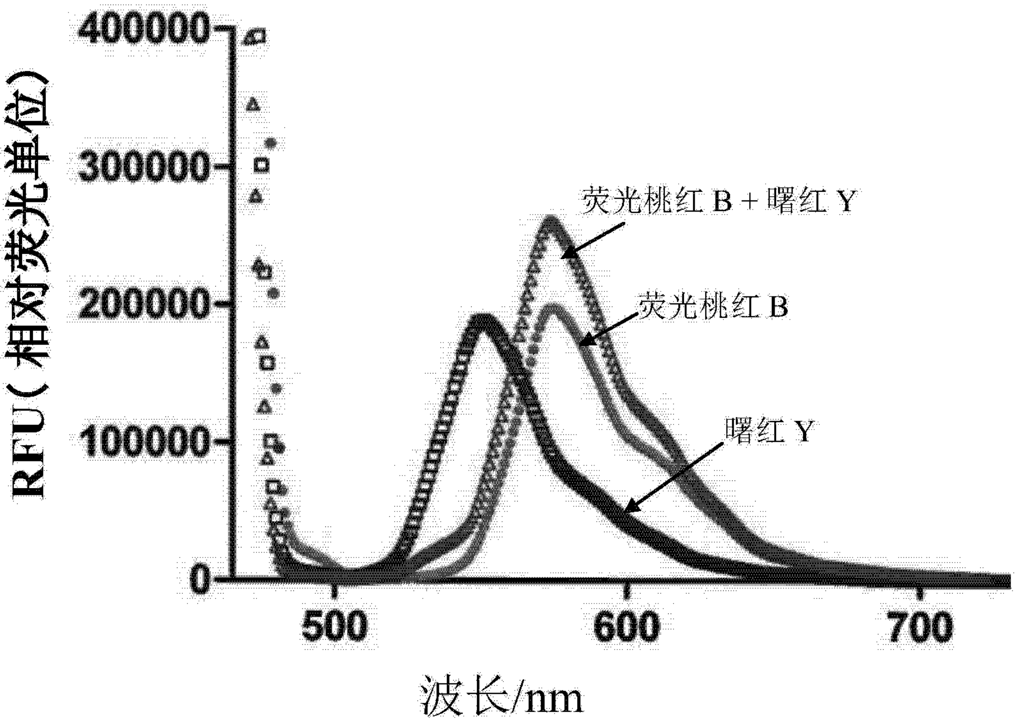
RFU（相对荧光单位）
400000
300000
200000
100000
0
500
600
700
波长/nm
荧光桃红 B + 曙红 Y
荧光桃红 B
曙红 Y

图 7B

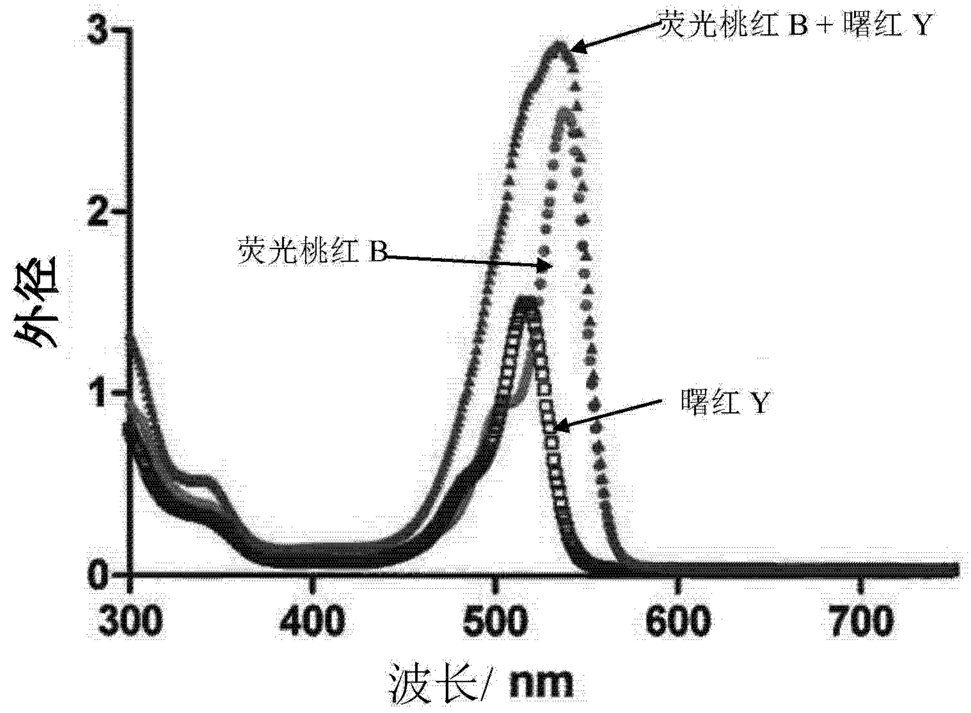
外径
3
2
1
0
300
400
500
600
700
波长/ nm
荧光桃红 B + 曙红 Y
荧光桃红 B
曙红 Y

