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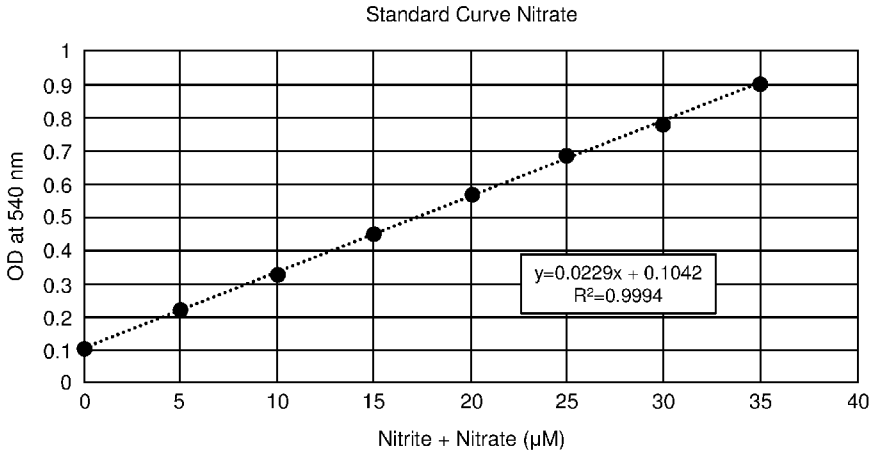

FIG. 1

(57) Abstract: Compositions that supplement and support nitric oxide availability to skin and methods of use are provided herein. An exemplary embodiment of the composition includes an adenosine, arginine, carnosine, niacinamide, and a magnesium ion source. The composition includes a first agent that is a precursor of nitric oxide, a second agent that facilitates production of nitric oxide by the first agent, and a third agent that antagonizes one or both of nucleotide activation of skeletal muscle ryanodine receptors and cellular calcium influx. The first, second and third agents are mixed to form a composition suitable for topical application to skin to provide reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, and/or an improved skin tone.

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[Continued on next page]

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TOPICAL COMPOSITION FOR HOMEOSTATIC DELIVERY OF NITRIC OXIDE AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to US Nonprovisional Patent Application No. 17/831,333 entitled "Topical Composition for Homeostatic Delivery of Nitric Oxide and Uses Thereof" filed on June 2, 2022.

TECHNICAL FIELD

[0002] The subject matter described herein relates to topical compositions that supplement and support availability of nitric oxide to skin and uses thereof.

BACKGROUND

[0003] The skin is the largest organ of the human body. Unfortunately, skin diseases and undesirable skin conditions are common and, according to studies, affect as many as one in three Americans at any given time. Treatment of conditions of the skin is important for both overall health, but also for appearance, the latter being associated with self-esteem and other important aspects of mental health. Common treatment methods for skin conditions include medicated creams and ointments, including agents such as antibiotics, antioxidants, retinoids, alpha hydroxy acids, peptides, and vitamins. Surveys indicate that the average American spends over $300 per year on skincare, possibly resulting in expenditures of more than $15,000 over a lifetime. The overall global market for skin care products is over 145.3 billion dollars per year.

[0004] A skin area may be a person's neck, décolleté, face, and/or back of hands. Compared to skin on the face, neck skin is thinner, more extensible, and subject to constant movement. Also, neck skin is highly exposed to environmental insults, and often neglected. Neck skin shows a more severe aging pattern including deeper wrinkles, skin sagging and laxity than other skin areas, and experiences suboptimal oxygen and nutrients. Due to having fewer sebaceous glands than the face, neck has an increasing need for moisturization support. As such, the neck area benefits most from a targeted skincare solution.

[0005] Nitric oxide plays a role in helping maintain homeostasis and balance in all cells of the body, including skin cells. Nitric oxide dilates blood vessels and increases blood flow. It contributes to vessel homeostasis by inhibiting vascular smooth muscle contraction. Nitric oxide stimulants induce formation of cyclic guanosine monophosphate (cGMP) in the vascular smooth muscle cells and through a series of activations (PKG) prevent the calcium influx, ultimately resulting in smooth muscle relaxation.

