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(57) Abstract: Provided are kits and methods for lightening a skin of a subject. The kit comprising a first container comprising a mask and a second container comprising a cosmetic composition, wherein the mask and the cosmetic composition are in no direct contact in the kit, wherein: (i) when the mask comprises an oxidizing activator then the cosmetic composition comprises a lignin peroxidase, (ii) when the mask comprises a lignin peroxidase then the cosmetic composition comprises an oxidizing activator.



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METHODS AND DEVICE FOR LIGHTENING SKIN COMPLEXION

FIELD AND BACKGROUND OF THE INVENTION

5 The present invention, in some embodiments thereof, relates to masks, kits and methods for lightening skin regions and, more particularly, but not exclusively, to kits which comprise lignin peroxidase and an oxidizing activator for lightening whole skin complexion.

 The color of human skin is governed by the quantity, quality, and distribution of
10 melanin, a pigment which is also present in plants and microorganisms.

 The synthesis of melanin initiates from the precursor L-tyrosine, which is transformed into a second precursor dopaquinone via the action of tyrosinase. Synthesis of melanin takes place in melanosomes, which are present in melanocyte cells present in the epidermal basal layer; synthesis of melanin in these cells is induced by ultraviolet
15 (UV) light. Following synthesis, melanin migrates to epidermal cells and is dispersed therein, where melanin is decolorized following dermal metabolism and then scaled off in the form of dirt at the time of skin renewal. Melanin has a clinical importance since it protects the skin from adverse effects caused by UV light. However, high levels of melanin can result in unwanted skin darkening, while the heterogeneous distribution
20 thereof can lead to chloasma and freckling which can be aesthetically displeasing.

 Skin lightening products have become increasingly popular in the past few years. The main purpose of skin lightening products is to lighten or whiten the skin or to treat pigmentation disorders such as chloasma, freckles, pregnancy marks and age spots. Several types of skin lightening products are presently available.

25 Products based on the degeneration and death of pigment cells typically include harsh chemicals, such as hydroquinone, 4-isopropylcatechol, and hydroquinone monobenzyl ether, that promote skin whitening, lightening or fade out skin pigmentation. Other agents which are commonly-available for skin-whitening include kojic acid and derivatives thereof, which inhibits melanin production, and a variety of
30 extracts such as Liquorice (Glycyrrhiza Glabra) which include Glabridin that decreases oxidization activity of the melanin creating cells in the skin in similar way as Kojic acid does.

Other lightening products are based on the inhibition of tyrosinase, the enzyme that transforms the precursor L-tyrosine into a second precursor dopaquinone. This group of products includes Arbutin, a glucose hydroquinone compound, which is capable of inhibiting tyrosinase by chelating copper ions thereby suppressing the tautomerization from Dopachrome to DHICA. Melanostat is another lightening product that acts through tyrosinase. Melanostat is a synthetic peptide that functions in deactivating melanogenesis in melanocytes.

Several antioxidant compounds that can inhibit the production of melanin are also utilized in lightening products. Since the synthesis of melanin involves an oxidation reaction, blocking the oxidation at various points from tyrosine/DOPA to melanin ultimately inhibits the synthesis of melanin. For example, L-Ascorbic (Vitamin C) acts as a reducing agent on melanin intermediates and blocks oxidative reactions; other antioxidants utilized by lightening products include bioflavonoids, which are typically extracted from mulberry or licorice.

However, the currently available skin whitening products are typically inefficient and may be harmful to the skin since a continuous external application of these products can lead to permanent leucoderma and side effects such as dyschromatosis and rash.

Masks designed for providing treatment to the skin are known in the art, such as SKII Facial Treatment Mask on the Japanese market. Treatment masks are particularly suitable for applying to the skin for delivering moisturizing agents and other benefit agents to the skin through a wet, typically aqueous, environment. Such masks are made of a substrate and a liquid soaked in the substrate, wherein the mask is adhered only very weakly to the skin, such that the mask is easily removed from the skin with practically no tension to the skin. These treatment masks can be distinguished from removal masks. Removal masks are those designed to firmly adhere to the skin and thereby remove dirt, clogs, and excess corneum on the surface and in the pores of skin upon peeling off the mask.

Consumers frequently use cosmetic products to care for their skin. Rough and/or broken skin and hyperpigmentations (such as age spots, freckles, and brown patches associated with sunlight exposure, skin aging or environmental damage to the human skin) are areas consumers typically seek to treat. Skin whitening is of particular interest in certain Asian populations.

U.S. Patent Application No. 20050013784 discloses a treatment mask comprising a water insoluble substrate and a liquid composition comprising a skin tone changing agent and a water-soluble thickening agent.

Treatment masks, which include hydrogen peroxide, are currently available from
5 Reviva Labs (e.g., Oxygen Mask, Green Papaya & Hydrogen Peroxide Oxygen Mask, REV-11305).

WO2004/052275 publication discloses methods of producing lignin peroxidase, and methods and cosmetic compositions suitable for skin and hair lightening as well as kits and an article-of-manufacturing including active ingredients for skin and hair
10 lightening.

Additional background art includes U.S. Patent Application No. 20040161435, 20090130040.

SUMMARY OF THE INVENTION

15 According to an aspect of some embodiments of the present invention there is provided a kit for lightening a skin of a subject, comprising a first container comprising a mask and a second container comprising a cosmetic composition, wherein the mask and the cosmetic composition are in no direct contact in the kit, wherein: (i) when the mask comprises an oxidizing activator then the cosmetic composition comprises a lignin
20 peroxidase,

(ii) when the mask comprises a lignin peroxidase then the cosmetic composition comprises an oxidizing activator.

According to an aspect of some embodiments of the present invention there is provided a kit for lightening a skin of a subject, comprising a first container comprising
25 a mask which comprises a hydrogen peroxide, and a second container comprising a cosmetic composition which comprising lignin peroxidase, wherein the mask and the cosmetic composition are in no direct contact in the kit, wherein:

(i) a concentration of the hydrogen peroxide in the mask is between 0.01-0.013 % (weight/weight);

30 (ii) a concentration of the lignin peroxidase in the cosmetic composition is between about 0.1% (weight/weight) to about 5% (weight/weight); and

(iii) wherein the mask and/or the cosmetic composition further comprises veratryl alcohol at a concentration in the range of about 0.1-0.2 % (weight/weight).

According to an aspect of some embodiments of the present invention there is provided a kit for lightening a skin of a subject, comprising a first container comprising
5 a mask which comprises a hydrogen peroxide, and a second container comprising a cosmetic composition which comprising lignin peroxidase, wherein the mask and the cosmetic composition are in no direct contact in the kit, wherein:

(i) a concentration of the hydrogen peroxide in the mask is between 0.01-0.013 % (weight/weight);

10 (ii) a concentration of the lignin peroxidase in the cosmetic composition is between about 0.5% (weight/weight) to about 2% (weight/weight); and

(iii) wherein the mask and/or the cosmetic composition further comprises veratryl alcohol at a concentration in the range of about 0.1-0.2 % (weight/weight).

According to an aspect of some embodiments of the present invention there is
15 provided a cosmetic method of lightening a skin of a subject, comprising:

(a) applying the lignin peroxidase on a skin region of the subject, while using the kit of some embodiments of the invention, and;

(b) applying the oxidizing activator in the kit on the skin region having the lignin peroxidase for a pre-determined time,

20 thereby lightening the skin of the subject.

According to an aspect of some embodiments of the present invention there is provided a mask comprising lignin peroxidase and a cosmetically acceptable carrier.

According to some embodiments of the invention, the kit further comprises an oxidizing mediator.

25 According to some embodiments of the invention, the cosmetic composition is in a form of a serum.

According to some embodiments of the invention, the cosmetic composition is in a form of a leave-on lotion, cream, gel or toner.

According to some embodiments of the invention, the mask further comprises a
30 cosmetically acceptable excipient.

According to some embodiments of the invention, the container comprising the oxidizing activator is sealed.

According to some embodiments of the invention, the container comprising the oxidizing activator is made of a material which is inert to oxidation by the oxidizing activator.

According to some embodiments of the invention, the container comprising the oxidizing activator is sealed by vacuum.

According to some embodiments of the invention, the mask comprises a solid support.

According to some embodiments of the invention, the solid support comprises an occlusive film.

According to some embodiments of the invention, the solid support comprises a non-occlusive film.

According to some embodiments of the invention, the mask is a rinse-off mask.

According to some embodiments of the invention, the mask comprises a material which hardens or polymerizes after a set period of time.

According to some embodiments of the invention, the material which hardens or polymerizes after a set period of time is made of at least two phases which are mixed prior to use.

According to some embodiments of the invention, the mask comprises a gelifying agent.

According to some embodiments of the invention, the mask comprises pH regulators.

According to some embodiments of the invention, the concentration of the lignin peroxidase in the cosmetic composition is selected from the range of about 0.1% (weight/weight) to about 5% (weight/weight).

According to some embodiments of the invention, the concentration of the lignin peroxidase in the cosmetic composition is selected from the range of about 0.5% (weight/weight) to about 2% (weight/weight).

According to some embodiments of the invention, the oxidizing mediator is veratryl alcohol.

According to some embodiments of the invention, the concentration of the veratryl alcohol in the cosmetic composition or the mask is between about 0.01 % (weight/weight) to about 1 % (weight/weight).

According to some embodiments of the invention, the oxidizing activator is hydrogen peroxide.

According to some embodiments of the invention, the concentration of the hydrogen peroxide in the mask is between about 0.001 % (weight/weight) to about 0.1 % (weight/weight).

According to some embodiments of the invention, the lignin peroxidase is isoenzyme H1 or a modified form of isoenzyme H2.

According to some embodiments of the invention, the lignin peroxidase is an extract of a *Phanerochaete chrysosporium* fungus.

According to some embodiments of the invention, the lignin peroxidase is recombinantly expressed.

According to some embodiments of the invention, the lignin peroxidase is encoded by a polynucleotide comprising a nucleic acid sequence set forth in SEQ ID NO:1.

According to some embodiments of the invention, the kit further comprising a third and a forth containers which comprise lignin peroxidase and an oxidizing activator, respectively.

According to some embodiments of the invention, the cosmetic method further comprising repeating step (b) daily for at least 5 days.

According to some embodiments of the invention, wherein step (a) of the cosmetic method is performed prior to step (b).

According to some embodiments of the invention, wherein step (b) of the cosmetic method is performed prior to step (a).

According to some embodiments of the invention, the cosmetic method further comprising:

(c) repeating steps (a) and (b) daily for at least 5 days.

According to some embodiments of the invention, the cosmetic method further comprising:

(c) repeating steps (a) and (b) daily for at least 14 days.

According to some embodiments of the invention, the cosmetic method further comprising applying a lotion comprising the lignin peroxidase.

According to some embodiments of the invention, the cosmetic method further comprising applying a lotion comprising the oxidizing activator.

According to some embodiments of the invention, the skin region comprises facial skin.

5 According to some embodiments of the invention, the skin region comprises a skin region exposed to the sun.

According to some embodiments of the invention, lightening the skin comprises lightening whole skin complexion.

10 According to some embodiments of the invention, the skin region comprises an uneven skin tone, dark spot(s), freckle(s), melasma, hyperpigmentation, skin discoloration, age spot(s), acne mark(s) and/or a scar.

According to some embodiments of the invention, the lignin peroxidase is sterile.

According to some embodiments of the invention, the lignin peroxidase is synthetic.

15 According to some embodiments of the invention, the lignin peroxidase is recombinantly expressed.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which
20 the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

25

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for
30 purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIGs. 1A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion following a single use of the serum and mask. FIG. 1A – photograph of an Asian woman before treatment
5 (before applying the serum and mask of some embodiments of the invention); FIG. 1B – photograph of the same Asian woman as in FIG. 1A after a single use of the serum and mask of some embodiments of the invention. Note the lightening of skin complexion following a single use of the serum and mask of some embodiments of the invention;

FIGs. 2A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion following a single
10 use of the serum and mask. FIG. 2A – photograph of an Asian woman before treatment (before applying the serum and mask of some embodiments of the invention); FIG. 2B – photograph of the same Asian woman as in FIG. 2A after a single use of the serum and mask of some embodiments of the invention. Note the lightening of skin complexion
15 following a single use of the serum and mask of some embodiments of the invention;

FIGs. 3A-B are histograms depicting quantitation of skin color in a subject treated the serum and mask of some embodiments of the invention before (baseline) and following a single treatment (a single use, 1 day). FIG. 3A – Mexameter index; FIG. 3B – Erythema index. * = results are significantly different (p value < 0.05). Note the
20 significant reduction in melanin content in the forehead and both cheeks (FIG. 3A) and the significant reduction in erythema in both cheeks (FIG. 3B) in a subject treated for one time with the serum and mask of some embodiments of the invention;

FIGs. 4A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion following 5 days
25 of use. FIG. 4A – photograph of an Asian woman at day 0 (before using the serum and mask of some embodiments of the invention, the same woman shown in FIGs. 2A-B); FIG. 4B – photograph of the same Asian woman as in FIG. 4A after using the serum and mask of some embodiments of the invention daily (one time per day) along with the advanced whitening lotion active enzyme according to some embodiments of the
30 invention and lotion activator according to some embodiments of the invention (one time per day) for 5 consecutive days. Note the lightening of skin complexion following 5 days of using the serum and mask of some embodiments of the invention;

FIGs. 5A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion following 5 days of use. FIG. 5A – photograph of an Asian woman at day 0 (before using the serum and mask of some embodiments of the invention); FIG. 5B – photograph of the same Asian woman as in FIG. 5A after using the serum and mask of some embodiments of the invention daily (one time per day) along with the advanced whitening lotion active enzyme according to some embodiments of the invention and activator lotion according to some embodiments of the invention (one time per day) for 5 consecutive days. Note the significant lightening of whole skin complexion following 5 days of treatment with the serum and mask of some embodiments of the invention;

FIGs. 6A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion. The mask and serum of some embodiments of the invention were applied daily for 5 consecutive days along with the advanced whitening lotion active enzyme according to some embodiments of the invention and activator according to some embodiments of the invention (one time per day) and the effect of the compositions on skin lightening was evaluated after 2 weeks from the first day of using the lightening compositions. FIG. 6A – photograph of an Asian woman at day 0 (baseline, before using the serum and mask of some embodiments of the invention); FIG. 6B – photograph of the same Asian woman as in FIG. 6A after two weeks. Note the significant lightening of whole skin complexion as well as the specific lightening of a pigmented scar (6 month old scar) after two weeks;

FIGs. 7A-B depict the composition of the advanced whitening lotion active enzyme according to some embodiments of the invention (FIG. 7A) and the composition of the advanced whitening lotion activator according to some embodiments of the invention (FIG. 7B).

FIG. 8 depicts the composition of the advanced facial wash according to some embodiments of the invention.

FIGs. 9A-B are images depicting the effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion, and pigmentation. The mask, serum, cleanser and lotion of some embodiments of the invention were applied daily over a 7 days period according to the treatment regimen summarized in Table 9 below (Example 3 of the Examples section which follows) and the effect of the

compositions on skin lightening was evaluated after 6 days from the first day of using the lightening compositions. FIG. 9A – photograph of a Caucasian woman at day 0 (baseline, before using the serum and mask of some embodiments of the invention); FIG. 9B – photograph of the same Caucasian woman as in FIG. 9A after 6 days. Note the significant lightening of whole skin complexion as well as the specific lightening of skin pigmentation on the right cheek after only 6 days of treatment.

FIGs. 10A-D are images depicting the remaining effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion, as follow up, 7 days after the treatment has concluded. The mask, serum, cleanser and lotion of some embodiments of the invention were applied daily over a 7 days period according to the treatment regimen summarized in Table 9 below (Example 3 of the Examples section which follows) and the effect of the compositions on skin lightening was evaluated after 2 weeks from the first day of using the lightening compositions. FIGs. 10A and 10C – photographs of a Caucasian woman at day 0 (baseline, before using the serum and mask of some embodiments of the invention); FIGs. 10B and 10D – photographs of the same Caucasian woman as in FIG. 9A after two weeks. The photographs clearly show that the effect remained visible after two weeks (7 days after treatment was stopped).

FIGs. 11A-B are images depicting the remaining effect of the serum and mask according to some embodiments of the invention on lightening of skin complexion, as follow up, 21 days after the treatment has concluded. The mask, serum, cleanser and lotion of some embodiments of the invention were applied daily over a 7 days period according to the treatment regimen summarized in Table 9 below (Example 3 of the Examples section which follows) and the effect of the compositions on skin lightening was evaluated after one month from the first day of using the lightening compositions. FIG. 11A – photograph of a Caucasian woman at day 0 (baseline, before using the serum and mask of some embodiments of the invention); FIG. 11B – photograph of the same Caucasian woman as in FIG. 11A after one month. The photographs clearly show that the effect remained visible after one month (21 days after treatment was stopped).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to masks, kits and methods for lightening skin regions and, more particularly, but not exclusively, to kits which comprise lignin peroxidase and an oxidizing activator for lightening whole skin complexion.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

The present inventors have designed lightening methods and kits which use a cosmetic composition and a mask one of which comprises a lignin peroxidase and the other comprises an oxidizing activator. These kits can be used for lightening whole skin complexion as well as specific skin regions-of-interest.

Thus, as shown in the Examples section which follows, the present inventors have designed a cosmetic composition (e.g., serum), which comprises lignin peroxidase, and a mask, which comprises an oxidizing activator (e.g., hydrogen peroxide) ("GENERAL MATERIALS AND METHODS"), which when applied on a skin region of a subject (e.g., whole facial skin) results in a significant whitening effect (Figures 1A-B and 2A-B, Example 1) which included reduction in both melanin content and erythema in the treated skin (Figures 3A-B, Example 1). Furthermore, a clinical study performed using the whitening serum and mask described in Tables 1-4 below and under "GENERAL MATERIALS AND METHODS" along with a whitening lotion which comprises lignin peroxidase and an oxidizing activator for 5 consecutive days resulted in significant lightening of whole skin complexion in Asian subjects following 5 days (Figures 4A-B, and 5A-B, Example 2), which significant even after two weeks (Figures 6A-B, Example 2). In addition, a further clinical test using the cleanser, serum, mask and lotion of some embodiments of the invention showed a significant effect on lightening skin complexion and reducing skin pigmentation after 7 days, 2 weeks and one month from initial treatment (Example 3, Figures 9-11). These results demonstrate the significant whitening effect of the serum and mask of some embodiments of the invention in whitening whole skin complexion as well as specific regions, such as of scarred skin or red spots on the skin.