图 8A

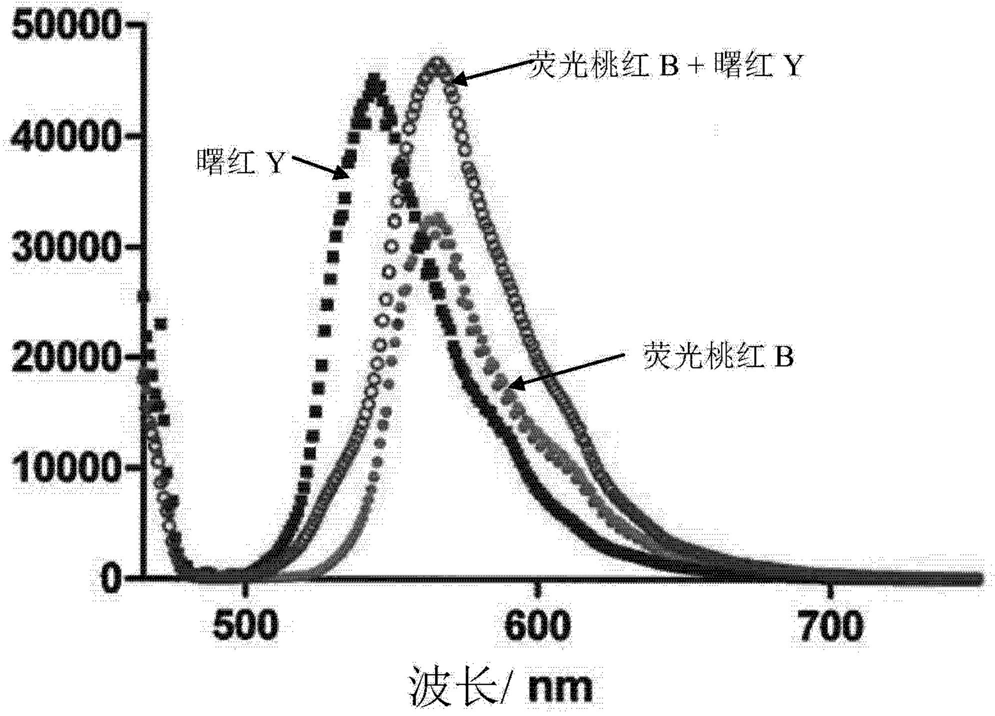
50000
40000
30000
20000
10000
0
500
600
700
波长/ nm
荧光桃红 B + 曙红 Y
曙红 Y
荧光桃红 B

图 8B

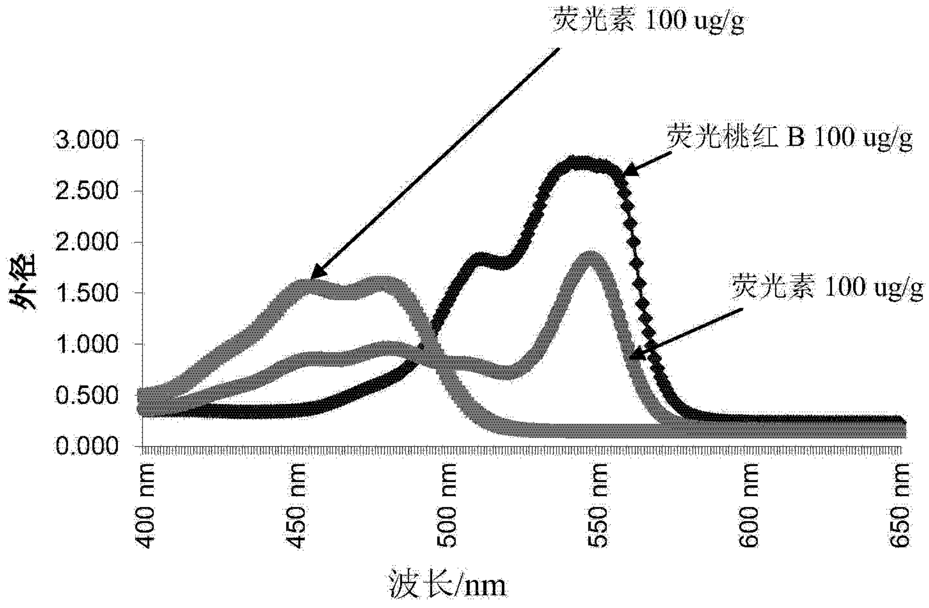
荧光素 100 ug/g
荧光桃红 B 100 ug/g
荧光素 100 ug/g
3.000
2.500
2.000
1.500
1.000
0.500
0.000
外径
400 nm
450 nm
500 nm
550 nm
600 nm
650 nm
波长/nm

图 10A

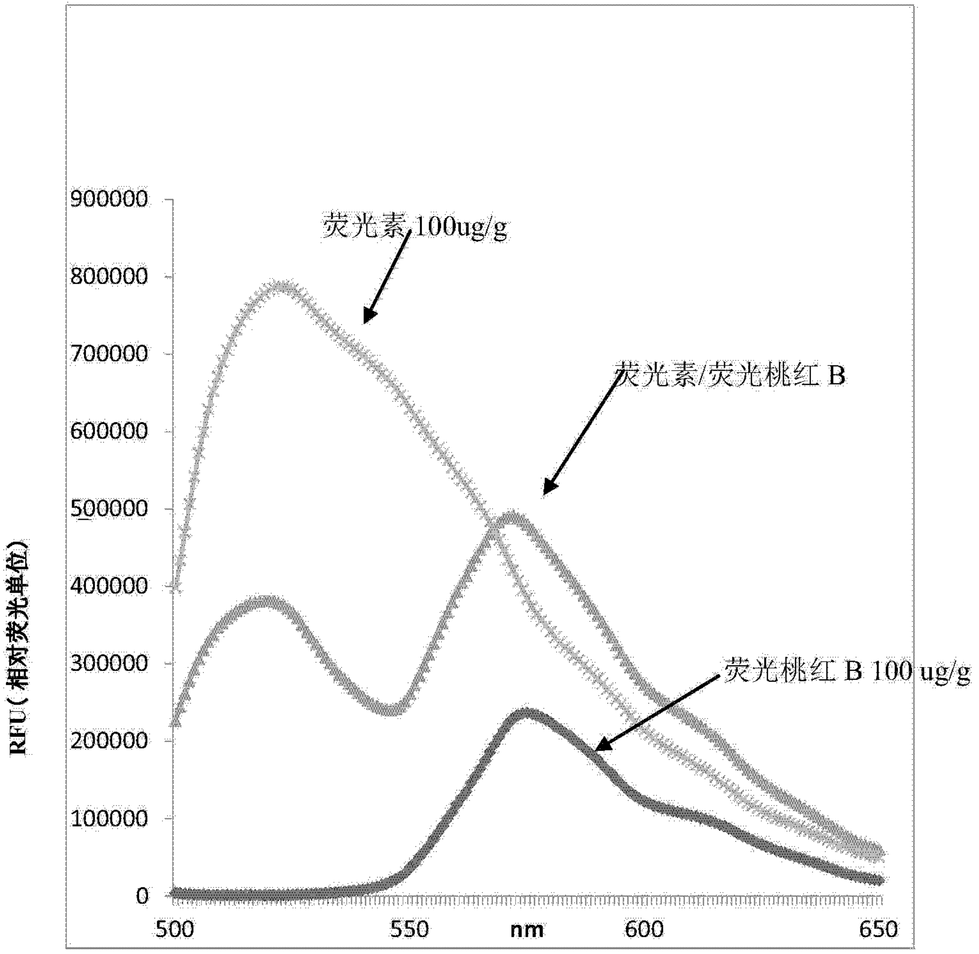
荧光素 100ug/g
荧光素/荧光桃红 B
荧光桃红 B 100 ug/g
RFU（相对荧光单位）
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
500
550
nm
600
650

图 10B

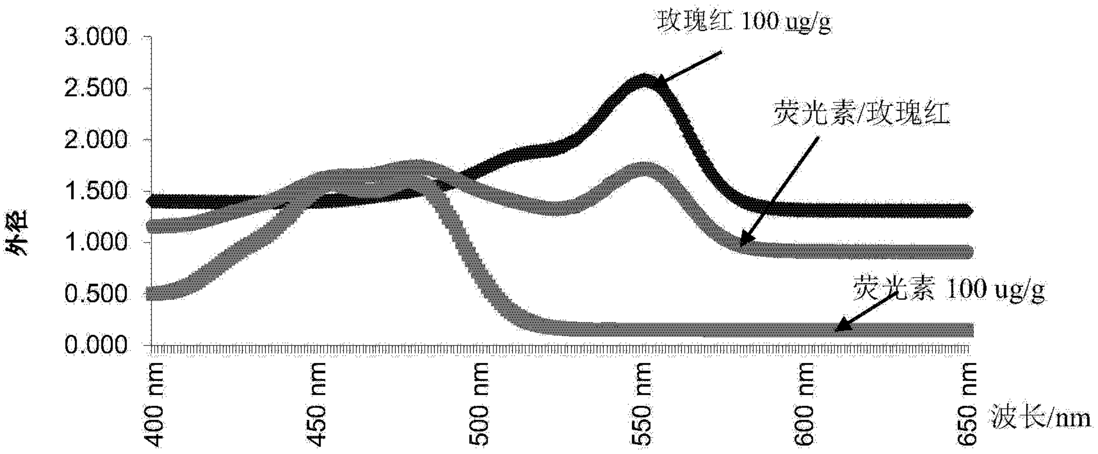
玫瑰红 100 ug/g
荧光素/玫瑰红
荧光素 100 ug/g
外径
3.000
2.500
2.000
1.500
1.000
0.500
0.000
400 nm
450 nm
500 nm
550 nm
600 nm
650 nm
波长/nm