According to an aspect of some embodiments of the invention there is provided a kit for lightening a skin of a subject, comprising a first container comprising a mask and a second container comprising a cosmetic composition, wherein the mask and the cosmetic composition are in no direct contact in the kit, wherein:

5 (i) when the mask comprises an oxidizing activator then the cosmetic composition comprises lignin peroxidase,

(ii) when the mask comprises lignin peroxidase then the cosmetic composition comprises an oxidizing activator.

As used herein the phrase "lignin peroxidase" refers to an enzyme, which plays
10 a major role in lignin degradation. Lignin peroxidase is able to catalyze the oxidation of substrates with high redox potential. This unique ability is consistent with a heme active site of low electron density, which is indicated by high redox potential [Cai and Tien (1993). J Biotechnol 30: 79-90].

Lignin peroxidase is produced in various organisms and the coding sequences of
15 the lignin peroxidase enzymes are available from GenBank via the Hypertext Transfer Protocol [://World Wide Web \(dot\) ncbi \(dot\) nlm \(dot\) nih \(dot\) gov/](http://WorldWideWeb.ncbi.nlm.nih.gov/). For example, lignin peroxidase from *Phanerochaete chrysosporium* (SEQ ID NO:1); *Mycobacterium tuberculosis* H37Rv [GenBank Accession No. NP_216416.1 (SEQ ID NO:2 (polypeptide) and SEQ ID NO:3 (polynucleotide)]; *Mycobacterium bovis* AF2122/97
20 [GenBank Accession No. NP_855586.1 (SEQ ID NO:4 (polypeptide) and SEQ ID NO:5 (polynucleotide)]; *Mycobacterium bovis* BCG str. Pasteur 1173P2 [GenBank Accession No. YP_978029.1 (SEQ ID NO:6 (polypeptide) and SEQ ID NO:7 (polynucleotide)]; *Mycobacterium bovis* BCG str. Tokyo 172 [GenBank Accession No. YP_002644977.1 (SEQ ID NO:8 (polypeptide) and SEQ ID NO:9 (polynucleotide)];
25 *Mycobacterium tuberculosis* H37Ra [GenBank Accession No. YP_001283231.1 (SEQ ID NO:10 (polypeptide) and SEQ ID NO:11 (polynucleotide)]; *Mycobacterium ulcerans* Agy99 [GenBank Accession No. YP_908214.1 (SEQ ID NO:12 (polypeptide) and SEQ ID NO:13 (polynucleotide)]; *Mycobacterium marinum* M [GenBank Accession No. YP_001848608.1 (SEQ ID NO:14 (polypeptide) and SEQ ID NO:15 (polynucleotide)];
30 *Mycobacterium tuberculosis* KZN 1435 [GenBank Accession No. YP_003032055.1 (SEQ ID NO:16 (polypeptide) and SEQ ID NO:17 (polynucleotide)]; *Mycobacterium tuberculosis* F11 [GenBank Accession No. YP_001287866.1 (SEQ ID

NO:18 (polypeptide) and SEQ ID NO:19 (polynucleotide)]; *P.chrysosporium* ligninase (CKG4) [GenBank Accession No. M18743.1 (SEQ ID NO:20 (polypeptide) and SEQ ID NO:21 (polynucleotide)]; *Phanerochaete chrysosporium* (anamorph: *Sporotrichum pruinosum*) [GenBank Accession No. M80213.1 (SEQ ID NO:22 (polypeptide) and
5 SEQ ID NO:23 (polynucleotide)]; *Phanerochaete sordida* ylpB [GenBank Accession No. AB455007.1 (SEQ ID NO:24 (polypeptide) and SEQ ID NO:25 (polynucleotide)]; *Phanerochaete chrysosporium* (anamorph: *Sporotrichum pruinosum*) [GenBank Accession No. M77508.1 (SEQ ID NO:26 (polypeptide) and SEQ ID NO:27 (polynucleotide)].

10 According to some embodiments of the invention, the lignin peroxidase enzyme used according to some embodiments of the invention is the lignin peroxidase H1 isoform, which exhibits melanin oxidation activities both *in vitro* and *in vivo* (WO2004/052275).

Several approaches can be used to produce the lignin peroxidase enzyme
15 utilized according to some embodiments of the present invention.

For example, lignin peroxidase isoenzyme H1 can be prepared from the fungus *Phanerochaete chrysosporium*. High levels of enzymatic activity of lignin peroxidase can be produced from the above fungus when grown in a stirred tank reactor (STR) fermentor while being immobilized on polyurethane foam or in suspension (Dosoretz et
20 al., 1993, Appl Environ Microbiol. 59: 1919-26).

According to some embodiments of the invention the fermentor is connected to a cooling system to maintain a culturing temperature of 37 °C and is stirred at speed of 50-300 rpm (rounds per minute), more preferably, 100-200 rpm, most preferably at 160 rpm. In order to increase the yield of lignin peroxidase activity the fermentor is aerated
25 at an aeration rate of 0.1-1 liter of air per liter of culture medium per minutes. According to presently preferred configurations the fermentor is aerated at an aeration rate of 0.2 liter of air per liter of culture medium per minute.

The *Phanerochaete chrysosporium* is cultured under culturing conditions devoid of manganese ions and containing glycerol as a source of carbon. According to some
30 embodiments of the invention, the glycerol is provided at a concentration range of 3-20 grams per liter. According to some embodiments of the invention the glycerol is provided at a concentration of 6 grams per liter.

During the purification process of lignin peroxidase isoenzyme H1 from the above fungus the enzymatic activity of the purified protein is been further tested by a change in absorbance at 310 nm that occurs due to the oxidation of veratryl alcohol to veratryl aldehyde.

5 Since lignin peroxidase isoenzyme H1 can result from a post-translational dephosphorylation of isoenzyme H2 (Kuan and Tien, 1989), the lignin peroxidase used according to some embodiments of the invention can be prepared by dephosphorylating the lignin peroxidase isoenzyme H2.

10 According to some embodiments of the invention, the lignin peroxidase can be recombinantly expressed using a nucleic acid construct designed to express the coding sequence of lignin peroxidase in a host cell. Non-limiting examples of such nucleic acid sequences are provided in SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25 and 27.

15 The nucleic acid construct (also referred to herein as an "expression vector") of some embodiments of the invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes, eukaryotes, or preferably both (e.g., shuttle vectors). In addition, typical cloning vectors may also contain a transcription and translation initiation sequence, transcription and translation terminator and a polyadenylation signal. By way of example, such constructs will typically include
20 a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

The nucleic acid construct of some embodiments of the invention typically includes a signal sequence for secretion of the peptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence
25 or the signal sequence of the polypeptide variants of some embodiments of the invention.

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA
30 polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for some embodiments of the invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, Enhancers and Eukaryotic Expression, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

Polyadenylation sequences can also be added to the expression vector in order to increase the efficiency of lignin peroxidase mRNA translation. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the polyadenylation site and a highly conserved sequence of six nucleotides, AAUAAA, located 11-30 nucleotides upstream. Termination and polyadenylation signals that are suitable for some embodiments of the invention include those derived from SV40.

In addition to the elements already described, the expression vector of some embodiments of the invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The expression vector of some embodiments of the invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

As mentioned hereinabove, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the polypeptides of some embodiments of the invention. These include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the coding sequence; yeast transformed with recombinant yeast expression vectors containing the coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the coding sequence. Mammalian expression systems can also be used to express the polypeptides of some embodiments of the invention.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A⁺, pMTO10/A⁺, pMAMneo-5, baculovirus pDSVE, and any other vector

allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

5 Various methods can be used to introduce the expression vector of some embodiments of the invention into stem cells. Such methods are generally described in Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., *Current Protocols in Molecular Biology*, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., *Somatic Gene*
10 Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., *Gene Targeting*, CRC Press, Ann Arbor Mich. (1995), *Vectors: A Survey of Molecular Cloning Vectors and Their Uses*, Butterworths, Boston Mass. (1988) and Gilboa et al. [*Biotechniques* 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lignin peroxidaseofection, electroporation and infection with recombinant viral vectors. In
15 addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lignin peroxidaseofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

20 Other than containing the necessary elements for the transcription and translation of the inserted coding sequence, the expression construct of some embodiments of the invention can also include sequences engineered to enhance stability, production, purification, yield or toxicity of the expressed peptide. For example, the expression of a fusion protein or a cleavable fusion protein comprising the
25 lignin peroxidase H1 or H2 protein of some embodiments of the invention and a heterologous protein can be engineered. Such a fusion protein can be designed so that the fusion protein can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the heterologous protein. Where a cleavage site is engineered between the lignin peroxidase H1 or H2 protein and the heterologous
30 protein, the lignin peroxidase protein can be released from the chromatographic column by treatment with an appropriate enzyme or agent that disrupts the cleavage site [e.g.,

see Booth et al. (1988) *Immunol. Lett.* 19:65-70; and Gardella et al., (1990) *J. Biol. Chem.* 265:15854-15859].

As mentioned hereinabove, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the polypeptides of some embodiments of the invention. These include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the coding sequence; yeast transformed with recombinant yeast expression vectors containing the coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the coding sequence. Mammalian expression systems can also be used to express the polypeptides of some embodiments of the invention.

Examples of bacterial constructs include the pET series of *E. coli* expression vectors [Studier et al. (1990) *Methods in Enzymol.* 185:60-89].

In yeast, a number of vectors containing constitutive or inducible promoters can be used, as disclosed in U.S. Pat. Application No: 5,932,447. Alternatively, vectors can be used which promote integration of foreign DNA sequences into the yeast chromosome.

In cases where plant expression vectors are used, the expression of the coding sequence can be driven by a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al. (1984) *Nature* 310:511-514], or the coat protein promoter to TMV [Takamatsu et al. (1987) *EMBO J.* 6:307-311] can be used. Alternatively, plant promoters such as the small subunit of RUBISCO [Coruzzi et al. (1984) *EMBO J.* 3:1671-1680 and Brogli et al., (1984) *Science* 224:838-843] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al. (1986) *Mol. Cell. Biol.* 6:559-565] can be used. These constructs can be introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach, 1988, *Methods for Plant Molecular Biology*, Academic Press, NY, Section VIII, pp 421-463.

Other expression systems such as insects and mammalian host cell systems which are well known in the art and are further described hereinbelow can also be used by some embodiments of the invention.

Recovery of the recombinant polypeptide is effected following an appropriate
5 time in culture. The phrase "recovering the recombinant polypeptide" refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification. Notwithstanding the above, polypeptides of some embodiments of the invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity
10 chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

According to some embodiments of the invention, the lignin peroxidase is
15 expressed in bacterial cells modified to express the lignin peroxidase as disclosed in US patent 5,200,338. For example, a bacterial cell, such as, *E. coli*, can be transformed with an expression vector including the lignin peroxidase coding sequence (SEQ ID NO:1) positioned under the regulatory control of a strong constitutive promoter (e.g., SP6). Following expression, the bacterial cells can be lysed and the lignin peroxidase
20 can be collected using chromatographic techniques (see, Billman-Jacobe, 1996, Curr. Opin. Biotechnol. 7: 500-4; Harris and Emtage, 1986, Microbiol. Sci. 3: 28-31, for further details).

The lignin peroxidase according to some embodiments of the invention can be extracted from mammalian cell lines such as HeLa cells. In this case the lignin
25 peroxidase coding sequence is positioned under a strong mammalian promoter (e.g., CMV) in a suitable expression vector (e.g., pCDNA3.1, Invitrogen Life Technologies, Frederick, MD, USA). Following transfection of HeLa cells with the expression vector, the lignin peroxidase expression product can be extracted from the cells or medium (e.g., by modifying the lignin peroxidase sequence to include a secretion signal) by
30 conventional purification and chromatography techniques (see Cunha and Aires-Barros, 2002. Mol. Biotechnol. 20: 29-40 for further details).

The oxidizing activator used in the mask or cosmetic composition of some embodiments of the invention is capable of oxidizing a substrate (*i.e.*, an electron acceptor).

According to some embodiments of the invention, the oxidizing activator is
5 hydrogen peroxide.

It should be noted that the oxidizing activator should not be in contact with the lignin peroxidase prior to applying onto the skin. This is to avoid pre-activation of the enzyme in the kit. Therefore the two are placed separately in the kit.

According to some embodiments of the invention the cosmetic composition
10 and/or the mask of some embodiments of the invention further comprise(s) an oxidizing mediator. The oxidizing mediators used according to some embodiments of the invention are small aromatic molecules or more specifically methoxylated compounds that increase the oxidative potential and the stability of the lignin peroxidase. Non-limiting examples of oxidizing mediators include, but are not limited to, phenolic
15 compounds, such as veratryl alcohol (Harvey et al., 1992, Biochem Soc Trans 20: 345-9), and veratrole (Ward et al., Enzyme and Microbial Technology (2002), 30: 490-498).

According to some embodiments of the invention, the oxidizing mediator is veratryl alcohol.

According to some embodiments of the invention the kit comprises a first
20 container comprising a mask which comprises an oxidizing activator and a second container comprising a cosmetic composition which comprises lignin peroxidase.

According to some embodiments of the invention, the mask comprises the oxidizing activator and an oxidizing mediator.

According to some embodiments of the invention, the cosmetic composition
25 comprises the lignin peroxidase and an oxidizing mediator.

Alternatively, according to some embodiments of the invention the kit comprising a first container comprising a mask which comprises lignin peroxidase and a second container comprising a cosmetic composition which comprising an oxidizing activator.

30 According to some embodiments of the invention, the mask comprises the lignin peroxidase and an oxidizing mediator.

According to some embodiments of the invention, the cosmetic composition comprises the oxidizing activator and an oxidizing mediator.

According to some embodiments of the invention, the kit further comprises a third and a forth containers which comprise lignin peroxidase and an oxidizing activator,
5 respectively.

For example, the third container can include a cosmetic composition (e.g., cream, lotion) which comprises the lignin peroxidase and the forth container can include a cosmetic composition (e.g., cream, lotion) which comprises the oxidizing activator. The third and forth containers can be separate containers, or can form two compartments
10 of a single container (as long as the different cosmetic compositions in the container's compartments are not in direct contact with each other). It should be noted that the third and/or forth container can further include an oxidizing mediator.

As used herein a "cosmetic composition" refers to a preparation which includes the active ingredients described hereinabove (e.g., lignin peroxidase, an oxidizing
15 activator and/or an oxidizing mediator) and additional chemical components such as physiologically suitable carriers and excipients. The purpose of a cosmetic composition is to facilitate administration of the active ingredient to an organism.

Hereinafter, the phrases "suitable carrier" used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological
20 activity and properties of the lignin modifying enzyme.

Herein the term "excipient" refers to an inert substance added to a cosmetic composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene
25 glycols.

The cosmetic composition may be applied in a local manner, for example, via administration of the cosmetic composition directly into a tissue (e.g., skin) region of a patient. Suitable routes of administration may, for example, include topical, subcutaneous and intradermal injections.

30 Cosmetic compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving,

granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Cosmetic compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more
5 physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations. Proper formulation is dependent upon the administration approach chosen.

For injection, the active ingredients of the cosmetic composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such
10 as Hank's solution, Ringer's solution, or physiological salt buffer.

Alternatively, the active ingredient may be in a powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

15 For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from *in vitro* assays. In addition, a dose can be formulated in tissue cultures systems or in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

20 Depending on the severity of the skin pigmentation disorder (e.g., chloasma, melasma, ochronosis and lentigo), the tone of skin color, skin complexion and/or the responsiveness of the skin, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the skin disorder is achieved.

25 The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

The lignin peroxidase included in the cosmetic composition or the mask of the some embodiments of the invention can be also provided at higher concentrations and
30 be prescribed by a physician as a pharmaceutical or a cosmetic composition to treat skin pigmentation disorders such as melasma, chloasma, ochronosis, and lentigo, and/or to lighten whole skin complexion.

Following is a non-limiting description of formulations incorporating a lignin peroxidase and formulated for skin lightening.

To optimize and control skin lightening, the lignin peroxidase is preferably included in a cosmetic composition or a mask which is formulated for skin lightening purposes.

Since the skin lightening cosmetic composition or mask of the some embodiments of the invention is utilized *in vivo*, the composition is preferably of high purity and substantially free of potentially harmful contaminants, e.g., at least National Food (NF) grade, generally at least analytical grade, and preferably at least pharmaceutical grade. To the extent that a given compound must be synthesized prior to use, such synthesis or subsequent purification shall preferably result in a product that is substantially free of any potentially contaminating toxic agents that may have been used during the synthesis or purification procedures.

Active ingredients

It should be noted, that unless otherwise specified, the concentration of the active ingredients within the cosmetic composition are expressed herein as weight per weight (w/w) percentages (%), *i.e.*, a weight of the active ingredient in a 100 gram (gr) of composition. With respect to the mask, the concentrations of the active ingredients within the mask are expressed as weight per weight percentages, *i.e.*, a weight of the active ingredient in a 100 grams of final preparation of a mask (e.g., of the masks devoid of solid support as is further explained hereinunder) or of the liquid portion of the mask in case of a mask made with a solid support (e.g., a cloth) soaked with a liquid.

Lignin peroxidase is included in the cosmetic composition or in the mask of the some embodiments of the invention at a concentration selected from a range of 0.05-20 % (w/w), e.g., from at least about 0.1 % (w/w) to no more than about 10 % (w/w), e.g., from about 0.1 – 5% (w/w), e.g., from about 0.1 – 1% (w/w), e.g., from the range of 0.5-5 % (w/w), 0.6-4 % (w/w), 0.6-3 % (w/w), 0.6-2.5 % (w/w), 0.65-2 % (w/w), e.g., 0.69%, 0.7%, 0.71%, 0.8%, 1.5% or 2% (w/w).

The concentration of lignin peroxidase can be also presented in units of active enzyme. One gram of lignin peroxidase equals to 3500 units.

For example, a concentration of 0.05% (w/w) lignin peroxidase (*i.e.*, 0.05 gram lignin peroxidase in 100 gram product (cream, serum or lotion)) equals to 175 units of lignin peroxidase in 100 gram product or to 1.75 units of lignin peroxidase in 1 gram product. Similarly, a concentration of 0.7% (w/w) of lignin peroxidase equals to 2450
5 units of lignin peroxidase in 100 gram product (cream, serum or lotion) or to 24.5 units of lignin peroxidase in 1 gram product. Similarly, a concentration of 20% (w/w) of lignin peroxidase equals to 70000 units of lignin peroxidase in 100 gram product (cream, lotion or serum) or 700 units of lignin peroxidase in 1 gram product.