图 10A

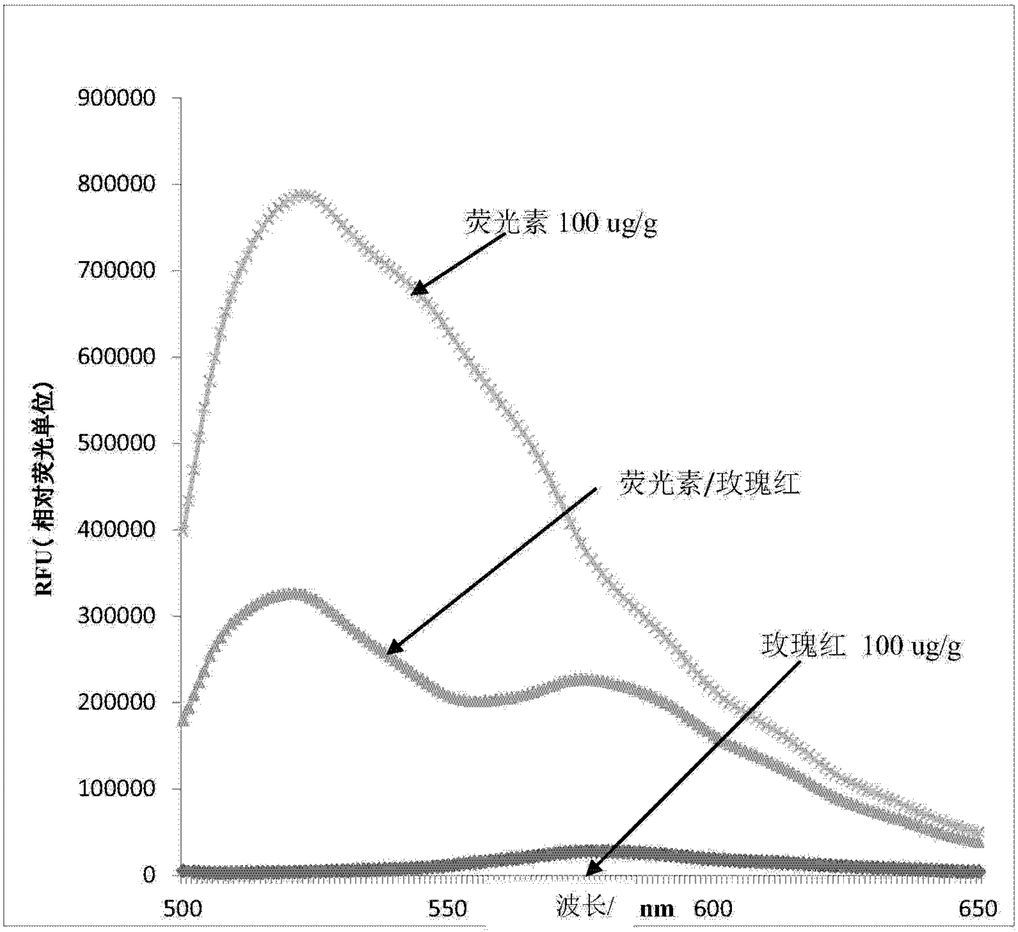
900000
800000
700000
600000
500000
400000
300000
200000
100000
0
RFU（相对荧光单位）
荧光素 100 ug/g
荧光素/玫瑰红
玫瑰红 100 ug/g
500
550
波长/ nm 600
650

图 10B

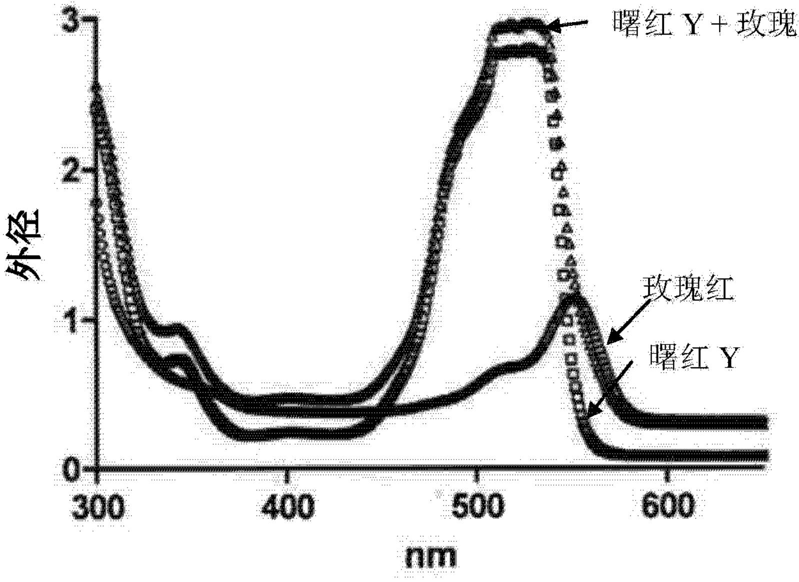
3
2
1
0
外径
300
400
500
600
nm
曙红 Y + 玫瑰红
玫瑰红
曙红 Y

图 11A

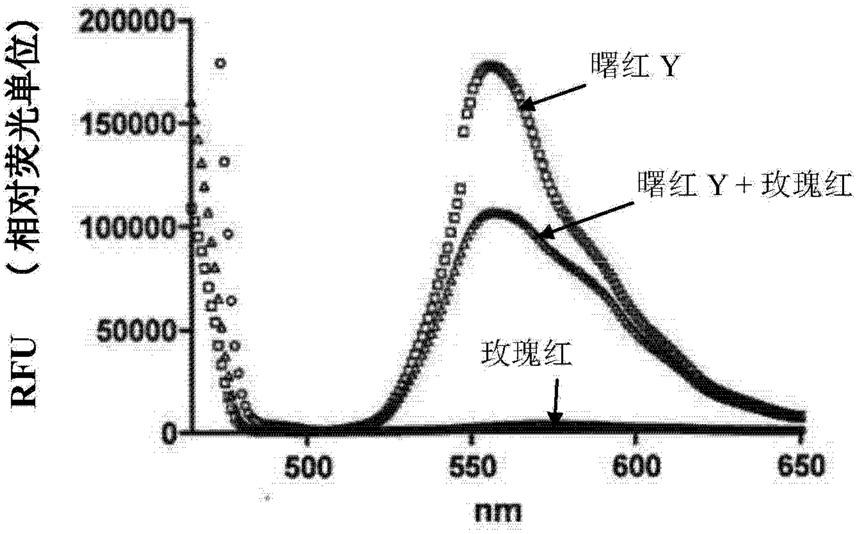
RFU （相对荧光单位）
200000
150000
100000
50000
0
500
550
600
650
nm
曙红 Y
曙红 Y + 玫瑰红
玫瑰红