According to some embodiments of the invention, the lignin peroxidase
10 included in the cosmetic composition or the mask of some embodiments of the invention is provided at a concentration of about 20-50 units per gram, *e.g.*, about 25-35 units per gram, *e.g.*, about 25 units per gram cosmetic composition or mask.

It should be noted that a preferred concentration of the lignin peroxidase is selected according to the specific use of the composition, thus, for lightening of whole
15 skin complexion, the concentration of lignin peroxidase can be about 0.6-0.8 % (w/w), while for freckle lightening, the concentration of lignin peroxidase can be about 1-2 % (w/w).

According to some embodiments of the invention, the concentration of hydrogen peroxide in the cosmetic composition or the mask of some embodiments of the invention is between 0.001% [weight per weight (w/w) percentage] to about 0.1%, *e.g.*,
20 in the range of 0.005-0.05%, 0.008-0.03%, 0.008-0.02%, 0.009-0.015%, 0.01-0.013, 0.011-0.013%, *e.g.*, 0.011%, 0.012%, 0.013% (w/w). According to some embodiments of the invention, the hydrogen peroxide included in the cosmetic composition or the mask of some embodiments of the invention is provided at a concentration of about
25 3.52 mmole/kg cosmetic composition or mask. Hydrogen peroxide is stable, but will decompose under neutral or alkaline conditions to form water and an active species of oxygen. The active species of oxygen are very reactive.

According to some embodiments of the invention, the container comprising the oxidizing activator is made of a material, which is inert to oxidation by the oxidizing
30 activator.

According to some embodiments of the invention, the container comprising the oxidizing activator is sealed.

According to some embodiments of the invention, the container comprising the oxidizing activator is sealed by vacuum.

The cosmetic composition or the mask comprising hydrogen peroxide is preferably buffered to a pH of about 5 or lower, e.g., pH of about 5-2, e.g., pH of about 5, about 4.5, about 4, about 3.5, about 3.0, about 2.5, about 2.0. It should be noted that hydrogen peroxide is stable at such pHs.

Any buffer capable of maintaining a pH of 5 or less may be employed. Thus, buffers employing acetic acid, tartaric acid, phosphoric acid or citric acid may be used by the skin lightening cosmetic composition or mask.

According to some embodiments of the invention, the hydrogen peroxide of some embodiments of the invention is stabilized at pH 3.5 with citric acid.

According to some embodiments of the invention, the veratryl alcohol comprised in the cosmetic composition or the mask of the some embodiments of the invention is diluted in water.

According to some embodiments of the invention, the concentration of veratryl alcohol in the cosmetic composition or the mask is between about 0.01 to about 1 % weight per weight (w/w), e.g., in the range of about 0.02-0.8%, e.g., about 0.05-0.5%, e.g., about 0.05-0.2%, 0.08-0.18%, e.g., about 0.1-0.15%, e.g., about 0.12-0.15, e.g., about 0.12%, 0.13%, 0.14%, 0.15% w/w. According to some embodiments of the invention, the veratryl alcohol included in the cosmetic composition or the mask of some embodiments of the invention is provided at a concentration of about 6 mmole/Kg cosmetic composition or mask.

Epidermal penetrants

In order to enhance the percutaneous absorption of the active ingredients (e.g., lignin peroxidase, oxidizing activator, and/or oxidizing mediator), one or more of a number of agents can be added to the cosmetic composition including, but not limited to, dimethylsulfoxide, dimethylacetamide, dimethylformamide, surfactants, azone, alcohol, acetone, propylene glycol, polyethylene glycol, and Butylene Glycol.

As illustrated under "GENERAL MATERIALS AND METHODS" in the Examples section which follows, the lignin peroxidase and/or the oxidizing mediator (veratryl alcohol) can be mixed with epidermal penetrants such as butylene glycol; the oxidizing activator (hydrogen peroxide) can be mixed with transcutol, in a manner and

concentration optimized for enhancing skin penetration of the lignin peroxidase. The butylene glycol used according to some embodiments of the invention is provided at a concentration of at least 1% (w/w) and the transcitol is provided at a concentration of at least 3% (w/w).

5 ***Carriers***

In addition to the pharmaceutically effective amount of an agent disclosed herein, the cosmetic composition or the mask of some embodiments of the invention also includes a dermatologically acceptable carrier.

10 The phrase "dermatologically acceptable carrier", refers to a carrier which is suitable for topical application onto the skin, *i.e.*, keratinous tissue, has good aesthetic properties, is compatible with the active agents of some embodiments of the invention and any other components, and is safe and non-toxic for use in mammals. An effective amount of carrier is selected from a range of about 50 % to about 99.99 %, preferably from about 80 % to about 99.9 %, more preferably from about 90 % to about 98 %, and
15 most preferably from about 90 % to about 95 %, by weight, of the composition.

Emulsions

20 The carrier utilized in the compositions or masks of some embodiments of the invention can be in a wide variety of forms. These include emulsion carriers, including, but not limited to, oil-in-water, water-in-oil, water-in-oil-in-water, and oil-in-water-in-silicone emulsions, a cream, an ointment, an aqueous solution, a lotion or an aerosol. As will be understood by the skilled artisan, a given component will distribute primarily into either the water or oil/silicone phase, depending on the water solubility/dispersibility of the component in the composition.

25 Emulsions according to some embodiments of the invention generally contain a pharmaceutically effective amount of an active ingredient disclosed herein and oil. The active ingredient (e.g., lignin peroxidase) and oils may be derived from animals, plants, or petroleum and may be natural or synthetic (*i.e.*, man-made). Preferred emulsions also contain a humectant, such as glycerin. Emulsions will preferably further contain from about 1 % to about 10 %, more preferably from about 2 % to about 5 %, of an
30 emulsifier, based on the weight of the carrier. Emulsifiers may be nonionic, anionic or cationic. Suitable emulsifiers are described in, for example, U.S. Pat. No. 3,755,560, issued to Dickert, et al. Aug. 28, 1973; U.S. Pat. No. 4,421,769, issued to Dixon, et al.,

Dec. 20, 1983; and McCutcheon's Detergents and Emulsifiers, North American Edition, pages 317-324 (1986).

The emulsion may also contain an anti-foaming agent to minimize foaming upon application to the keratinous tissue. Anti-foaming agents include high molecular weight silicones and other materials well known in the art for such use.

Suitable emulsions may have a wide range of viscosities, depending on the desired product form. Exemplary low viscosity emulsions, which are preferred, have a viscosity of about 50 centistokes or less, more preferably about 10 centistokes or less, most preferably about 5 centistokes or less. The emulsion may also contain an anti-foaming agent to minimize foaming upon application to the keratinous tissue. Anti-foaming agents include high molecular weight silicones and other materials well known in the art for such use.

One type of emulsion is a water-in-silicone emulsion. Water-in-silicone emulsions contain a continuous silicone phase and a dispersed aqueous phase. Preferred water-in-silicone emulsions of the present invention comprise from about 1 % to about 60 %, preferably from about 5% to about 40%, more preferably from about 10 % to about 20 %, by weight of a continuous silicone phase. The continuous silicone phase exists as an external phase that contains or surrounds the discontinuous aqueous phase described hereinafter.

The continuous silicone phase may contain a polyorganosiloxane oil. A preferred water-in-silicone emulsion system is formulated to provide an oxidatively stable vehicle for delivery of a pharmaceutically effective amount of an agent disclosed herein. The continuous silicone phase of these preferred emulsions comprises between about 50 % and about 99.9 % by weight of organopolysiloxane oil and less than about 50 % by weight of a non-silicone oil. In an especially preferred embodiment, the continuous silicone phase comprises at least about 50 %, preferably from about 60 % to about 99.9 %, more preferably from about 70 % to about 99.9 %, and even more preferably from about 80 % to about 99.9 %, polyorganosiloxane oil by weight of the continuous silicone phase, and up to about 50 % non-silicone oils, preferably less about 40 %, more preferably less than about 30 %, even more preferably less than about 10 %, and most preferably less than about 2 %, by weight of the continuous silicone phase. These useful emulsion systems may provide more oxidative stability over extended

periods of time than comparable water-in-oil emulsions containing lower concentrations of the polyorganosiloxane oil. Concentrations of non-silicone oils in the continuous silicone phase are minimized or avoided altogether so as to possibly further enhance oxidative stability of the active compound of the invention in the compositions. Water-in-silicone emulsions of this type are described in U.S. Pat. No. 5,691,380 to Mason et al., issued Nov. 25, 1997.

The organopolysiloxane oil for use in the composition may be volatile, non-volatile, or a mixture of volatile and non-volatile silicones. The term "nonvolatile" as used in this context refers to those silicones that are liquid under ambient conditions and have a flash point (under one atmospheric of pressure) of or greater than about 100 degrees Celsius. The term "volatile" as used in this context refers to all other silicone oils. Suitable organopolysiloxanes can be selected from a wide variety of silicones spanning a broad range of volatilities and viscosities. Examples of suitable organopolysiloxane oils include polyalkylsiloxanes, cyclic polyalkylsiloxanes, and polyalkylarylsiloxanes, which are known to those skilled in the art and commercially available.

The continuous silicone phase may contain one or more non-silicone oils. Concentrations of non-silicone oils in the continuous silicone phase are preferably minimized or avoided altogether so as to further enhance oxidative stability of the pharmaceutically effective agent in the compositions. Suitable non-silicone oils have a melting point of about 25 °C or less under about one atmosphere of pressure. Examples of non-silicone oils suitable for use in the continuous silicone phase are those well known in the chemical arts in topical personal care products in the form of water-in-oil emulsions, e.g., mineral oil, vegetable oils, synthetic oils, semisynthetic oils, etc.

Useful topical compositions of the present invention comprise from about 30 % to about 90 %, more preferably from about 50 % to about 85 %, and most preferably from about 70 % to about 80 % of a dispersed aqueous phase. The term "dispersed phase" is well-known to one skilled in the art it implies that the phase exists as small particles or droplets that are suspended in and surrounded by a continuous phase. The dispersed phase is also known as the internal or discontinuous phase. The dispersed aqueous phase is a dispersion of small aqueous particles or droplets suspended in and surrounded by the continuous silicone phase described hereinbefore. The aqueous

phase can be water, or a combination of water and one or more water soluble or dispersible ingredients. Nonlimiting examples of such optional ingredients include thickeners, acids, bases, salts, chelants, gums, water-soluble or dispersible alcohols and polyols, buffers, preservatives, sunscreens, agents, colorings, and the like.

5 The topical compositions of some embodiments of the invention typically comprise from about 25 % to about 90 %, preferably from about 40 % to about 80 %, more preferably from about 60 % to about 80 %, water in the dispersed aqueous phase by weight of the composition.

10 The water-in-silicone emulsions of some embodiments of the invention comprise an emulsifier. In a preferred embodiment, the composition contains from about 0.1 % to about 10 % emulsifier, more preferably from about 0.5 % to about 7.5 %, most preferably from about 1 % to about 5 %, emulsifier by weight of the composition. The emulsifier helps disperse and suspend the aqueous phase within the continuous silicone phase.

15 A wide variety of emulsifying agents can be employed herein to form the preferred water-in-silicone emulsion. Known or conventional emulsifying agents can be used in the composition, provided that the selected emulsifying agent is chemically and physically compatible with essential components of the composition, and provides the desired dispersion characteristics. Suitable emulsifiers include silicone emulsifiers, e.g., organically modified organopolysiloxanes, also known to those skilled in the art as
20 silicone surfactants, non-silicon-containing emulsifiers, and mixtures thereof, known by those skilled in the art for use in topical personal care products.

Useful emulsifiers include a wide variety of silicone emulsifiers. These silicone emulsifiers are typically organically modified organopolysiloxanes, also known to those
25 skilled in the art as silicone surfactants. Suitable emulsifiers are described, for example, in McCutcheon's, Detergents and Emulsifiers, North American Edition (1986), published by Allured Publishing Corporation; U.S. Pat. No. 5,011,681 to Ciotti et al., issued Apr. 30, 1991; U.S. Pat. No. 4,421,769 to Dixon et al., issued Dec. 20, 1983; and U.S. Pat. No. 3,755,560 to Dickert et al., issued Aug. 28, 1973.

30 Other preferred topical carriers include oil-in-water emulsions, having a continuous aqueous phase and a hydrophobic, water-insoluble phase ("oil phase") dispersed therein. Examples of suitable carriers comprising oil-in-water emulsions are

described in U.S. Pat. No. 5,073,371 to Turner, D. J. et al., issued Dec. 17, 1991, and U.S. Pat. No. 5,073,372, to Turner, D. J. et al., issued Dec. 17, 1991. An especially preferred oil-in-water emulsion, containing a structuring agent, hydrophilic surfactant and water, is described in detail hereinafter.

5 A preferred oil-in-water emulsion comprises a structuring agent to assist in the formation of a liquid crystalline gel network structure. Without being limited by theory, it is believed that the structuring agent assists in providing rheological characteristics to the composition which contribute to the stability of the composition. The structuring agent may also function as an emulsifier or surfactant. Preferred compositions of this invention comprise from about 0.5 % to about 20 %, more preferably from about 1 % to about 10 %, most preferably from about 1 % to about 5 %, by weight of the composition, of a structuring agent. The preferred structuring agents of the present invention are selected from the group consisting of stearic acid, palmitic acid, stearyl alcohol, cetyl alcohol, behenyl alcohol, stearic acid, palmitic acid, the polyethylene glycol ether of stearyl alcohol having an average of about 1 to about 21 ethylene oxide units, the polyethylene glycol ether of cetyl alcohol having an average of about 1 to about 5 ethylene oxide units, and mixtures thereof.

 A wide variety of anionic surfactants are also useful herein. See, e.g., U.S. Pat. No. 3,929,678, to Laughlin et al., issued Dec. 30, 1975. In addition, amphoteric and zwitterionic surfactants are also useful herein.

 The preferred oil-in-water emulsions comprise from about 0.05 % to about 10 %, preferably from about 1 % to about 6 %, and more preferably from about 1 % to about 3 % of at least one hydrophilic surfactant which can disperse the hydrophobic materials in the water phase (percentages by weight of the topical carrier). The surfactant, at a minimum, must be hydrophilic enough to disperse in water. Suitable surfactants include any of a wide variety of known cationic, anionic, zwitterionic, and amphoteric surfactants. See, McCutcheon's. Detergents and Emulsifiers, North American Edition (1986), published by Allured Publishing Corporation; U.S. Pat. No. 5,011,681 to Ciotti et al., issued Apr. 30, 1991; U.S. Pat. No. 4,421,769 to Dixon et al. issued to Dec. 20, 1983; and U.S. Pat. No. 3,755,560. The exact surfactant chosen depends upon the pH of the composition and the other components present. Preferred are cationic surfactants, especially dialkyl quaternary ammonium compounds, examples

of which are described in U.S. Pat. No. 5,151,209 to McCall et al. issued to Sep. 29, 1992; U.S. Pat. No. 5,151,210 to Steuri et al., issued to Sep. 29, 1992; U.S. Pat. Nos. 5,120,532; U.S. Pat. No. 4,387,090; U.S. Pat. No. 3,155,591; U.S. Pat. No. 3,929,678; U.S. Pat. No. 3,959,461; McCutcheon's, Detergents & Emulsifiers (North American
5 edition 1979) M.C. Publishing Co.; and Schwartz, et al., Surface Active Agents, Their chemistry and Technology, New York: Interscience Publishers, 1949.

Alternatively, other useful cationic emulsifiers include amino-amides. Nonlimiting examples of these cationic emulsifiers include stearamidopropyl PG-dimonium chloride phosphate, behenamidopropyl PG dimonium chloride,
10 stearamidopropyl ethyldimonium ethosulfate, stearamidopropyl dimethyl (myristyl acetate) ammonium chloride, stearamidopropyl dimethyl cetearyl ammonium tosylate, stearamidopropyl dirnethyl ammonium chloride, stearamidopropyl dimethyl ammonium lactate, and mixtures thereof.

The preferred oil-in-water emulsion comprises from about 25 % to about 98 %, preferably from about 65 % to about 95 %, more preferably from about 70 % to about
15 90 % water by weight of the topical carrier.

The CTFA Cosmetic Ingredient Handbook, Second Edition (1992) describes a wide variety of non-limiting cosmetic ingredients commonly used in the skin care industry, which are suitable for use in the compositions of the present invention.
20 Examples of these ingredient classes include: abrasives, absorbents, aesthetic components such as fragrances, pigments, colorings/colorants, essential oils, skin sensates, astringents, etc. (e.g., clove oil, menthol, camphor, eucalyptus oil, eugenol, menthyl lactate, witch hazel distillate), anti-acne agents, anti-caking agents, antifoaming agents, antimicrobial agents (e.g., iodopropyl butylcarbamate), antioxidants, binders,
25 biological additives, buffering agents, bulking agents, chelating agents, chemical additives, colorants, cosmetic astringents, cosmetic biocides, denaturants, drug astringents, external analgesics, film formers or materials, e.g., polymers, for aiding the film-forming properties and substantivity of the composition (e.g., copolymer of eicosene and vinyl pyrrolidone), opacifying agents, pH adjusters, propellants, reducing
30 agents, sequestrants, skin-conditioning agents (e.g., humectants, including miscellaneous and occlusive), skin soothing and/or healing agents (e.g., panthenol and derivatives (e.g., ethyl panthenol), aloe vera, pantothenic acid and its derivatives,

allantoin, bisabolol, and dipotassium glycyffhizinate), skin treating agents, thickeners, and vitamins and derivatives thereof.

The cosmetic composition can be applied directly to the skin. Alternatively, it can be delivered via normal skin application by various transdermal drug delivery systems which are known in the art, such as transdermal patches that release the composition into the skin in a time released manner. Other drug delivery systems known in the arts include pressurized aerosol bottle, iontophoresis or sonophoresis. Iontophoresis is employed to increase skin permeability and facilitate transdermal delivery. U.S. Pat. Nos. 5,667,487 and 5,658,247 discloses an ionosonic apparatus suitable for the ultrasonic-iontophoretically mediated transport of therapeutic agents across the skin. Alternatively, or in addition, lignin peroxidaseosomes or micelles may also be employed as a delivery vehicle.

Topical compositions

As used herein the phrase "topical application" refers to a composition-of-matter which is formulated for applying or spreading onto the surface of the skin.

The cosmetic composition can be formulated in any of a variety of forms utilized by the cosmetic industry for skin application including solutions, serum, lotions, sprays, creams, ointments, salves, gels, leave-on lotion, cream, toner, etc., as described below.

According to some embodiments of the invention, the cosmetic composition is formulated viscous enough to remain on the treated skin area, does not readily evaporate, and/or is not easily removed by rinsing with water.

As used herein the term "serum" refers to an aqueous solution which is formulated for application on a skin of a subject.

According to some embodiments of the invention, the serum is a thickened aqueous cosmetic solution. According to some embodiments of the invention, the serum is a clear to slight hazy aqueous solution.

According to some embodiments of the invention, the serum is formulated to remain on the skin and does not need to be removed by water, soap and/or cleansers.

According to some embodiments of the invention, upon application of the serum to the skin, the serum is absorbed rapidly leaving a cosmetically elegant silky smooth skin feel.

According to some embodiments of the invention, the serum comprises the lignin peroxidase for application on the skin.

According to some embodiments of the invention, the serum is covered with a mask (a cosmetic mask).

5 Methods for preparing compositions having such properties are well known to those skilled in the art, and are described in detail in Remington's Pharmaceutical Sciences, 1990 (supra); and Pharmaceutical Dosage Forms and Drug Delivery Systems, 6th ed., Williams & Wilkins (1995).

Carriers

10 The topical compositions of the subject invention, including but not limited to serum, lotions and creams, may comprise a dermatologically acceptable emollient. Such compositions preferably contain from about 2% to about 50% of the emollient. As used herein, "emollient" refers to a material useful for the prevention or relief of dryness, as well as for the protection of the skin. A wide variety of suitable emollients
15 are known and may be used herein. See, e.g., Sagarin, Cosmetics, Science and Technology, 2nd Edition, Vol. 1, pp. 3243 (1972), which contains numerous examples of materials suitable as an emollient. A preferred emollient is glycerin. Glycerin is preferably used in an amount of from or about 0.001 to or about 20 %, more preferably from or about 0.01 to or about 10 %, most preferably from or about 0.1 to or about 5 %, e.g., 3 %.
20

Lotions and creams according to the present invention generally comprise a solution carrier system and one or more emollients. Lotions typically comprise from about 1 % to about 20 %, preferably from about 5 % to about 10 % of emollient; from about 50 % to about 90 %, preferably from about 60 % to about 80 % water; and a
25 pharmaceutically effective amount of an agent described herein. A cream typically comprises from about 5 % to about 50 %, preferably from about 10 % to about 20 % of emollient; from about 45 % to about 85 %, preferably from about 50 % to about 75 % water; and a pharmaceutically effective amount of an agent described herein.

The topically applied cosmetic composition of the present invention may also
30 include additional components which are added, for example, in order to enrich the cosmetic compositions with fragrance and skin nutrition factors.

Such components are selected suitable for use on human keratinous tissue without inducing toxicity, incompatibility, instability, allergic response, and the like within the scope of sound medical judgment. In addition, such optional components are useful provided that they do not unacceptably alter the benefits of the active compounds of the invention.

As used herein the term "mask" refers to a cosmetic mask which comprises the active ingredient as described above and which is applied on a skin in order to lighten the skin of the subject.

As described above, the mask according to some embodiments of the invention comprises an oxidizing activator (e.g., hydrogen peroxide), with or without the oxidizing mediator.

According to some embodiments of the invention, when the mask comprises the oxidizing activator, the container comprising the mask has a shading package.

According to some embodiments of the invention, when the mask comprises the oxidizing activator, the container comprising the mask is stored in a vacuumed package.

According to some embodiments of the invention, the mask comprises lignin peroxidase with or without the oxidizing mediator.

According to an aspect of some embodiments of the invention, there is provided a mask comprising lignin peroxidase and a cosmetically acceptable carrier.

According to some embodiments of the invention, the lignin peroxidase comprised in the mask is sterile.

According to some embodiments of the invention, the lignin peroxidase comprised in the mask is synthetic.

According to some embodiments of the invention, the lignin peroxidase comprised in the mask is recombinantly expressed.

According to some embodiments of the invention, the lignin peroxidase comprised in the mask is highly pure, of a pharmaceutically grade, of a clinically grade and/or of a cosmetically grade.

According to some embodiments of the invention the mask further comprises a cosmetically acceptable excipient.

Various types of cosmetic masks are known in the cosmetic industry. These include, but are not limited to a mask having a solid support, a rinse-off mask, a peeled-off mask, or a mask which hardens or polymerizes after a set period of time.

A solid support-mask can be made of a water insoluble substrate and a liquid composition comprising the active ingredient.

As used herein the phrase "water-insoluble substrate" refers to a substrate that does not dissolve in or readily break apart upon immersion in water. The water-insoluble substrate is the implement or vehicle for delivering the liquid composition to the skin.

A wide variety of materials can be used as the substrate. The following non-limiting characteristics are desirable: (i) sufficient wet strength for use, (ii) sufficient abrasivity, (iii) sufficient thickness, (iv) appropriate size, (v) air permeability, and (vi) hydrophobicity.

Non-limiting examples of suitable substrates which meet the above criteria include nonwoven substrates, woven substrates, hydroentangled substrates, air entangled substrates, natural sponges, synthetic sponges, polymeric netted meshes, and the like.

The phrase "nonwoven" as used herein refers to a layer comprised of fibers which are not woven into a fabric but rather are formed into a sheet, mat, or pad layer. The fibers can either be random (i.e., randomly aligned) or they can be carded (i.e., combed to be oriented in primarily one direction). Furthermore, the nonwoven substrate can be composed of a combination of layers of random and carded fibers.

Nonwoven substrates may be comprised of a variety of materials both natural and synthetic. By "natural", it is meant that the materials are derived from plants, animals, insects or byproducts of plants, animals, and insects. By "synthetic", it is meant that the materials are obtained primarily from various man-made materials or from natural materials which have been further altered. The conventional base starting material is usually a fibrous web comprising any of the common synthetic or natural textile-length fibers, or mixtures thereof.

Non-limiting examples of natural materials which can be used in the mask of some embodiments of the invention include silk fibers, keratin fibers (e.g., wool fibers, camel hair fibers, and the like) and cellulosic fibers (e.g., wood pulp fibers, cotton

fibers, hemp fibers, jute fibers, flax fibers, and mixtures thereof).

Non-limiting examples of synthetic materials which can be used in the mask of some embodiments of the invention include acetate fibers, acrylic fibers, cellulose ester fibers, modacrylic fibers, polyamide fibers polyester fibers, polyolefin fibers, polyvinyl alcohol fibers, rayon fibers, polyurethane foam, and mixtures thereof. Examples of some of these synthetic materials include acrylics such as acrilan, creslan, and the acrylonitrile-based fiber, orlon; cellulose ester fibers such as cellulose acetate, arnel, and acele; polyamides such as nylons (e.g., nylon 6, nylon 66, nylon 610, and the like), polyesters such as fortrel, kodel, and the polyethylene terephthalate fiber, dacron; polyolefins such as polypropylene, polyethylene; polyvinyl acetate fibers; polyurethane foams and mixtures thereof. These and other suitable fibers and the nonwoven materials prepared therefrom are generally described in Riedel, "Nonwoven Bonding Methods and Materials," Nonwoven World (1987); The Encyclopedia Americana, vol. 11, pp. 147-153, and vol. 26, pp. 566-581 (1984); U.S. Pat. No. 4,891,227, to Thaman et al., issued Jan. 2, 1990; and U.S. Pat. No. 4,891,228 which are all incorporated by reference herein in their entirety.

Nonwoven substrates made from natural materials consist of webs or sheets commonly formed on a fine wire screen from a liquid suspension of the fibers. See C. A. Hampel et al., The Encyclopedia of Chemistry, third edition, 1973, pp 793-795 (1973); The Encyclopedia Americana, vol. 21, pp. 376383 (1984); and G. A. Smook, Handbook of Pulp and Paper Technologies. Technical Association for the Pulp and Paper Industry (1986); which are incorporated by reference herein in their entirety.

Substrates made from natural materials which can be used in the mask of some embodiments of the invention can be obtained from a wide variety of commercial sources such as AIRTEX.RTM., an embossed airlaid cellulosic layer having a base weight of about 71 gsy (grams per square yard), i.e., about 85 gsm (grams per square meter), available from James River, Green Bay, Wis.; and WALKISOFT.RTM., an embossed airlaid cellulosic having a base weight of about 75 gsy (about 90 gsm), available from Walkisoft U.S.A., Mount Holly, N.C.

Nonwoven substrates made from synthetic materials which can be used in the mask of some embodiments of the invention can also be obtained from a wide variety of commercial sources such as NOVONET.RTM. 149-616, a thermo-bonded grid

patterned material containing about 100% polypropylene, and having a basis weight of about 50 gsy (about 60 gsm), available from Veratec, Inc., Walpole, Mass.; NOVONET.RTM. 149-801, a thermo-bonded grid patterned material containing about 69% rayon, about 25% polypropylene, and about 6% cotton, and having a basis weight of about 75 gsy (about 90 gsm), available from Veratec, Inc. Walpole, MA; NOVONET 149-191, a thermo-bonded grid patterned material containing about 69% rayon, about 25% polypropylene, and about 6% cotton, and having a basis weight of about 100 gsy (about 120 gsm), available from Veratec, Inc. Walpole, Mass.; KEYBAK.RTM. 951V, a dry formed apertured material, containing about 75% rayon, about 25% acrylic fibers, and having a basis weight of about 43 gsy (about 51 gsm) available from PGI/Chicopee, Dayton, NJ; KEYBAK.RTM. 1368, an apertured material, containing about 75% rayon, about 25% polyester, and having a basis weight of about 39 gsy (about 47 gsm), available from PGI/Chicopee, Dayton, N.J., RMT-90, a 3-layer substrate having a pulp layer as an inner layer with outer layers respectively made of the combination of rayon and polyester, and RFP-90, a 3-layer substrate having a combined PET/PE layer as an inner layer with outer layers of rayon and polyester, both available from Daiwabo K. K.

The nonwoven layer can be prepared by a variety of processes including hydroentanglement, thermally bonding or thermo-bonding, and combinations of these processes. Methods of making nonwoven substrates are well known in the art. Generally, these nonwoven substrates can be made by air-laying, water-laying, meltblowing, coforming, spunbonding, or carding processes in which the fibers or filaments are first cut to desired lengths from long strands, passed into a water or air stream, and then deposited onto a screen through which the fiber-laden air or water is passed.

Processes for preparing hydroentangled webs are well known in the art. See, for example, Evans; U.S. Pat. No. 3,485,786; issued Dec. 23, 1969; Kalwarres; U.S. Pat. No. 2,862,251 and Griswold, U.S. Pat. No. 3,025,585, all of which describe hydroentangling procedures generally and all of which are herein incorporated by reference. See also U.S. Pat. No. 5,674,591; James et al; issued Oct. 7, 1997 which specifically describes a hydroentangling process, including the apparatus used in the process, which can be used to prepare the patterned web. U.S. Pat. No. 5,674,591 is incorporated herein in its entirety.

Among the water-insoluble substrate described hereinbefore, fluid-entangled (hydroenrangled), nonwoven, flexible substrates are preferably used in the present invention, in view of providing softness, loftness, strength while being used, and cloth-like texture. Any fluid-entangled, nonwoven, flexible substrate that is known or is otherwise suitable for application to the skin can be As used herein the phrase "fluid-entangled" refers to the manufacturing process for entangling a fibrous web by using a fluid jet on a fibrous web to obtain the desired fiber and void configuration within the resulting fluid-entangled substrate, to thereby produce an art recognized, fluid-entangled, nonwoven, flexible substrate. Fluid-entangled, nonwoven, flexible substrates and the fluid entangling techniques for making them are well known in the substrate arts, preferred examples of such substrates and fluid entangling techniques being described in U.S. Pat. Nos. 5,142,752 (Greenway et al.) and U.S. Pat. Nos. 5,281,461 (Greenway et al.), U.S. Pat. No. 3,485,786 (Evans); U.S. Pat. No. 2,862,251 (Kalwarres), and U.S. Pat. No. 3,025,585 (Griswald); U.S. Pat. No. 5,674,591 (James et al.); U.S. Patent Application No. 20050013784, each of which patents and patent applications are incorporated herein by reference in their entirety.

The resulting layer, regardless of its method of production or composition, is then subjected to at least one of several types of bonding operations to anchor the individual fibers together to form a self-sustaining web. Moreover, the substrates of the present invention can consist of a single layer or multiple layers. In addition, multilayered substrates can include films and other nonfibrous materials. In one embodiment the substrate may also be laminated with polymeric film on the substrate, coating the substrate, or heat sealing the substrate. The resulting substrate with the laminated polymeric film, coating or heat scaling comprises an occluded side on one side of the substrate, which faces away from the skin, and a skin facing side that is positioned on the skin surface. By having a substrate with an occluded side, the substrate acquires low air permeability. By "low air permeability" it is meant that the side of the substrate having the film, coating or heat sealing allows very little air to enter into the substrate and very little vapor to escape from the substrate. Preferably the air permeability is less than about 5 mg/cm.sup.2/min, more preferably between about 0.01 mg/cm.sup.2/min and about 4.8 mg/cm.sup.2/min. The air permeability can be measured by taking the weight of a fully saturated sample of the substrate and weighing

the substrate after it is exposed to the atmosphere.

The substrate is flexible enough such that, when soaked with the liquid composition, readily fits along the skin, yet strong enough so that it does not easily tear or crumble upon use. According to some embodiments of the invention, the substrate
5 has a thickness of from about 100 μm to about 1 cm, e.g., from about 300 μm to about 3 mm, depending on the material for making the substrate, and use and characteristic of the product. In some embodiments, a cotton substrate having a relatively thick thickness of about 2 mm is preferred for providing a natural, lofty, cloth-like appearance, and for soaking an abundant amount of the liquid composition. In some embodiments, a
10 substrate made of layers of substrates, wherein the layer to be applied to the face is made of hydrophilic material, and having a relatively thin thickness of about 0.5 mm, is preferred for providing a fresh, soft appearance, and for soaking just about the amount of liquid composition comprising the active ingredient.

The solid support of the mask can be for example, an occlusive film, such as a
15 film made of a woven textile (e.g., 20% to 100% woven cotton), a non-woven textile (e.g., 20% to 100% non woven polyester substrate) or a paper or other wood derived product (e.g., 20% to 100% paper or other wood derived product).

Additionally or alternatively, the occlusive film can made of a plastic.

Additionally or alternatively, the solid support can be a non-occlusive film, made
20 of a woven textile or a non-woven textile, or from a porous plastic.

The solid-support mask can be a re-usable mask or a single-use-disposable mask. For example, the re-usable mask can be impregnated or soaked with the liquid comprising the active ingredient(s) by the subject (e.g., by the subject in need of skin lightening or by a physician or nurse), by pouring pre-determined aliquots of the liquid
25 comprising the active ingredient on the solid support.

The mask can be a disposable mask which covers the entire face and is pre-moistened or impregnated with the active ingredient.

The disposable mask can be made of an absorbent material, which is at least partially absorbs the active ingredient (e.g., lignin peroxidase, oxidizing activator, or
30 oxidizing mediator). The absorbent material may have an oval shape with openings corresponding in approximate size and position to human eyes, nose and mouth and radial cuts to allow the shaping of the flat material around the three dimensional

contours of a face, and being enclosed in the sealed container, essentially as described in U.S. Patent Application No. 20060182704, which is fully incorporated herein in its entirety by reference.

According to some embodiments of the invention, the mask (e.g., the absorbent
5 material) is lined with a disposable plastic liner.

According to some embodiments of the invention, the solid-support mask is in a freeze-drying form.

Methods of preparing a mask in a freeze-drying form are known in the art and further described in U.S. Patent Application No. 20050244482, which is fully
10 incorporated herein in its entirety by reference.

For example, a mask cloth can be doused with a solution comprising the active ingredient and then the mask is freeze-dried. It should be noted, that prior to freeze-drying the mask, additional agents can be added, such as a thickener in pure water, and/or additional active ingredients. A skin care solution can be also prepared and be
15 applied on the face mask when being used. The freeze-dried mask can be stored in a vacuumed shading package.

For example, the active ingredient can be attached to the mask freeze-drying form and a skin care solution, can be applied to the face mask when being used. Non-limiting examples of such skin care substances include Hyaluronic Acid, Liquoric,
20 Witch Hazel Extract, and other nourishing materials which are not easily oxidized.

According to some embodiments of the invention, the when the mask comprises the lignin peroxidase enzyme, the skin care lotion can include the oxidizing activator.

According to some embodiments of the invention, the mask, which comprises the active ingredient is devoid of a solid support. Non-limiting examples of such a mask
25 include, but are not limited to, a rinse-off, a creamy mask and a mask that hardens or polymerizes on the skin.

Methods of preparing a creamy mask are known in the art, and described for example in U.S. Patent Application No. 20060292108, which is fully incorporated herein by its entirety. For example, a creamy mask can be prepared by mixing a water
30 phase into a powder phase at room temperature while stirring. Mixing is continued for several minutes (e.g., 15 to 20 minutes) at high speed till the final composition becomes soft creamy mask.

As used herein the phrase “rinse-off mask” refers to a cosmetic composition formulated in order to be applied to the skin in a relatively thick layer and left for a certain period of time, generally from a few minutes to several tens of minutes, and in order to be, at the end of this period of time, rinsed off with water or also simply wiped off.

Methods of preparing a rinse-off mask are known in the art and also described in U.S. patent Application No. 20090130040, which is fully incorporated herein by its entirety. For example, the rinse-off mask can be prepared by an absorbent polymer (e.g., a polymer or copolymer of acrylamide, methacrylamide, N-vinylpyrrolidone, vinyl acetate, vinyl alcohol, acrylate ester or allyl alcohol; or a polymer or copolymer of acrylic acid; or a polymer or copolymer of methacrylic acid and salts thereof, or a polymer or copolymer obtained from a cationic monomer of dialkylaminoalkyl acrylate or methacrylate, dialkylaminoalkylacrylamide, dialkylaminoalkylmethacrylamide, diallylamine, methyldiallylamine, and quaternary ammonium or acid salts thereof) at a concentration from 0.1% to 5% by weight, and at least one film-forming agent, a gelling/thickening agent, at a concentration of between 0.5% and 10% by weight, with respect to the total weight of the final composition.

According to some embodiments of the invention, the mask comprises a material which hardens or polymerizes after a set period of time. Such a mask can be applied to the skin using known methods such as using a spatula or other similar utensil.

Methods of preparing a mask which hardens or polymerizes after a set period of time are known in the art and also described in U.S. patent Application No. 20050249765, which is fully incorporated herein by its entirety. For example, the mask which hardens or polymerizes after a set period of time can be prepared from two different phases, capable of being mixed together prior to application. Usually, the two phases are thoroughly and/or vigorously mixed for a minimum time of 45 seconds. According to some embodiments of the invention, the two phases do not harden or polymerize if not mixed together.