图 11B

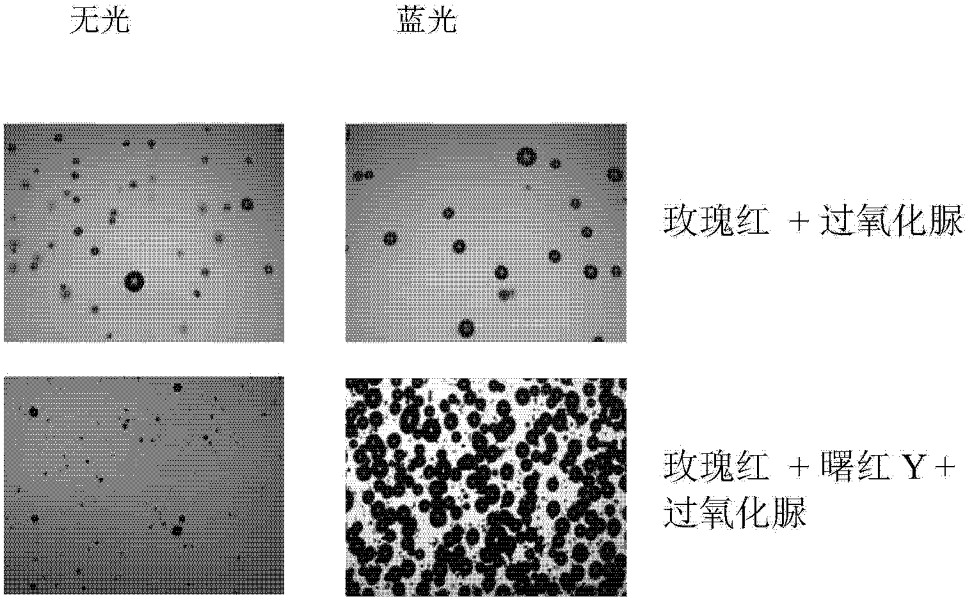
无光
蓝光
玫瑰红 + 过氧化脲
玫瑰红 + 曙红 Y +
过氧化脲

图 12

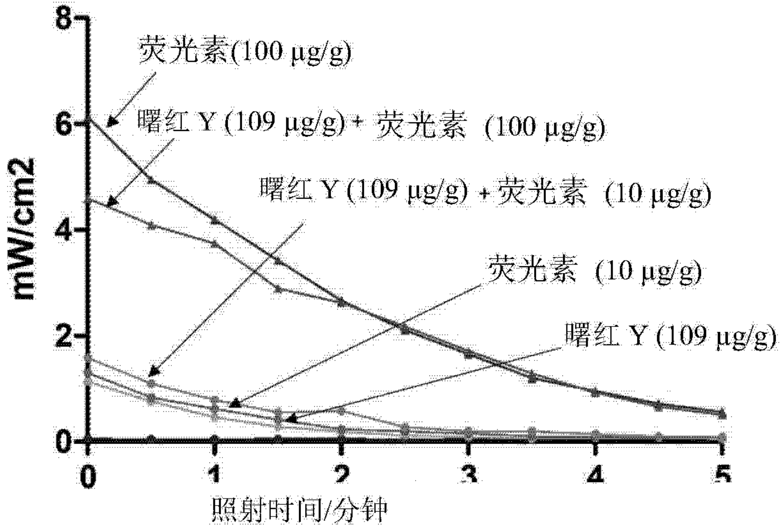
8
6
4
2
0
mW/cm2
荧光素(100 μg/g)
曙红 Y (109 μg/g) + 荧光素 (100 μg/g)
曙红 Y (109 μg/g) + 荧光素 (10 μg/g)
荧光素 (10 μg/g)
曙红 Y (109 μg/g)
0
1
2
3
4
5
照射时间/分钟

图 13A

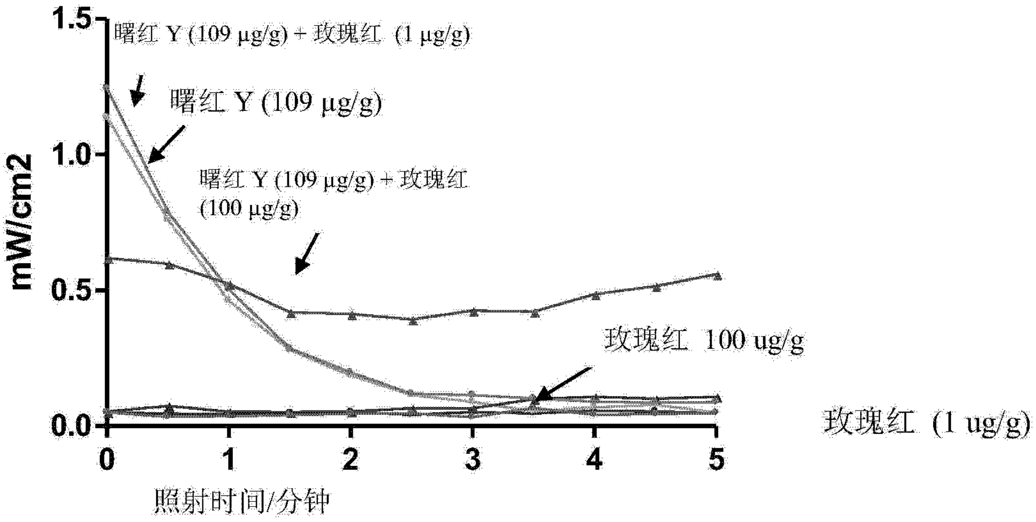
1.5
1.0
0.5
0.0
mW/cm2
曙红 Y (109 μg/g) + 玫瑰红 (1 μg/g)
曙红 Y (109 μg/g)
曙红 Y (109 μg/g) + 玫瑰红 (100 μg/g)
玫瑰红 100 ug/g
玫瑰红 (1 ug/g)
0
1
2
3
4
5
照射时间/分钟