For example, one phase may be in a form of a suspension with a creamy appearance, and the second phase may be in a form of a gel. Following is a non-limiting example of such phases: a first phase may comprise Liquid paraffin 39.21% Calcium sulphate 21.00% Diatomaceous earth 12.50% Algin 8.00% Styrene/isoprene

hydrogenated copolymer 5.40% Capric/caprylic triglyceride 3.60% Glycine soja 3.00% Magnesium oxide 3.00% CI 77891 3.00% Sodium phosphate 0.80% Phenoxyethanol 0.10% Methylparaben 0.06% Propylparaben 0.01% Ethylparaben 0.01% Butylparaben 0.01% Perfume (sage oil) 0.30%; and the second phase may comprise Water 93.46%
5 Algin 5.70% Imidazolidinyl urea 0.50% Sodium methylparaben 0.12% Mica 0.05% Titanium oxide 0.05% Methylchloroisothiazolinone 11.5 ppm Methylisothiazolinone 3.5 ppm Perfume (sage oil) 0.02%.

The first phase can be in a form of a suspension, of intense white colour, with a sage aroma, and a creamy appearance, with a density (measured at 20.degree. C.) in the
10 region of 1160 to 1170 g/l, a Brookfield viscosity of >500000, a mechanical stability of 30 min/3000 rpm without separation and thermal stability of 24 h/45.degree. C. without separation. The second phase can be in the form of a gel, of faded white colour, with a sage aroma, and a pearly appearance, with a density (measured at 20.degree. C.) in the region of 1020 to 1025 g/l, a pH of 7.00 to 8.50, with a Brookfield viscosity of >40000,
15 a mechanical stability of 30 min/3000 rpm without separation and a thermal stability of 24 h/45.degree. C. without separation.

The mask according to some embodiments of the invention comprises a gelifying agent. Non-limiting examples of suitable gelifying agents include acrylic polymers, high dispersability ETD class acrylic polymers (e.g., selected in amounts by
20 weight comprised of between 0.1% and 1.5% with respect to the weight of the cosmetic excipients); celluloses, such as hydroxyethylcellulose (e.g., selected in amounts by weight comprised of between 0.1% and 1% with respect to the weight of the cosmetic excipients); gums, such as tragacanth gum or xanthan gum (e.g., selected in amounts by weight comprised of between 0.1% and 1% with respect to the weight of the cosmetic
25 excipients).

The mask according to some embodiments of the invention comprises additional agents such as pH regulators (acidity regulators, selected from among cosmetically acceptable organic or inorganic bases); a humectant agent (such as polyol, a glycol, glycerine and/or propylene glycol, in amounts by weight comprised of between 1% and
30 20%); chelating agents or sequestrants, non-ionic surfactants (such as PEG 7 glyceryl cocoate, PEG 6 triglyceryl caproic glycerides or polyquaternum 7, in amounts by weight comprised of between 0.5% and 6% with respect to the weight of the cosmetic

excipients). Additional agents which may be included in the mask are described in U.S. Patent Application No. 20050226899, which is fully incorporated herein in its entirety by reference.

The mask according to some embodiments of the invention further comprises a
5 substance having a moisturizing effect (e.g., glycerol) at a concentration of between 0.001% and 5% by weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises a substance having a calming or soothing or relaxing effect (e.g., a glycyrrhizate or a glycyrrhizate potassium salt), at a concentration ranging between 0.001% and 5% by
10 weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises a substance having a slimming effect (e.g., xanthine) at a concentration ranging between 0.001% and 5% by weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises a
15 substance for stimulating skin microcirculation (e.g., ruscogenin), at a concentration ranging between 0.001% and 5% by weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises a substance having a seoregulating activity for the care of the skin (e.g., zinc oxide, an
20 organic zinc salt, zinc gluconate, zinc salicylate, or zinc pidolate), at a concentration ranging between 0.01% and 10% by weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises a substance having an activity for combating free radicals (e.g., tocopherol ester, or an
25 alpha-tocopherol ester), at a concentration ranging between 0.001% and 5% by weight, with respect to the total weight of the composition.

The mask according to some embodiments of the invention further comprises at least one refreshing agent (e.g., menthol or menthoxypropanediol).

The active ingredients included in the cosmetic or topical compositions of some
30 embodiments of the invention are suitable for skin lightening via the oxidation of melanin. However, the oxidation reaction can be controlled and stopped when desired

by the addition of reducing reagents. These reagents can be formulated in a separate cosmetic composition and be applied on the skin when desired.

Compositions of the present invention may, if desired, be presented in a dispenser device or a kit, along with appropriate instructions for use and labels indicating FDA approval for use in skin lightening.

According to an aspect of some embodiments of the invention, there is provided a kit for lightening a skin of a subject, comprising a first container comprising a mask which comprises a hydrogen peroxide, and a second container comprising a cosmetic composition which comprising lignin peroxidase, wherein said mask and said cosmetic composition are in no direct contact in the kit, wherein:

(i) a concentration of said hydrogen peroxide in said mask is between 0.01-0.013 % (weight/weight);

(ii) a concentration of said when said lignin peroxidase in said cosmetic composition is between about 0.1% to about 5 % (w/w), e.g., between about 0.5% (weight/weight) to about 2% (weight/weight); and

(iii) wherein said mask and/or said cosmetic composition further comprises veratryl alcohol at a concentration in the range of about 0.1-0.2 % (weight/weight).

Following is a non-limiting list of formulations, which can be used for the preparation of the serum (Table 1 below), mask (Table 1 below), facial wash (Table 2 below), cream (Table 3 below) or lotion (Table 4 below) of some embodiments of the invention.

Table 1
Composition of the Mask and Serum according to some embodiments of the invention

<i>Active Serum</i>		<i>Activator Mask</i>	
<i>INCI name</i>	<i>w/w %</i>	<i>INCI name</i>	<i>w/w %</i>
Water	75 – 100	Water	75 – 100
Glycerine	5 – 10	Glycerine	5 – 10
Polyacrylate-13	0.1 – 1	Tribehenin	1 – 5
Ligninase (lignin peroxidase)	0.1 – 5	Dimethicone	0.1 – 1
Dimethicone	0.1 – 1	Citric Acid	0.1 – 1
Dicaprylyl Carbonate	0.1 – 1	Polyacrylate-13	0.1 – 1
Cetyl Alcohol	0.1 – 1	Sodium Levulinate	0.1 – 1
Stearyl Alcohol	0.1 – 1	Cetyl Alcohol	0.1 – 1
Phenoxyethanol	0.1 – 1	Glycol Stearate	0.1 – 1
Portulaca Oleracea	0.1 – 1	Phenoxyethanol	0.1 – 1
Polyisobutene	0.1 – 1	Polyisobutene	0.1 – 1

<i>Active Serum</i>		<i>Activator Mask</i>	
<i>INCI name</i>	<i>w/w %</i>	<i>INCI name</i>	<i>w/w %</i>
Butylene Glycol	0.1 – 1	Portulaca Oleracea	0.1 – 1
Veratryl Alcohol	0.1 – 1	Sodium Anisate	0.1 – 1
Pullulan	0 – 0.1	Butylene Glycol	0.1 – 1
Polysorbate 20	0 – 0.1	Sodium Citrate	0.1 – 1
PEG-4 Laurate	0 – 0.1	Trehalose	0 – 0.1
PEG-4 Dilaurate	0 – 0.1	Pullulan	0 – 0.1
PEG-4	0 – 0.1	Polysorbate 20	0 – 0.1
Iodopropynyl Butylcarbamate	0 – 0.1	Hydrogen Peroxide	0 – 0.1

Table 1. Provided are the ranges of concentrations of the ingredients of the active serum and activator mask according to some embodiments of the invention. Note that the concentrations of the above described materials (ingredients) can be varied depending on the regulations for use of these materials for cosmetic purposes. For example, the concentration Iodopropynyl Butylcarbamate (IPBC) can be between 0-0.01% (w/w). PEG = polyethylene glycol.

Table 2
Composition of Advanced Facial Wash according to some embodiments of the invention

<i>INCI name</i>	<i>w/w %</i>
Aqua	25 - 50
Propanediol	10 - 25
Glycerine	10 - 25
Stearic Acid	10 - 25
Myristic Acid	5 - 10
Potassium Hydroxide	5 - 10
Lauric Acid	1 - 5
Decyl Glucoside	1 - 5
Glycol Distearate	1 - 5
Glyceryl Stearate	1 - 5
PEG-100 Stearate	1 - 5
Synthetic Beeswax	1 - 5
Potassium Cocoyl Glycinate	0 - 0.1
BHT	0 - 0.1
Ligninase (lignin peroxidase)	0 - 0.1

Table 2: Provided are the ranges of concentrations of the ingredients of the facial wash according to some embodiments of the invention. Note that the concentrations of the above described materials (ingredients) can be varied depending on the regulations for use of these materials for cosmetic purposes. INCI = International Nomenclature of Cosmetic Ingredients; BHT- Butylated hydroxytoluene.

Table 3
Composition of Advanced Lightening Cream (enzyme & activator) according to some embodiments of the invention)

<i>Enzyme Cream</i>		<i>Activator Cream</i>	
<i>INCI name</i>	<i>w/w %</i>	<i>INCI name</i>	<i>w/w %</i>
Water	50 – 75	Water	50 – 75

<i>Enzyme Cream</i>		<i>Activator Cream</i>	
<i>INCI name</i>	<i>w/w %</i>	<i>INCI name</i>	<i>w/w %</i>
Glycerine	5 – 10	Glycerine	5 – 10
Butyrospermum Parkii Butter	1 – 5	Butyrospermum Parkii Butter	1 – 5
Dimethicone	1 – 5	Dimethicone	1 – 5
Dicaprylyl Carbonate	1 – 5	Dicaprylyl Carbonate	1 – 5
Trehalose	1 – 5	Trehalose	1 – 5
Caprylic Capric Triglyceride	0.1 – 1	Caprylic Capric Triglyceride	0.1 – 1
Cetyl Alcohol	0.1 – 1	Cetyl Alcohol	0.1 – 1
Jjoba Ester	0.1 – 1	Jjoba Ester	0.1 – 1
Stearyl Alcohol	0.1 – 1	Stearyl Alcohol	0.1 – 1
Ligninase (lignin peroxidase)	0.1 – 5	Polyacrylate-13	0.1 – 1
Polyacrylate-13	0.1 – 1	Cetyl Palmitate	0.1 – 1
Cetyl Palmitate	0.1 – 1	Myristyl Myristate	0.1 – 1
Myristyl Myristate	0.1 – 1	Phenoxyethanol	0.1 – 1
Phenoxyethanol	0.1 – 1	Polyisobutene	0.1 – 1
Polyisobutene	0.1 – 1	Pullulan	0.1 – 1
Pullulan	0.1 – 1	Citric Acid	0 – 0.1
Veratryl Alcohol	0 – 0.1	Hydrogen Peroxide	0 – 0.1
Polysorbate 20	0 – 0.1	Polysorbate 20	0 – 0.1
PEG- 4 Laurate	0 – 0.1	PEG- 4 Laurate	0 – 0.1
PEG-4 Dilaurate	0 – 0.1	PEG-4 Dilaurate	0 – 0.1
PEG-4	0 – 0.1	PEG-4	0 – 0.1
Iodopropynyl Butylcarbamate	0 – 0.1	Iodopropynyl Butylcarbamate	0 – 0.01

Table 3: Provided are the ranges of concentrations of the ingredients of the lightening cream (enzyme and activator) according to some embodiments of the invention. Note that the concentrations of the above described materials (ingredients) can be varied depending on the regulations for use of these materials for cosmetic purposes. For example, the concentration of Iodopropynyl Butylcarbamate (IPBC) can be between 0-0.01% (w/w). INCI = International Nomenclature of Cosmetic Ingredients;

Table 4

Composition of Advanced Lightening Lotion (Enzyme & Activator) according to some embodiments of the invention

Enzyme Lotion		Activator Lotion	
INCI name	w/w %	INCI name	w/w %
Water	75 – 100	Water	75 – 100
Glycerine	5 – 10	Glycerine	5 – 10
Propylene Glycol	1 – 5	Propylene Glycol	1 – 5
Dicaprylyl Carbonate	0.1 – 1	Dicaprylyl Carbonate	0.1 – 1
Dimethicone	0.1 – 1	Dimethicone	0.1 – 1
Ligninase (lignin peroxidase)	0.1 – 5	Polyacrylate-13	0.1 – 1
Polyacrylate-13	0.1 – 1	Cetyl Alcohol	0.1 – 1
Cetyl Alcohol	0.1 – 1	Paraffinum Liquidum	0.1 – 1
Paraffinum Liquidum	0.1 – 1	Stearyl Alcohol	0.1 – 1
Stearyl Alcohol	0.1 – 1	Phenoxyethanol	0.1 – 1
Phenoxyethanol	0.1 – 1	Polyisobutene	0.1 – 1
Polyisobutene	0.1 – 1	Polysorbate 20	0 – 0.1
Polysorbate 20	0 – 0.1	PEG- 4 Laurate	0 – 0.1
PEG- 4 Laurate	0 – 0.1	PEG-4 Dilaurate	0 – 0.1
PEG-4 Dilaurate	0 – 0.1	Citric Acid	0 – 0.1
Veratryl Alcohol	0 – 0.1	Hydrogen Peroxide	0 – 0.1
PEG-4	0 – 0.1	PEG-4	0 – 0.1
Iodopropynyl Butylcarbamate (IPBC)	0 – 0.1	Iodopropynyl Butylcarbamate	0 – 0.01

Table 4: Provided are the ranges of concentrations of the ingredients of the lightening lotion (enzyme and activator) according to some embodiments of the invention. Note that the concentrations of the above described materials (ingredients) can be varied depending on the regulations for use of these materials for cosmetic purposes. For example, the concentration Iodopropynyl Butylcarbamate (IPBC) can be between 0-0.01% (w/w). INCI = International Nomenclature of Cosmetic Ingredients;

Some specific formulations of the serum, mask, cream, lotion and/or facial wash of some embodiments of the invention are marketed under the ELURE™ (Syneron Medical Ltd.).

According to an aspect of some embodiments of the invention, there is provided a cosmetic method of lightening a skin of a subject, comprising:

(a) applying the lignin peroxidase on a skin region of the subject, while using the kit of some embodiments of the invention, and;

(b) applying the oxidizing activator in the kit on the skin region having the lignin peroxidase for a pre-determined time, thereby lightening the skin of the subject.

As used herein, the phrase "lightening a skin" refers to reducing the tone or color of skin by reducing the pigmentive quality or concentration of melanin pigment
5 contained therein.

As used herein, the phrase "subject" refers to mammals, typically human beings.

According to some embodiments of the invention, the subject has excess skin pigmentation, or skin imperfections such as freckles etc.

According to some embodiments of the invention, the subject is of an Asian
10 population.

According to some embodiments of the invention, the subject is of a Caucasian population.

As used herein the phrase "pre-determined time" refers to a time period needed for obtaining the lightening effect on the treated skin.

According to some embodiments of the invention, the pre-determined time lasts
15 from a few seconds to a few tens of minutes, or even hours, e.g., from about 10 seconds to about 3 hours, e.g., from about 30 seconds to about 1 hour, from about 1 minute to about 30 minutes, e.g., for about 2-25 minutes, e.g., for about 5-25 minutes, e.g., about 5-20 minutes, e.g., about 10-20 minutes, e.g., about 11-19, about 12-18, about 13-17,
20 about 14-16, e.g., about 15 minutes.

According to some embodiments of the invention, the method further comprising repeating step (b) daily for at least 5 days.

According to some embodiments of the invention, the method further comprising repeating step (b) daily for 5 days.

According to some embodiments of the invention, the method further comprising
25 repeating step (b) daily for about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 days.

According to some embodiments of the invention, the method further comprising repeating step (b) daily for at least 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks.

According to some embodiments of the invention, wherein step (a) is performed prior to step (b).

5 According to some embodiments of the invention, wherein step (b) is performed prior to step (a).

According to some embodiments of the invention, the method further comprising: (c) repeating steps (a) and (b) daily for at least 5 days.

10 According to some embodiments of the invention, the method further comprising: (c) repeating steps (a) and (b) daily for 5 days.

According to some embodiments of the invention, the method further comprising repeating steps (a) and (b) daily for about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 days.

15 According to some embodiments of the invention, the method further comprising: (c) repeating steps (a) and (b) daily for at least 14 days.

According to some embodiments of the invention, the method further comprising repeating steps (a) and (b) daily for at least 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks.

It should be noted that after completion of a single session during which the lightening compositions are applied daily, e.g., for 1-5 days, the subject in need thereof can repeat the process using the lightening kit of some embodiments of the invention. The repeated treating (lightening) session can occur immediately, or following an intermediate period (interval) during which the cosmetic composition and the mask are not used; or following an intermediate period (interval) during which none of the lightening compositions described above (e.g., serum, mask and lotion) are used.

25 According to some embodiments of the invention, the method further comprising: (c) repeating steps (a) and (b) daily for 5 days, and

(d) repeating step (c) at least twice in pre-determined intervals during which no skin lightening compositions are applied on the skin.

30 It should be noted that the intervals can be of a few days, weeks or months, depending on the degree of skin lightening desired.

According to some embodiments of the invention, the method further comprising: (c) repeating steps (a) and (b) daily for 5 days, and

(d) repeating step (c) at least twice in pre-determined intervals during which only the lightening lotion/cream with the lignin peroxidase and oxidizing activator are applied daily on the skin.

5 It should be noted that the doses of the active ingredients can vary during treatment.

For example, if a subject is known to have multiple skin allergies or highly sensitive skin then in order to test the sensitivity of the subject to the active ingredients included in the compositions and masks of some embodiments of the invention, the first time the cosmetic composition and/or mask is applied on the subject's skin a low dose (e.g., 1-10% of the optimal concentrations) is employed, while the repeating applications can be at optimal concentrations as described above.

10 Additionally or alternatively, the first application of the cosmetic composition and/or mask of some embodiments of the invention can include higher doses of the active ingredient(s) (e.g., 2-10 folds higher concentrations than optimal concentrations), while the repeating applications of the cosmetic composition and/or mask of some embodiments of the invention can include optimal or less than optimal concentrations of the active ingredient(s).

According to some embodiments of the invention, the first application of the higher doses of the active ingredient(s) is performed under clinical supervision, e.g., at the Dermatologist's office/clinic.

20 According to some embodiments of the invention, the additional applications of the active ingredients are performed in the subject's home.

According to some embodiments of the invention, the method further comprising applying a lotion or cream comprising the lignin peroxidase.

25 It should be noted that the lotion/cream comprising the lignin peroxidase can be added before applying the serum and/or after applying the serum.

Additionally or alternatively, the lotion/cream comprising the lignin peroxidase can be added before applying the mask and/or after applying the mask.

According to some embodiments of the invention, the method further comprising applying a lotion or cream comprising the oxidizing activator.

30 It should be noted that the lotion/cream comprising the oxidizing activator can be added before applying the serum and/or after applying the serum.

Additionally or alternatively, the lotion/cream comprising the oxidizing activator can be added before applying the mask and/or after applying the mask.

According to some embodiments of the invention, lotion/cream is applied daily in the morning and the serum and mask are applied daily in the evening.

5 It should be noted that the skin region can be the whole skin of the subject, the whole skin covering a specific organ or tissue of the subjects or only a portion or part thereof. For example, the mask can cover part of the face such as with a buccal mask.

The cosmetic compositions/masks of some embodiments can be employed on a skin region of the subject, including, but not limited to, a facial skin, neck, forehead,
10 arms, hands, legs, abdomen, and back.

According to some embodiments of the invention, the skin region comprises facial skin.

According to some embodiments of the invention, the skin region comprises a visible portion of the skin.

15 According to some embodiments of the invention, the skin region comprises a skin region exposed to the sun.

According to some embodiments of the invention, lightening the skin comprises lightening whole skin complexion and/or changing skin tone (e.g., to a more white appearance).

20 According to some embodiments of the invention, the skin region comprises an uneven skin tone, a mottled skin tone, redness, dark spot(s), freckle(s), melasma, hyperpigmentation, skin discoloration, age spot(s), acne mark(s) and/or a scar.

As used herein the term "about" refers to $\pm 10\%$.

25 The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

The term "consisting of means "including and limited to".

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the
30 additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination

in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various
5 embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

10

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized
15 in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons,
20 Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659
25 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are
30 extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219;

5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., Eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

GENERAL MATERIALS AND METHODS

Table 5 below, provides details on the materials used to generate the serum according to some embodiments of the invention.

Table 5

Raw Material Trade Name	CTFA/INCI Name	CAS No.	Activity of Material	Constituents & Carry Over Items With %	Function Of Material	Supplier	Origin
Purified Water	Purified Water	7732-18-5	100%		Vehicle	In-house	
Supercel V Glycerin, USP	Glycerin	56-81-5	99.7%	Glycerin	Humectant	P&G Chemicals	Plant
Extract of <i>Phanerochaete Chrysosporium</i>	Ligninase		3×10^5 units/L		Skin Whitening agent, Skin conditioning agent, humectant	Rakuto Biotechnologies	Plant
Seppiphus 400	Polyisobutene Polysorbate 20 Polyacrylate-13	9003-27-4 9005-64-5	23-25% 3-8% 50-70%		Solvent Surfactant/emulsifier	Seppic	Synthetic
Portulaca Extract	Portulaca Oleracea Extract	90083-07-1		Portulaca Oleracea Butylene Glycol Water	Film former Anti-inflammatory, Skin Conditioning Agent, Humectant	Bioland	Plant
Cetiol CC	Dicaprylyl Carbonate	1680-31-5	100%		Emollient	Cognis	Plant

Raw Material Trade Name	CTFA/INCI Name	CAS No.	Activity of Material	Constituents & Carry Over Items With %	Function Of Material	Supplier	Origin
Dow Corning Fluid 200 200 CS	Dimethicone	9006-65-9 9016-00-6 63148-62-9	100%		Emollient	Dow Corning	Synthetic
Lanette C16 98-100 MY	Cetyl Alcohol	36653-82-4	100%		Opacifier, Emulsifier	Cognis	Plant
Lanette C1898-100MY	Stearyl Alcohol	112-92-5	100%		Opacifier Emulsifier	Cognis	Plant
Phenoxy ethy alcohol aerosol	Phenoxyethanol	122-99-6	99.0% min	Phenoxy ethanol	Microbial Preservative	Synrise	Synthetic
Veratryl Alcohol	3,4-dimethoxybenzyl alcohol	93-03-8	100%		Skin Conditioning Agent, Redox Mediator	Sigma	Synthetic
Pullulan	Pullulan	9057-02-7	100%		Skin lubricity	Hayashibara	plant
Glycol L	Isododecyl Biscyclobutane PEG-4	55406-53-6 112-607 25322-08-3	9.1-10.3%		Microbial Preservative	Lonza	Synthetic
	PEG-4 Laurate	9004-51-3		Approx 40%			
	PEG-4 Dilaurate	10108-24-4 9005-02-01		approx 40%			

Concentrations of active ingredients:***Lignin peroxidase (LIP) in whitening lotion or serum according to some embodiments of the invention:***

25 lignin peroxidase enzyme units per gram lotion/serum, or 2500 enzyme units per 100
 5 gram lotion/serum.

Raw material: LIP water solution at a concentration of 3,500,000 units per 1000
 gram solution. For every 100 gram whitening lotion or serum according to some
 embodiments of the invention, 0.71 milliliters or grams of such a LIP solution are taken
 (0.71 % (w/w)).

10 Every 0.71 ml or grams of LIP solution contains 2485 enzyme units.

Veratryl alcohol:

100 grams of whitening lotion or serum according to some embodiments of the
 invention contains 0.1 grams of veratryl alcohol (0.1% (w/w))(6 mmole/Kg
 lotion/serum)

Hydrogen peroxide:

100 grams of activator lotion or mask according to some embodiments of the
 invention contains 0.012 grams of hydrogen peroxide (0.012% (w/w)) (3.52 mmole/Kg
 lotion/serum).

Preparation of active white serum according to some embodiments of the

20 ***invention*** - Serum was prepared using the following reagents: Water (84.56%),
 Glycerine (10%), Ligninase (Extract of Phanerochaete chrysosporium) (25 Units/gram,
 0.71%), Polyacrylate-13 (0.7%), Dimethicone (0.5%), Dicaprylyl Carbonate (0.5%),
 Cetyl Alcohol (0.5%), Stearyl Alcohol (0.5%), Phenoxyethanol (0.5%), Portulaca
 Oleracea (0.375%), Polyisobutene (0.25%), Butylene Glycol (0.225%), Veratryl Alcohol
 25 (0.14%), Pullulan (0.1%), Polysorbate 20 (0.05%), PEG-4 Laurate (0.04%), PEG-4
 Dilaurate (0.04%), PEG-4 (0.01%), Iodopropynyl Butylcarbamate (0.01%). All
 percentages are weight/weight (W/W).

* Note that the concentration of ligninase can be changed from 0.5% to 2%.

Preparation of activator mask according to some embodiments of the invention

30 - The activating mask was prepared from the following reagents: Water (84.07%),
 Glycerine (8%), Tribehenin (2%), Dimethicone (1%), Citric Acid (1%), Polyacrylate-13
 (0.7%), Sodium Levulinate (0.5%), Cetyl Alcohol (0.5%), Glycol Stearate (0.5%),

Phenoxyethanol (0.5%), Polyisobutene (0.25%), Portulaca Oleracea (0.25%), Sodium Anisate (0.2%), Butylene Glycol (0.15%), Sodium Citrate (0.12%), Trehalose (0.1%), Pullulan (0.1%), Polysorbate 20 (0.05%), Hydrogen Peroxide (0.012%). All percentages are weight/weight (W/W).

5 A nonwoven material is put in a sachet, soaked with the above described mixed mask reagents and sealed.

Preparation of Whitening Lotion Active Enzyme & Activator – Figure 7A describes formulation of a whitening lotion with active enzyme (lignin peroxidase, LIP) according to some embodiments of the invention, and Figure 7B describes a
10 formulation of a whitening lotion with activator according to some embodiments of the invention.

Preparation of Advanced Facial Wash - Figure 8 describes the preparation of a facial wash with lignin peroxidase according to some embodiments of the invention (used in the test described in Example 2 of the Examples section which follows).

15 *Preparation of the advanced facial wash according to some embodiments of the invention (used in the study described in Example 3 below)* – Table 2 hereinabove.

EXAMPLE 1

LIGHTENING EFFECT OF THE SERUM AND MASK OF SOME EMBODIMENTS OF THE INVENTION

20

The active white serum and activator mask according to some embodiments of the invention were applied on Asian subjects and the effect of the serum and mask was monitored by photography of the study's faces before (baseline) and after a single use of the serum and mask, as follows.

25 *Inclusion Criteria* - 25 to 55 year-old Asian females in good general health; Regular user of facial moisturizers; Willing & able to follow all study directions, accept skin examination and commit to all follow-up visits; Has completed the informed consent process; Willing to avoid direct sun exposure.

Exclusion Criteria - Subjects having a history of sensitivity to cosmetic
30 products, toiletries or its components; having facial skin disorders (such as inflammation, eczema, psoriasis, skin cancer, severe acne, skin lesions, etc.); having concurrent therapy or disease which could interfere with evaluations or increase risk to

the subject; having or had been using oral retinoids or steroids within the last 6 months; having or had been using topical retinoids, alpha-hydroxy and/or beta-hydroxy acids, hydroquinone or any whitening preparation in any form (cleanser, toner, facial lotion or cream) within the last 3 months; having or had been undergoing dermatologist facial treatments or procedures within the last 3 months; having or had been involved in another facial skin care clinical investigation within the last 3 months; Pregnant or nursing female; and subjects having many outdoor activities.

Lightening Serum – An adequate amount of Lightening Serum (which includes lignin peroxidase and the oxidizing mediator) was applied on the face and spread evenly to thoroughly cover entire face. The serum was used in combination with the Activator Treatment Mask. Following the application of the serum, the subject had to wait 1 minute before applying the Bio-Activator Treatment Mask. Contact with eyes was avoided.

Activator Treatment Mask – The mask was used 1 minute after using the Lightening Serum. The cloth mask (which includes the oxidizing activator) was placed on the face using fingertips to position the mask. The mask covered the entire face and was left on the face for at least 15 minutes.

Results

As shown in Figures 1A-B and 2A-B a single use of the serum and mask according to some embodiments of the invention resulted in a lightening effect on whole skin complexion. No irritation or discomfort were associated with the use of the serum and mask of some embodiments of the invention.

Quantitative measurements of the skin complexion before and after a single use of the serum and mask described under “GENERAL MATERIALS AND METHODS” above, revealed significant reduction in melanin concentration as measured using the mexameter index (Figure 3A) or in erythema intensity as detected using the erythema index (Figure 3B). These measurements conclusively show that the serum and mask of some embodiments of the invention are effective in lightening skin complexion and color even after a single use.

EXAMPLE 2**A 5 DAYS INDICATIVE STUDY TO ASSESS THE IMMEDIATE, SHORT-TERM
FACIAL SKIN WHITENING RELATED TO THE USE OF SERUM & MASK
REGIMEN**

5 **Objectives:** To evaluate comparative efficacy after 1 treatment and application over a consecutive 5-day period a Serum/Mask Regimen on facial skin whitening of Asian subjects via instrumental measurements and subject-self-assessment.

Study Population

10 A minimum of 5 subjects were recruited. Participation of the subjects was purely voluntary. A subject who was admitted into the study, but subsequently determined not to meet the admission criteria was terminated from the study and replaced by an eligible subject in order to reach the minimum number of subjects.

15 **Inclusion Criteria** - 25 to 55 year-old Asian females in good general health; Regular user of facial moisturizers; Willing & able to follow all study directions, accept skin examination and commit to all follow-up visits; Has completed the informed consent process; Willing to avoid direct sun exposure.

20 **Exclusion Criteria** - Subjects having a history of sensitivity to cosmetic products, toiletries or its components; having facial skin disorders (such as inflammation, eczema, psoriasis, skin cancer, severe acne, skin lesions, etc.); having concurrent therapy or disease which could interfere with evaluations or increase risk to the subject; having or had been using oral retinoids or steroids within the last 6 months; having or had been using topical retinoids, alpha-hydroxy and/or beta-hydroxy acids, hydroquinone or any whitening preparation in any form (cleanser, toner, facial lotion or cream) within the last 3 months; having or had been undergoing dermatologist facial treatments or procedures within the last 3 months; having or had been involved in another facial skin care clinical investigation within the last 3 months; Pregnant or nursing female; and subjects having many outdoor activities.

25 **Procedures** - Subjects were evaluated at baseline, after 1 treatment, on day 6 and after two weeks.

30 **Global facial evaluations included:** instrumental measurement, dryness, and photography.

Instrumental Measurement - Subjects were evaluated at baseline, after 1st treatment at Derm's Clinic and on day 6. Instrumental evaluations included:

Mexameter – Assessment of melanin and erythema. Four measurements were taken on one side of the face (cheekbone).

5 **Digital Photography** – Assessment of overall fairness using white light. Subject positioning and light intensity settings of subsequent images were matched with baseline images to ensure objective analysis. Subjects were asked to arrive 30 minutes before the evaluation time in order to acclimatize to study evaluation room conditions.

10 **Skin measurement** sites on the face were identified at baseline, and transparency paper was used as templates to aid in locating each test site.

All instrumental measurements were taken in a climate-controlled room with a temperature of 20-24 °C and a relative humidity of 40-60 %. Subjects were asked to arrive at the testing center 30 minutes before the evaluation time in order to adjust to the conditions.

15 **The test included the use of the following cosmetic products:** Advanced Facial Wash according to some embodiments of the invention, Lotion according to some embodiments of the invention, SPF 15 Lotion, Active White Serum, Activator Treatment Mask according to some embodiments of the invention.

Instructions for use with each of the whitening products:

20 ***Advanced Whitening Lotion Active Enzyme and Activator according to some embodiments of the invention for face and neck*** - Wash the face using facial wash according to some embodiments of the invention. On first use, activate the pumps by pressing them few times. Step 1: Pump an adequate amount of active Enzyme (comprising lignin peroxidase) spreading it evenly to thoroughly cover your cleaned
25 face. Step 2: Wait 1 minute then pump and apply an equal amount of Bio-activator (comprising veratryl alcohol). The whitening lotion can be used daily, morning and evening. Contact with eyes should be avoided.

30 ***Advanced Facial Wash according to some embodiments of the invention*** - Wet face and squeeze a small amount of advanced facial wash according to some embodiments of the invention into palm and work into a rich leather massage gently on face and rinse thoroughly. Use twice daily. Contact with eyes should be avoided.

Lightening Serum - Use this product at night in place of your usual night facial moisturizer for 4 consecutive nights. Apply on face an adequate amount of Lightening Serum and spread evenly to thoroughly cover entire face. The serum is used in combination with the Bio-Activator Treatment Mask. Wait 1 minute after application of the lightening serum product before applying the Bio-Activator Treatment Mask. Contact with eyes should be avoided. If irritation develops, discontinue use.

Activator Treatment Mask - Use this product at night for 4 consecutive nights, 1 minute after applying the Lightening Serum. Tear open sachet and take out the mask. Unfold mask. 3. Gently place cloth mask on your face using fingertips to position the mask. Cover mask over the entire face. Use fingertips to make sure it fits well, especially around nose area. Leave the mask on the face for at least 15 minutes. Remove the mask and then gently massage the face. There is no need to wash it off. Discard cloth mask after use. Contact with eyes should be avoided. If irritation develops, discontinue use.

Each study consisted of 2 monadic cells and treatment over a 6-day period according to Table 6, below.

Table 6

<i>Day</i>	<i>Product Administration</i>	<i>Test</i>	<i>Measurement</i>	<i>Results</i>
Do - Baseline Morning	Derm Office Derm Office	None Wash + Serum + Mask SPF 15	Yes Yes None	Fo F1
D1 AM PM	Home Home	Wash + Lotion + SPF 15 Wash + Serum + Mask	None	
D2 AM PM	Home Home	Wash + Lotion + SPF 15 Wash + Serum + Mask	None	
D3 AM PM	Home Home	Wash + Lotion + SPF 15 Wash + Serum + Mask	None	
D4 AM PM	Home Home	Wash + Lotion + SPF 15 Wash + Serum + Mask	None	

<i>Day</i>	<i>Product Administration</i>	<i>Test</i>	<i>Measurement</i>	<i>Results</i>
D5 AM	Home Derm Office	Wash + SPF 15	None Yes	F2

Table 6. "Derm" – Dermatologist.

Table 7, hereinbelow, summarizes the study's activities.

5

Table 7

Baseline, Day 0	Pre-screening
	Photography Release Forms
	Subject Assignment
	Clinical Assessments
	Instrumental Measurements
	Product Dispensation
	Diary Sheet Dispensation
Day 6	Self-Assessment Questionnaire
	Clinical Assessments
	Instrumental Measurements
	Product Return
	Diary Sheet Submission

Table 7.

Results

10

The serum and mask of some embodiments of the invention exhibit a significant lightening effect on whole skin complexion – As shown in Figures 4A-B and 5A-B following 5 days of applying the lightening serum and mask, along with the lightening lotion on subjects' faces there was a significant lightening of whole skin complexion.

15

The mask and serum of some embodiments of the invention were applied for 5 consecutive days and the effect of the compositions on skin lightening was evaluated after 2 weeks from the first day of using the lightening compositions. As shown in Figures 6A-B the lightening effect remained even after 2 weeks from beginning of treatment.

20

Significant lightening of pigmented scars using the serum and mask of some embodiments of the invention – The mask and serum of some embodiments of the invention were applied for 5 consecutive days and the effect of the compositions on skin lightening was evaluated after 2 weeks from the first day of using the lightening compositions. As shown in Figures 6A-B, a significant lightening of a pigmented scar

(6 month old scar) was observed after 2 weeks from the first day of using the lightening compositions of some embodiments of the invention.

EXAMPLE 3

5 **THE LONG TERM EFFECT OF A 6-DAYS USE OF SERUM, MASK AND**
LOTION OF SOME EMBODIMENTS OF THE INVENTION FOR LIGHTENING
SKIN COMPLEXION

The following study was designed as an open study to measure the effect application of a lightening serum according to some embodiments of the invention followed by an activator mask according to some embodiments of the invention on facial skin, as compared with baseline. The objectives of the study were to evaluate efficacy of the treatment, after application of a Lightening Serum, followed with Mask once a day, over a 7-day period, on facial skin of Caucasian subjects. Measurement of the effect was done via dermatological grading, photograph and subject-self-assessment against baseline.

The serum, mask, facial wash, lotion and cream used in the study include ingredients that are commercially available and have passed strict quality and safety standards. The Serum + Mask samples used at the doctor's office have passed microbial testing.