图 13B

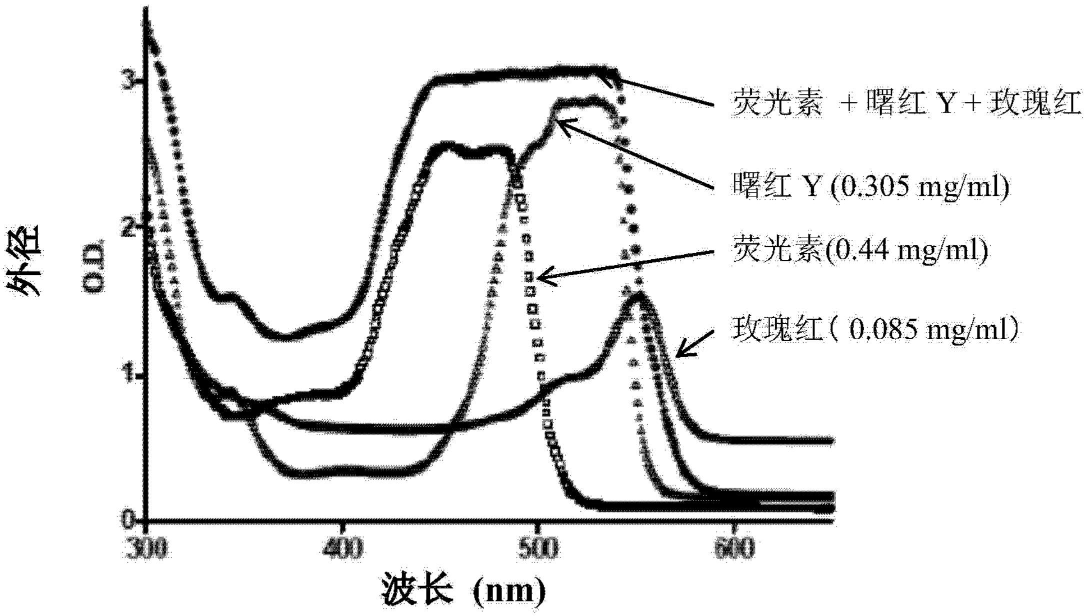
外径
O.D.
3
2
1
0
300
400
500
600
波长 (nm)
荧光素 + 曙红 Y + 玫瑰红
曙红 Y (0.305 mg/ml)
荧光素(0.44 mg/ml)
玫瑰红(0.085 mg/ml)

图 14A

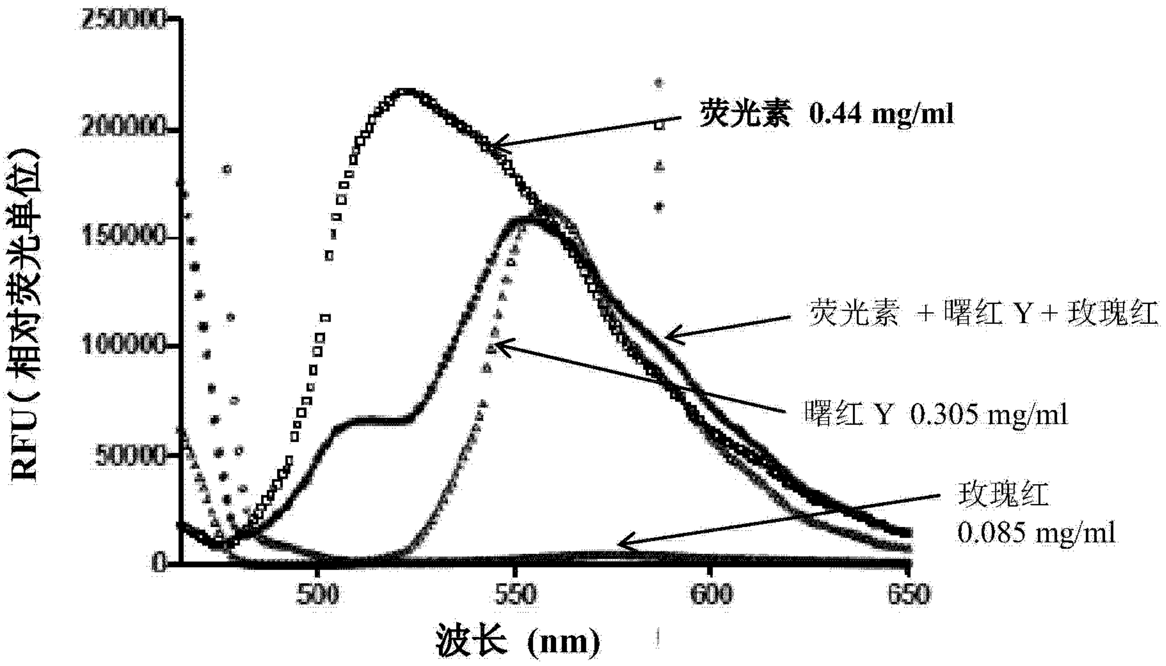
250000
200000
150000
100000
50000
0
RFU（相对荧光单位）
500
550
600
650
波长 (nm)
荧光素 0.44 mg/ml
荧光素 + 曙红 Y + 玫瑰红
曙红 Y 0.305 mg/ml
玫瑰红
0.085 mg/ml