20 **I. Treatment regimen**

Treatment at the clinic D0 (day 0) and D6 (day 6) -

Morning: use of cleanser (the facial wash according to some embodiments of the invention) followed by application of the serum of some embodiments of the invention, followed by application of the mask according to some embodiments of the invention + SPF15.

Evening: use of cleanser (the facial wash according to some embodiments of the invention) followed by application of the lotion according to some embodiments of the invention.

Treatment at home D1 (day 1) to D5 (day 5):

30 Morning: use of cleanser (the facial wash according to some embodiments of the invention) followed by application of the lotion of some embodiments of the invention + SPF 15.

Evening: use of cleanser (the facial wash according to some embodiments of the invention) followed by application of the serum of some embodiments of the invention, followed application of the mask of some embodiments of the invention.

II. Study design

5 This is an open study enrolling a total of 10 subjects as shown in Table 8 below:

Table 8

Study Investigation	Location	No. of Test Sites	No. of Subjects
Doctor's Clinic	Israel	1	10

Table 8.

10 The study consisted of one cell and treatment over a 7-day period as detailed in Table 9 below.

Table 9

<i>Day</i>	<i>Product Application</i>	<i>Treatment Location</i>	<i>Treatment No.</i>	<i>Treatment (Cell T)</i>	<i>Assessment & photography</i>
0	Dermatologist	Dermatologist Clinic	0	AM: None (Baseline) Cleanser + Serum + Mask + SPF 15	Yes, F0
	Self	At home	1	PM: Cleanser + Lotion	Yes, F1
1	Self	At home	2	AM: Cleanser + Lotion + SPF 15 PM: Cleanser + Serum + Mask	None
2	Self		3	AM: Cleanser + Lotion + SPF 15 PM: Cleanser + Serum + Mask	None
3	Self		4	AM: Cleanser + Lotion + SPF 15 PM: Cleanser + Serum + Mask	None
4	Self		5	AM: Cleanser + Lotion + SPF 15 PM: Cleanser + Serum + Mask	None
5	Self		6	AM: Cleanser + Lotion + SPF 15 PM: Cleanser + Serum + Mask	None
6	Self	At home	7	AM: Cleanser + SPF 15	Yes, F2
	Dermatologist	Dermatologist Clinic		Cleanser + Serum + Mask + SPF 15	

Table 9.

15 ***Directions of using the tested compounds:***

The facial wash (cleanser) of some embodiments of the invention - Wet face and squeeze a small amount of advanced facial wash into palm and work into a rich leather massage gently on face and rinse thoroughly. Use twice daily. Warning: Avoid contact with the eyes.

The facial lightening lotion dual chamber container of some embodiments of the invention: Wash your face using facial wash. On first use, activate the pumps by pressing them few times. Step 1: Pump an adequate amount of active enzyme spreading it evenly to thoroughly cover your cleaned face. Step 2: Wait 1 minute then pump and
5 apply an equal amount of activator. Wait for 1 minute after applying whitening lotion. For best results use daily, morning and evening. Warning: Avoid contact with eyes.

Instructions for participants in the study:

On Days 0 and 6 - In the morning at home:

- (1) Cleanse your face by using the cleanser (facial wash according to some
10 embodiments of the invention) as you normally do with a cleanser in the morning.
- (2) Use the SPF15 lotion as you normally do with a moisturizer.

On Days 0 and 6 - At dermatologist clinic:

- (1) Dermatologist asks subject to cleanse her face by using the cleanser as she normally does. Dermatologist evenly applies the serum of some embodiments of the invention on
15 the subject's face. Wait 1 minute after application of this product before applying the activator treatment mask.
- (2) Tear open sachet and take out the Mask.
- (3) Unfold mask.
- (4) Gently place cloth mask on subject face using fingertips to position the mask.
- 20 (5) Cover mask over the entire face. Use fingertips to make sure it fits well, especially around nose area.
- (6) Leave the mask on the face for at least 15 minutes. Remove the mask and then gently massage the face. There is no need to wash it off. Discard cloth mask after use.

On Days 0 and 6 - In the evening at home:

- 25 (1) Cleanse your face by using the cleanser as you normally do with a cleanser in the evening.
- (2) Apply the whitening lotion on face.

On days 1 to 5 – at home:

In the morning at home:

- 30 (1) Cleanse your face by using the whitening cleanser as you normally do with a cleanser in the morning.
- (2) Use the SPF15 lotion as you normally do with a moisturizer.

In the evening at home:

(1) Cleanse your face by using the cleanser as you normally do with a cleanser in the evening.

(2) Apply serum on face as trained by dermatologist. Wait 1 minute after application of this product before applying the Bio-Activator Treatment Mask.

(3) Tear open sachet and take out the Mask.

(4) Unfold mask.

(5) Gently place cloth mask on subject face using fingertips to position the mask.

(6) Cover mask over the entire face. Use fingertips to make sure it fits well, especially around nose area.

(7) Leave the mask on the face for at least 15 minutes. Remove the mask and then gently massage the face. There is no need to wash it off. Discard cloth mask after use.

III. Subject selection

Study Population - A minimum of 10 subjects in the dermatologist clinics. Any subject who was admitted into the study, but subsequently determined not to meet the admission criteria was terminated from the study and replaced by an eligible subject, if necessary to reach the minimum number of subjects.

Inclusion Criteria - 25 to 55 year-old females in good general health. Regular user of facial moisturizers; willing and able to follow all study directions, accept skin examination and commit to all follow-up visits; has completed the informed consent process; willing to avoid direct sun exposure.

Exclusion Criteria - Has a history of sensitivity to cosmetic products, toiletries or its components; suffers facial skin disorders (such as inflammation, eczema, psoriasis, skin cancer, severe acne, skin lesions, etc.); has concurrent therapy or disease which could interfere with evaluations or increase risk to the subject; currently or has been using oral retinoid or steroids within the last 6 months; currently or has been using topical retinoid, alpha-hydroxy and/or beta-hydroxy acids, hydroquinone or any whitening preparation in any form (cleanser, toner, facial lotion or cream) within the last 3 months; currently or has been undergoing dermatologist facial treatments or procedures within the last 3 months; currently or has been involved in another facial skin care clinical investigation within the last 3 months; pregnant or nursing female; with many outdoor activities.

IV. Procedures

Pre-Study - Participation of the subjects was purely voluntary. Before being entered into the study, the subjects were pre-screened for the criteria indicated in the subject selection section, using a screening form according to the inclusions and
5 exclusions criteria. Only subjects, who met the requirements of this section and have completed the informed consent and medical history forms have entered into the study.

Continued compliance of the subjects with the inclusion / exclusion criteria was checked on the subsequent visit using the same screening form.

During the baseline visit, each subject was given one set of test products to be
10 used for five (5) consecutive days. In addition, subjects completed a diary sheet in which they recorded the time of using the compound, if they were exposed to the sun, or if they were not able to use the compounds and the reason for that.

V. Clinical Measurements - The subjects were evaluated at baseline after 1 treatment, on Day 6, on day 14 (2 weeks) and on day 28 (1 month). Global facial
15 evaluations included: Overall Fairness; Overall Evenness; Mottled Hyperpigmentation; Lentigines; Sallowiness; Overall Roughness; Overall Clarity; Erythema; Edema; Dryness; and Scaling.

Overall Fairness, Overall Evenness, Mottled Hyperpigmentation, Lentigines, Sallowiness, Overall Roughness, and Overall Clarity were rated using a 10-pt. scale,
20 where 0 indicates perfect condition and 9 indicates the worst condition of each of these parameters. Skin reactions (erythema, edema, dryness and scaling) was evaluated at each visit using the scale: 0 = absence of symptoms; 1 = mild; 2 = moderate; 3 = marked; and 4 = severe.

VI. Instrumental Measurements - Subjects were evaluated at baseline, after 1st
25 treatment at Dermatologist's Clinic, on Day 6, on day 14 (2 weeks) and on day 28 (1 month).

Instrumental evaluations included:

Digital Photography – Assessment of overall fairness using white light. Subject positioning and light intensity settings of subsequent images were matched with
30 baseline images to ensure objective analysis.

Subjects were asked to arrive 30 minutes before the evaluation time in order to acclimatize to study evaluation room conditions.

Self-Assessment - Each subject accomplished a self-assessment questionnaire at the testing center or dermatologist clinics on Day 6 based on the following attributes: overall rating; leaves skin soft/smooth; leaves skin supple; lightens dark spots; makes skin tone even; makes skin looking radiant; makes skin fairer; makes spots / freckles lighter; reduces yellowness of skin; is non-irritating to the skin.

All attributes were assessed using the 5-point scale: Excellent; very good; good; fair; poor.

Table 10, hereinbelow summarizes the activities performed in this study.

Table 10
Summary of Activities

Baseline, Day 0	Pre-screening
	Completion of Informed Consent, Medical History and Photography Release Forms
	Subject Assignment #1
	Clinical Assessments
	Photography
	Mask + Serum/Lotion application
	Clinical Assessments #2
	Photography
	Product Dispensation
	Diary Sheet Dispensation
Day 6	Self-Assessment Questionnaire
	Mask + Serum/Lotion application
	Clinical Assessments
	Photography
	Product Return
	Diary Sheet Submission

Table 10.

Statistical analysis - Parametric and non-parametric tests are performed for each parameter - clinical grading, instrumental readings and consumer self-assessment—comparing subsequent time point readings to baseline readings. A significance probability of $p < 0.05$ is used to determine if there is an improvement versus baseline.

Results of clinical/cosmetic study

The serum and mask of some embodiments of the invention exhibit a significant lightening effect on whole skin complexion and reduction of skin's redness— As shown in Figures 9A-B following 7 days of applying the lightening serum and mask, along with the lightening lotion, and lightening cleanser on subjects' faces there was a significant lightening of whole skin complexion and in reduction of skin pigmentation (e.g., red spots over the skin).

In addition, as shown in Figures 10A-D, following 2 weeks from the first day, which is one week after completion of the treatment (ceasing of using the whitening compounds of some embodiments of the invention) there was still a significant effect of the tested compounds on skin lightening and reduction of skin pigmentation, suggesting a long term effect of the serum and mask of some embodiments of the invention in skin lightening.

Furthermore, as shown in Figures 11A-B, the long term effect remained even one month after the initial treatment, which is in fact 21 days after ceasing the treatment with the serum and mask of some embodiments of the invention.

Altogether, these results show that the serum and mask according to some embodiments of the invention are effective in lightening whole skin complexion, reducing skin pigmentation, as well as lightening scars on the skin.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. A kit for lightening a skin of a subject, comprising a first container comprising a mask and a second container comprising a cosmetic composition, wherein said mask and said cosmetic composition are in no direct contact in the kit, wherein:

(i) when said mask comprises an oxidizing activator then said cosmetic composition comprises a lignin peroxidase,

(ii) when said mask comprises a lignin peroxidase then said cosmetic composition comprises an oxidizing activator.

2. The kit of claim 1, further comprises an oxidizing mediator.

3. The kit of claim 1, wherein said cosmetic composition is in a form of a serum.

4. The kit of claim 1, wherein said cosmetic composition is in a form of a leave-on lotion, cream, gel or toner.

5. The kit of claim 1, wherein said mask further comprises a cosmetically acceptable excipient.

6. The kit of any of claims 1-5, wherein said container comprising said oxidizing activator is sealed.

7. The kit of any of claims 1-5, wherein said container comprising said oxidizing activator is made of a material which is inert to oxidation by said oxidizing activator.

8. The kit of any of claims 6 and 7, wherein said container comprising said oxidizing activator is sealed by vacuum.

9. The kit of any of claims 1-8, wherein said mask comprises a solid support.
10. The kit of claim 9, wherein said solid support comprises an occlusive film.
11. The kit of claim 9, wherein said solid support comprises a non-occlusive film.
12. The kit of any of claims 1-8, wherein said mask is a rinse-off mask.
13. The kit of any of claims 1-5, wherein said mask comprises a material which hardens or polymerizes after a set period of time.
14. The kit of claim 13, wherein said material which hardens or polymerizes after a set period of time is made of at least two phases which are mixed prior to use.
15. The kit of any of claims 1-8 and 12-14, wherein said mask comprises a gelifying agent.
16. The kit of any of claims 1-15, wherein said mask comprises pH regulators.
17. The kit of any of claims 1-16, wherein a concentration of said lignin peroxidase in said cosmetic composition is selected from the range of about 0.5% (weight/weight) to about 2% (weight/weight).
18. The kit of any of claims 2-16, wherein said oxidizing mediator is veratryl alcohol.

19. The kit of claim 18, wherein a concentration of said veratryl alcohol in said cosmetic composition is between about 0.01 % (weight/weight) to about 1 % (weight/weight).

20. The kit of any of claims 1-19, wherein said oxidizing activator is hydrogen peroxide.

21. The kit of claim 20, wherein a concentration of said hydrogen peroxide in said mask is between about 0.001 % (weight/weight) to about 0.1 % (weight/weight).

22. The kit of any of claims 1-21, wherein said lignin peroxidase is isoenzyme H1 or a modified form of isoenzyme H2.

23. The kit of any of claims 1-22, wherein said lignin peroxidase is an extract of a *Phanerochaete chrysosporium* fungus.

24. The kit of any of claims 1-22, wherein said lignin peroxidase is recombinantly expressed.

25. The kit of claim 24, wherein said lignin peroxidase is encoded by a polynucleotide comprising a nucleic acid sequence set forth in SEQ ID NO:1.

26. The kit of any of claims 1-25, further comprising a third and a forth containers which comprise lignin peroxidase and an oxidizing activator, respectively.

27. A cosmetic method of lightening a skin of a subject, comprising:

(a) applying said lignin peroxidase on a skin region of the subject, while using the kit of claims 1-26, and;

(b) applying said oxidizing activator in said kit on said skin region having said lignin peroxidase for a pre-determined time,
thereby lightening the skin of the subject.

28. The cosmetic method of claim 27, further comprising repeating step (b) daily for at least 5 days.
29. The cosmetic method of claim 27, wherein step (a) is performed prior to step (b).
30. The cosmetic method of claim 27, wherein step (b) is performed prior to step (a).
31. The cosmetic method of claim 27, further comprising:
(c) repeating steps (a) and (b) daily for at least 5 days.
32. The cosmetic method of claim 27, further comprising:
(c) repeating steps (a) and (b) daily for at least 14 days.
33. The cosmetic method of any of claims 27-32, further comprising applying said lotion comprising said lignin peroxidase.
34. The cosmetic method of any of claim 27-32, further comprising applying said lotion comprising said oxidizing activator.
35. The kit of any of claims 1-26 or the method of any of claims 27-34, wherein said skin region comprises facial skin.
36. The kit of any of claims 1-26 or the method of any of claims 27-34, wherein said skin region comprises a skin region exposed to the sun.
37. The kit of any of claims 1-26 or the method of any of claims 27-34, wherein said lightening said skin comprises lightening whole skin complexion.

38. The kit of any of claims 1-26 or the method of any of claims 27-34, wherein said skin region comprises an uneven skin tone, dark spot(s), freckle(s), melasma, hyperpigmentation, skin discoloration, age spot(s), acne mark(s) and/or a scar.

39. A kit for lightening a skin of a subject, comprising a first container comprising a mask which comprises a hydrogen peroxide, and a second container comprising a cosmetic composition which comprising lignin peroxidase, wherein said mask and said cosmetic composition are in no direct contact in the kit, wherein:

(i) a concentration of said hydrogen peroxide in said mask is between 0.01-0.013 % (weight/weight);

(ii) a concentration of said when said lignin peroxidase in said cosmetic composition is between about 0.1% (weight per weight) to about 5% (weight per weight); and

(iii) wherein said mask and/or said cosmetic composition further comprises veratryl alcohol at a concentration in the range of about 0.1-0.2 % (weight/weight).

40. The kit of claim 39, wherein said cosmetic composition is in a form of a serum.

41. The kit of claim 39, wherein said concentration of said lignin peroxidase in said cosmetic composition is between 0.5% (weight/weight) to about 2% (weight/weight).