图 14B

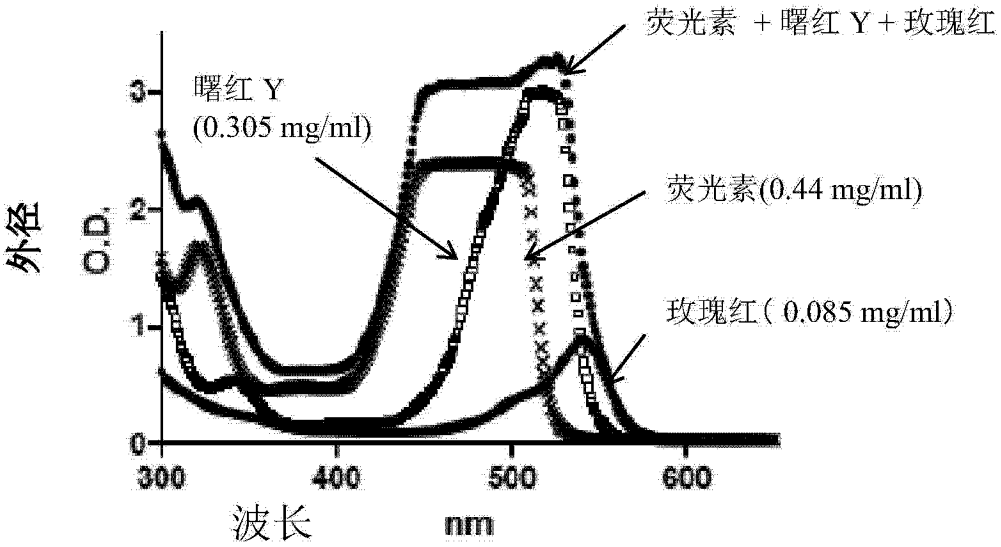
荧光素 +曙红 Y+玫瑰红
曙红 Y
(0.305 mg/ml)
荧光素(0.44 mg/ml)
玫瑰红(0.085 mg/ml)
外径
O.D.
3
2
1
0
300
400
500
600
波长
nm

图 15A

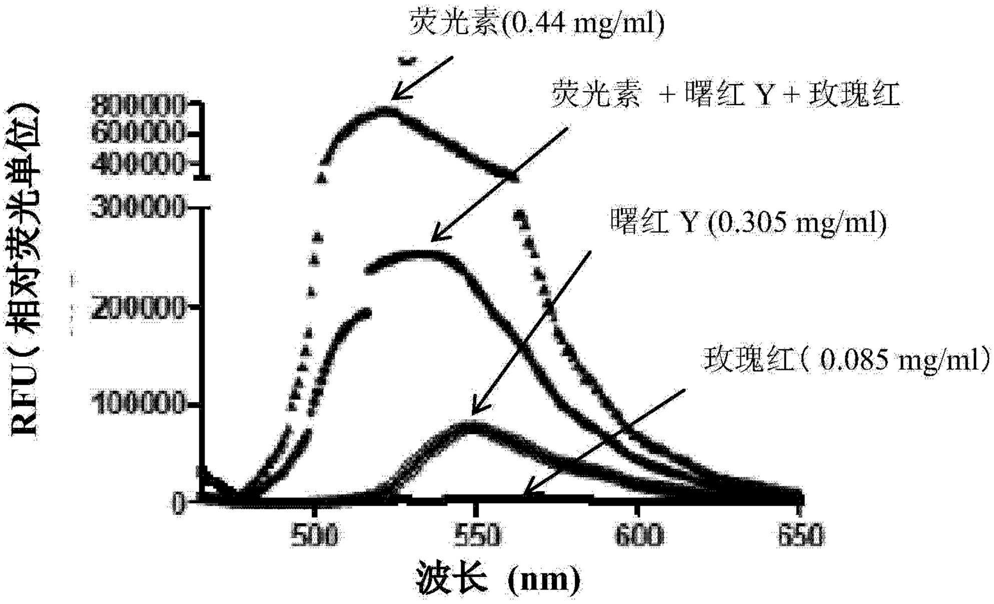
荧光素(0.44 mg/ml)
荧光素 +曙红 Y+玫瑰红
曙红 Y (0.305 mg/ml)
玫瑰红(0.085 mg/ml)
RFU(相对荧光单位)
800000
600000
400000
300000
200000
100000
0
500
550
600
650
波长 (nm)