42. A mask comprising lignin peroxidase and a cosmetically acceptable carrier.

43. The mask of claim 42, wherein said lignin peroxidase is sterile.

44. The mask of claim 42, wherein said lignin peroxidase is synthetic.

45. The mask of claim 42, wherein said lignin peroxidase is recombinantly expressed.



Before Treatment

FIG. 1A



Immediately After 1
Treatment of Serum + Mask

FIG. 1B

Subject No. 1000



Untreated baseline

FIG. 2A



After 1 treatment of serum + mask

FIG. 2B

Subject No. 1006

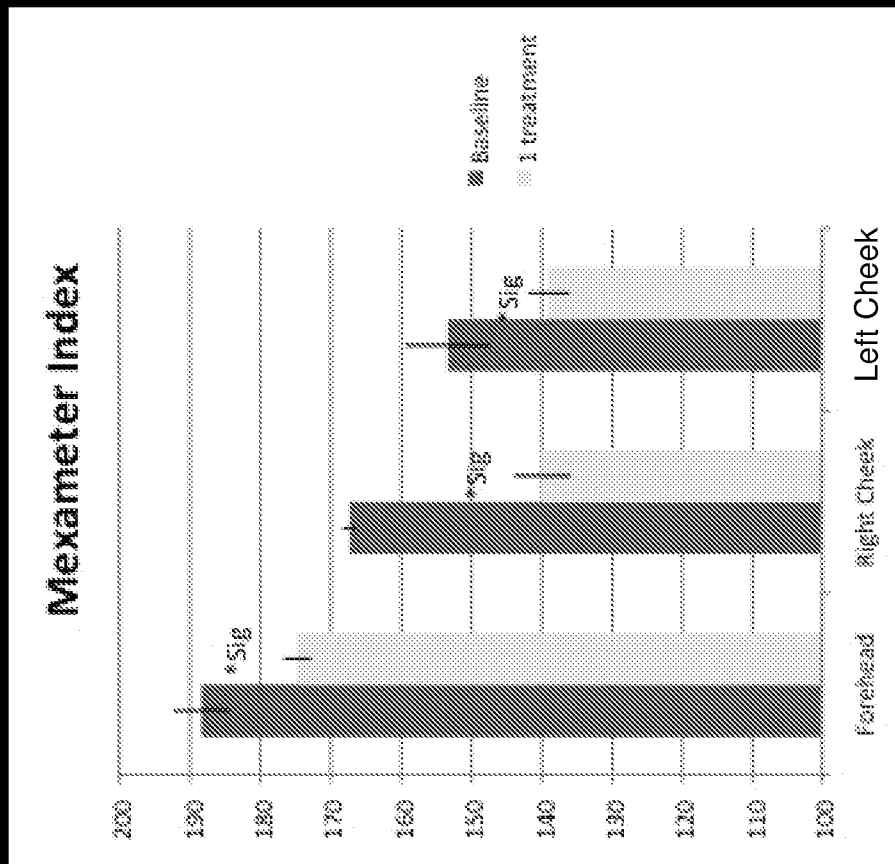


FIG. 3A

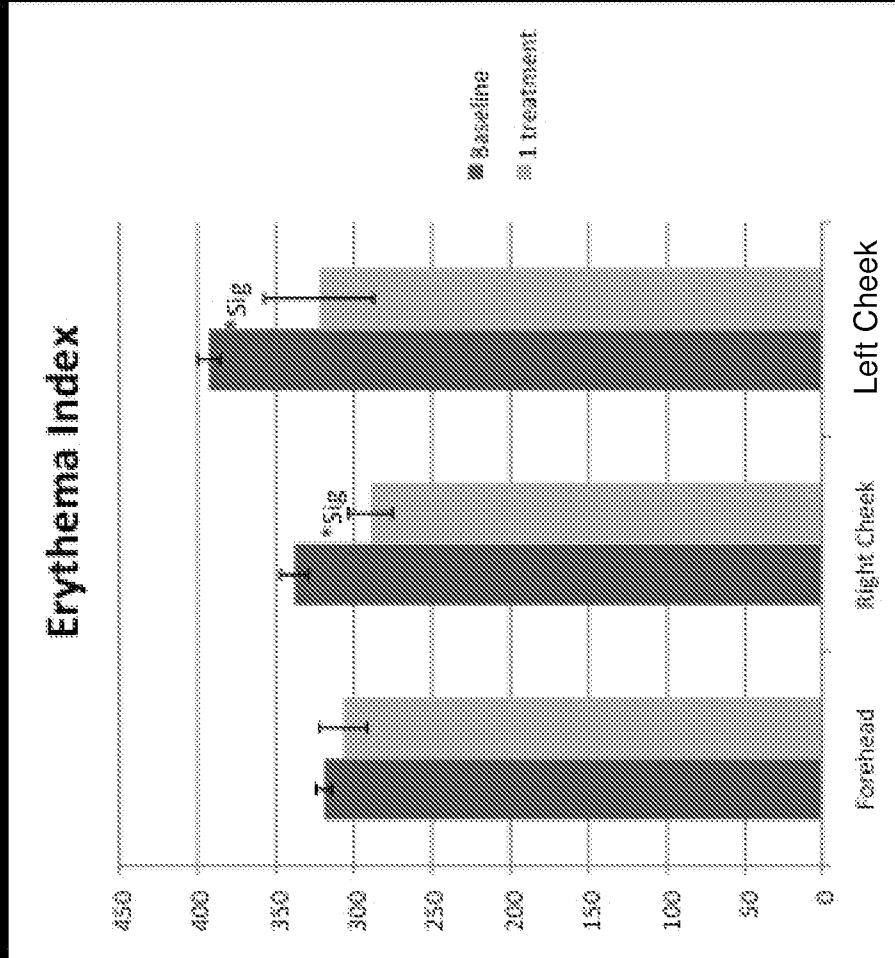


FIG. 3B

Subject No. 1000

FIG. 4A



Day 0

FIG. 4B



Day 5

Subject No. 1001

FIG. 5A



Day 0

FIG. 5B



Day 5



RM No.	Raw Material Trade Name	Wt/Wt RM	INCI Name	Activity of Material	Specs	Wt/Wt Ingredient	CAS Number	Constituents & Carry Over Items	Function of Material	Supplier (Distributor)	Origin (Plant Animal Synthetic)
1	Purified Water	84.19	Water	1		84.13	7732-18-5		Vehicle	In-house	Synthetic
2	Moon K Glycerin, USP	8.00	Glycerin	99.70% min		7.98	56-81-5	Water 0.30% max	Humectant	P&G Chemicals (EAC Chemicals)	Plant
3	Propylene Glycol, USP	2.00	Propylene Glycol	99.50% min		1.99	57-55-6	Water 0.50% max	Humectant	Shell Eastern Petroleum Pte (Eumice Inc)	Synthetic
4	Dow Corning Fluid 200 cst	1.00	Dimethicone	100%		1.00	141-42-8 141-43-9 9006-65-9 9016-00-6 63148-62-9		Emollient	Xiameter (Edward Keller DKSH)	Synthetic
5	Cetiol CC	1.00	Dicaprylyl Carbonate	100%		1.00	1680-31-5		Emollient	Cognis (Asia Pacific Kimika Inc)	Plant
6	Drakeol 19, USP	0.50	Liquid Paraffin	100%		0.50	8012-95-1 8020-835 8042-47-5		Emollient	Peureco (Chemcentral)	Mineral
7	Lanelle C1635-100MY	0.50	Cetyl Alcohol	100%		0.50	36653-82-4		Opacifier, Emulsifier	Cognis (Asia Pacific Kimika Inc)	Plant
8	Lanelle C1835-100MY	0.50	Stearyl Alcohol	100%		0.50	112-92-5		Opacifier, Emulsifier	Cognis (Asia Pacific Kimika Inc)	Plant
9	Seppius 400	1.00	Polyisobutene Polycarbonate 20 Polyacrylate-13	25% 5.0% 70% 99%	23-25% 1-8% 50-70% min	0.25 0.05 0.70	9003-27-4 9005-64-5		Solvent, Emulsifier, Film former	Seppic (Eurochem Inc)	Synthetic
10	Pheroxy ethyl alcohol Arosol	0.50	Phenoxyethanol	99%		0.50	122-99-6		Microbial Preservative	Synrise (Synrise Philippines)	Synthetic
11	Veratril Alcohol	0.10	Veratril Alcohol	100%		0.10	93-03-8		Skin Conditioning Agent, Skin softener	Sigma (Chermine)	Synthetic
12	Up (Extract of <i>Phanerochaete chrysosporium</i>)	0.71	Ligninase	100%	3x10 ⁶ units per Liter	0.71			Skin Whitening Active, Skin Conditioning Agent-humectant	Rakuto Bio-Technologies	Synthetic
13	Glyzool L	0.10	Iodopropenyl Butyrcarbonate PEG-4 PEG-4 Laurate PEG-4 Diamate	10% 10.3% 10% 40% 40%	9.1-10.3% ~10% ~10% ~40% ~40%	0.01 0.01 0.04 0.04	55406-53-6 112-607 25322-68-3 9004-81-3 10108-24-4 9005-02-01		Microbial Preservative	Lonza (BNC Ingredients)	Synthetic
TOTAL		100.00				100.00					

FIG. 7A

RM No.	Raw Material Trade Name	w/w% RM	INCI Name	Activity of Material	Specs	w/w% Ingredient	CAS Number	Constituents & Carry Over Items	Function of Material	Supplier (Distributor)	Origin (Plant, Animal, Synthetic)
1	Purified Water	84.53	Water	1		84.64	7732-18-5		Vehicle	In-house	
2	Mon K Glycerin, USP	8.00	Glycerin	99.70%	min	7.98	56-81-5	Water 0.30% max	Humectant	P&G Chemicals (EAC Chemicals)	Plant
3	Propylene Glycol, USP	2.00	Propylene Glycol	99.50%	min	1.99	57-55-6	Water 0.50% max	Humectant	Shell Eastern Petroleum Pte (Eunice Inc)	Synthetic
4	Dow Corning Fluid 200 cst	1.00	Dimethicone	100%		1.00	141-62-8 141-63-9 9006-65-9 9016-00-6 63148-62-9		Emollient	Xiameter (Edward Keller DASH)	Synthetic
5	Cetiol CC	1.00	Dicaprylyl Carbonate	100%		1.00	1680-31-5		Emollient	Cognis (Asia Pacific Kinda Inc)	Plant
6	Drakeol 19, USP	0.50	Liquid Paraffin	100%		0.50	8012-95-1 8016-51-5 8042-47-5		Emollient	Petroco (Chemcentral)	Mineral
7	Lanette C1635-100 MY	0.50	Cetyl Alcohol	100%		0.50	36653-82-4		Opacifier, Emulsifier	Cognis (Asia Pacific Kinda Inc)	Plant
8	Lanette C1838-100MY	0.50	Squalyl Alcohol	100%		0.50	112-92-5		Opacifier, Emulsifier, Solvent	Cognis (Asia Pacific Kinda Inc)	Plant
9	Seppius 400	1.25	Polyisobutene Polysorbate 20 Polysorbate-13	25% 5% 70%	23-25% 5-8% 50-70%	0.313 0.06 0.875	9003-27-4 9005-64-5		Solvent Emulsifier Film former	Seppic (Eurochem Inc)	Synthetic
10	Phenoxy ethy alcohol Arsol	0.50	Phenoxyethanol	99%	min	0.50	122-99-6		Microbial Preservative	Syntise (Syntise Philippines)	Synthetic
11	Citric Acid Solution, 50%	0.08	Citric Acid	50%		0.04	93-03-8		Skin Conditioning Agent, Roix mediator	Shibei City Changyun Biochemical Col, Ltd (Vilchem)	Synthetic
12	Hydrogen Peroxide, 30%	0.04	Hydrogen Peroxide	30%		0.012			Skin Whitening Active, Skin Conditioning Agent-humectant	Sigma-Aldrich (Chemline)	Synthetic
13	Glycocal L	0.10	Iodopropynyl Biguanide PEG-4 PEG-4 Lactate PEG-4 Diuretic	10% 10% 40% 40%	9.1-10.3% ~10% ~40% ~40%	0.01 0.01 0.04 0.04	55406-53-6 112-607 25322-88-3 9004-81-3 10108-24-4 9005-02-01		Microbial Preservative	Lonza (BNC Ingredients)	Synthetic
TOTAL		100.00				100.00					

FIG. 7B

RM No.	Raw Material Trade Name	w/w% RM	INCI Name	Activity of Material	Specs	w/w% Ingredient	CAS Number	Constituents & Carry Over Items	Function of Material	Supplier (Distributor)	Origin (Plant, Animal, Synthetic)
1	Purified Water	23.54	Water	100%			32.14 7732-18-5		Vehicle	In-house	
2	Superol V, Glycerin 99.7%	16.00	Glycerin	99.70%	min		15.95 56-81-5	Water 0.30% max	Humectant	P&G Chemicals (EAC Chemicals)	Plant
3	Zemea	20.00	Propanediol	100%			20.00 504-63-2 26264-14-2		Humectant	Du Pont (Du Pont Asia)	Plant
4	Potassium Hydroxide	4.75	Potassium Hydroxide	100%			4.75 1310-58-3		Soap Base/ Neutralization	Chem Supply (Alyson's)	Synthetic
5	Stearic Acid	9.50	Stearic Acid	99%	min		9.41 57-11-4		Soap Base/ Cleansing	Emery Oleochemicals (EAC Chemicals)	Plant
6	Edenor C1499	6.50	Myristic Acid	99%	min		6.44 544-63-8		Soap Base/ Cleansing	Emery Oleochemicals	Plant
7	Edenor C1299	3.50	Lauric Acid	99%	min		3.47 143-07-7		Soap Base/ Cleansing	Emery Oleochemicals (EAC Chemicals)	Plant
8	Amilite GCK-12	3.00	Sodium Cocoyl Glutamate	30%			0.90 68187-30-4		Surfactant/Cleansing	Ajinomoto (Actichem)	Plant
9	Plantaren 2000 UP	7.50	Decyl Glucoside	50%			3.75 58846-77-8 68515-73-1 141464-42-8		Surfactant/Cleansing	Cognis	Plant
10	Atacel 165	2.20	Glyceryl Stearate PEG-100 Stearate	50% 50%			1.10 123-94-4 1.10 9004-99-3		Surfactant/Cleansing	Croda (BNC Ingredients)	Plant
11	Synchrowax BB4	1.00	Synthetic Beeswax	100%			1.00 71243-51-1		Stabilizer, Skin conditioner	Croda (BNC Ingredients)	Plant
12	Citrol EGMS	2.50	Glycol Distearate	100%			627-83-8 91031-31-1		Stabilizer, Pearlizer	Croda (BNC Ingredients)	Plant
13	Vulkanox BHT	0.01	BHT	100%			0.01 128-37-0		Antioxidant	Vulkanox (CCT Chemicals)	Synthetic
14	Extract of Phanerochaete Chrysosporium	0.005	Ligninase (Extract of Phanerochaete Chrysosporium)	100%			0.01		Skin Whitening Active, skin conditioning agent, humectant	Lonza (RBT)	Plant
TOTAL		100.00					100.00				

FIG. 8

After 6 day

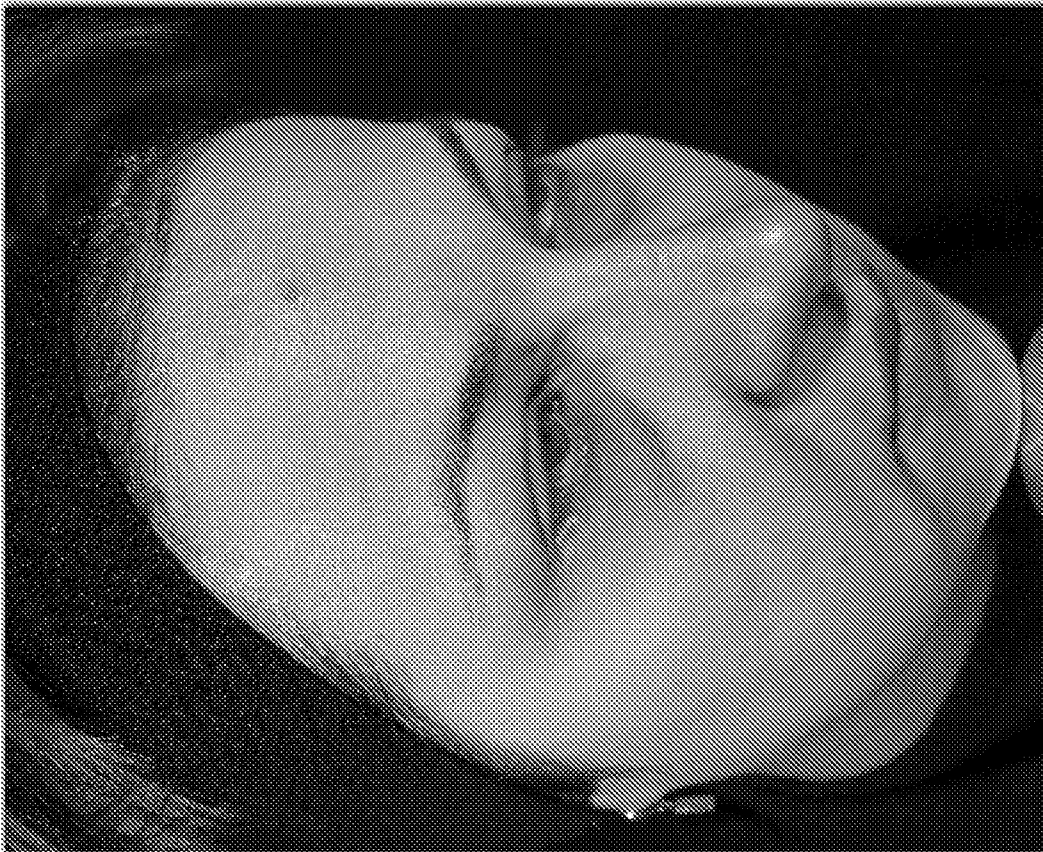


FIG. 9B

Baseline

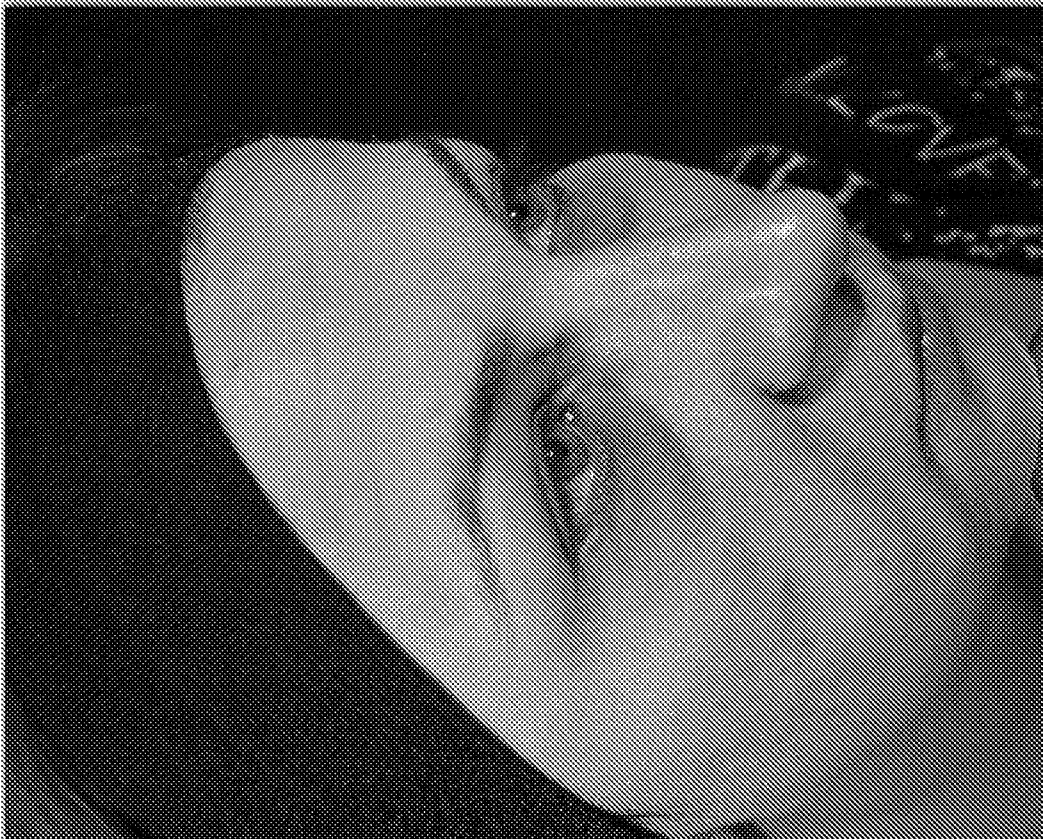


FIG. 9A

After 2 weeks



FIG. 10B

Baseline



FIG. 10A

After 2 weeks



FIG. 10D

Baseline



FIG. 10C

Baseline



FIG. 11A

1 month after



FIG. 11B