图 15B

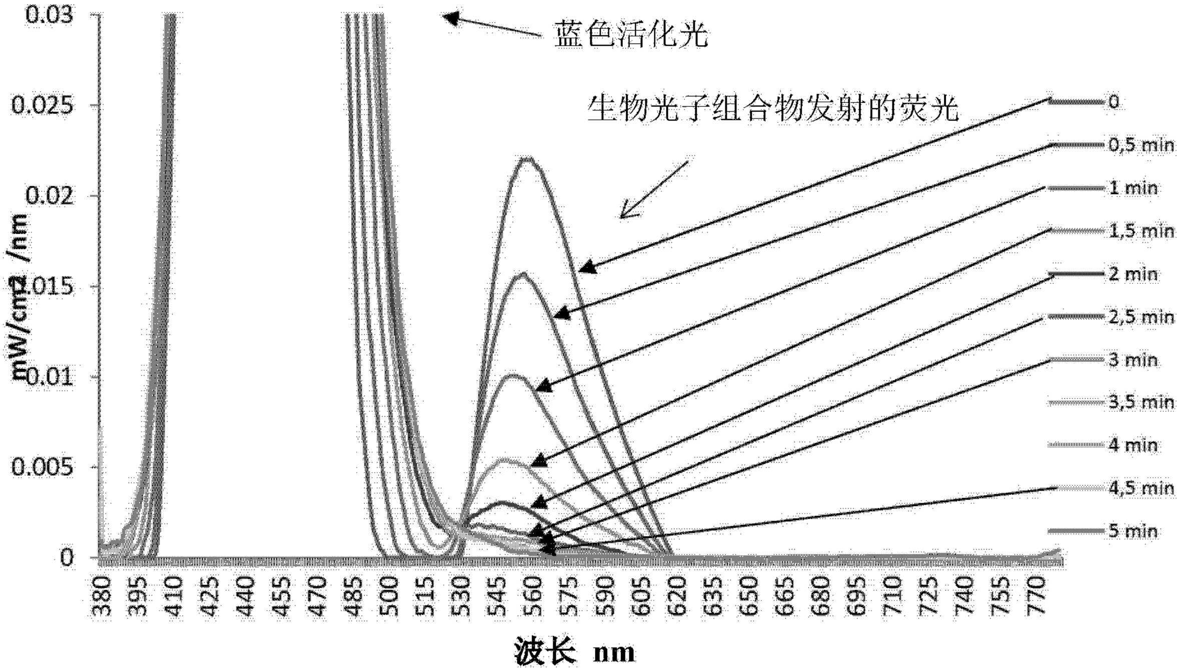
0.03
0.025
0.02
0.015
0.01
0.005
0
mW/cm2 /nm
蓝色活化光
生物光子组合物发射的荧光
0
0,5 min
1 min
1,5 min
2 min
2,5 min
3 min
3,5 min
4 min
4,5 min
5 min
380
395
410
425
440
455
470
485
500
515
530
545
560
575
590
605
620
635
650
665
680
695
710
725
740
755
770
波长 nm

图 16

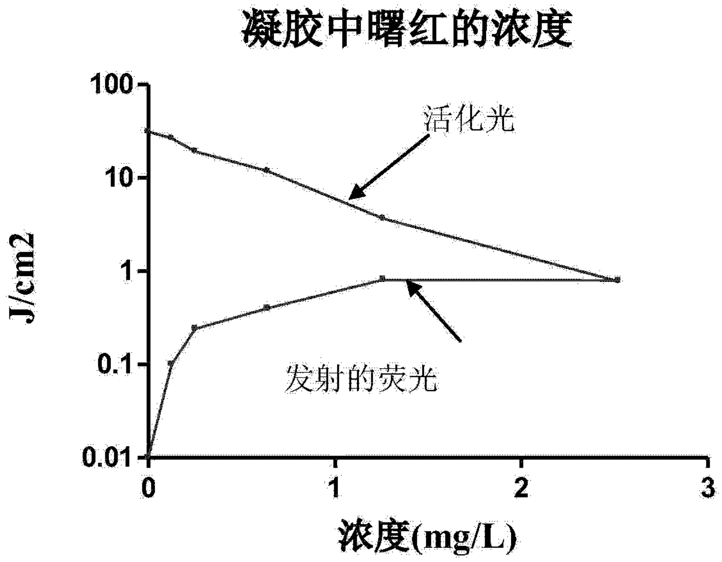
凝胶中曙红的浓度
100
10
1
0.1
0.01
J/cm2
活化光
发射的荧光
0
1
2
3
浓度(mg/L)

图 17A

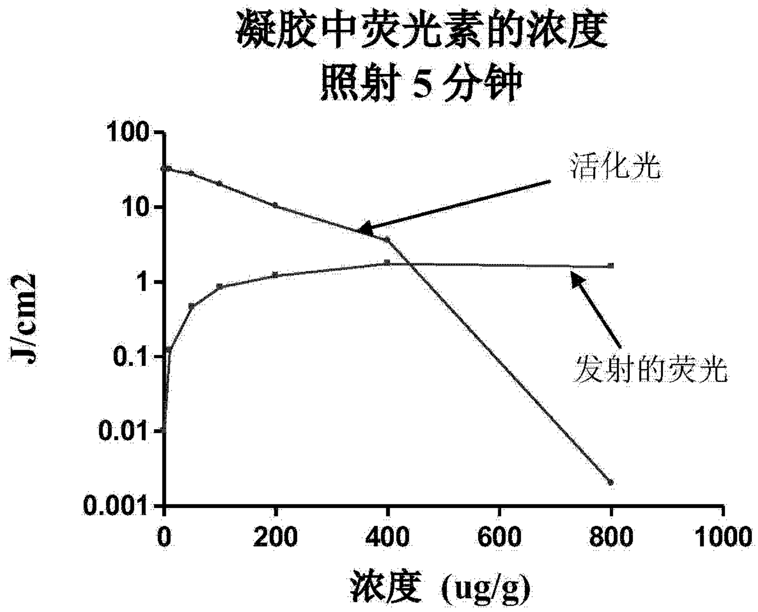
凝胶中荧光素的浓度
照射 5 分钟
J/cm2
100
10
1
0.1
0.01
0.001
0
200
400
600
800
1000
浓度 (ug/g)
活化光
发射的荧光

图 17B

Abstract

The present disclosure provides biophotonic compositions and methods useful in phototherapy. In particular, the biophotonic compositions of the present disclosure comprise at least two xanthene dyes. The biophotonic compositions and the methods of the present disclosure are useful for promoting wound healing and skin rejuvenation, as well as treating acne and other skin disorders.