[0006] Youthful-acting skin cells require nitric oxide to open up nutrient and oxygen channels in the skin. The flow of nutrients and oxygen to skin cells is critical to dermal health and the body's natural healing process. Aging is associated with deficits in nitric oxide availability, contributing to a 40% reduction in blood flow to the skin between the ages of 20 to 70 years. Reduction in nitric oxide contributes to signs of skin aging such as fine lines and wrinkles, crepiness, hyperpigmentation and dryness. As a result, cells function less efficiently, making them more vulnerable to accelerated skin aging (Eunjoo K, et al. *Skin Res and Technol.* 2013 Aug;19(3):236-41; Ryan T, et al. *Micron.* 2004;35(3):161–71; Bruch-Gerharz D. *J Invest Dermatol.* 1998 Jan;110(1):1-7.; Gad MZ. *J Adv Res.* 2010;1:169-177; Bentov I, et al. *Microvasc Res.* 2015 July;100:25-31).

BRIEF SUMMARY

[0007] The present disclosure provides topical compositions/skincare products that supplement and support nitric oxide availability to skin. The following aspects and embodiments thereof described and illustrated below are meant to be exemplary and illustrative, not limiting in scope.

[0008] Compositions that supplement and support nitric oxide availability to skin and methods of use are provided herein. An exemplary embodiment of the composition includes an adenosine, arginine, carnosine, niacinamide, and a magnesium ion source. The composition includes a first agent that is a precursor of nitric oxide, a second agent that facilitates production of nitric oxide by the first agent, and a third agent that antagonizes one or both of nucleotide activation of skeletal muscle ryanodine receptors and cellular calcium influx. The first, second and third agents are mixed to form a composition suitable for topical application to skin to provide reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, and/or an improved skin tone.

[0009] In one aspect, the present disclosure provides a composition comprising a first agent that is a precursor of nitric oxide, a second agent that facilitates production of nitric oxide by the first agent, and a third agent that antagonizes (i) nucleotide activation of skeletal muscle ryanodine receptors or (ii) cellular calcium influx. The first, second and third agents are mixed to form a composition suitable for topical application to skin.

[0010] In some embodiments, the first agent may be selected from the group consisting of arginine, citrulline, nitroglycerin, amyl nitrite, and sildenafil. In certain embodiments, the first agent may be L-arginine. By way of non-limiting example, L-arginine is present in the composition in an amount of about 0.1-1 wt%.

[0011] In some embodiments, the second agent may be niacinamide, carnosine, or both. In certain embodiments, the composition may comprise about 1.5-6 wt% niacinamide and/or about 0.05-0.2 wt% carnosine.

[0012] In some embodiments, the third agent may antagonize nucleotide activation of skeletal muscle ryanodine receptors. In certain embodiments, the third agent may be adenosine. By way of non-limiting example, the composition may comprise about 0.01-0.5 wt% adenosine.

[0013] In some embodiments, the third agent may antagonize cellular calcium influx. In certain embodiments, the third agent may be a source of magnesium. In certain embodiments, the source of magnesium may be a magnesium salt. By way of non-limiting example, the magnesium salt may be magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, or magnesium oxide. In certain embodiments, the composition may comprise about 0.1-1 wt%, 0.4-0.8 wt% or 0.4-0.6 wt% of a magnesium source or magnesium ion source.

[0014] In some embodiments, any one of the compositions disclosed above and herein may further comprise an extracellular matrix component. In certain embodiments, the extracellular matrix component may be selected from proteins/glycoproteins, such as proteoglycans and glycosaminoglycans, including, but not limited to, collagen, elastin, fibronectin, hyaluronic acid or lectin, or combinations thereof. In certain embodiments, the extracellular matrix component has an average molecular weight of about 1,000-60,000 daltons, or about 1,000-40,000 daltons, or about 1,000-30,000 daltons, or about 1,000-25,000 daltons, or about 1,000-20,000 daltons, or about 1,000-15,000 daltons, or about 1,000-10,000 daltons.

[0015] In some embodiments, any one of the compositions described above and herein may further comprise a cosmeceutically acceptable vehicle.

[0016] In some embodiments, any one of the compositions described above and herein may be an emulsion. In certain embodiments, the emulsion may be an oil-in-water emulsion.

[0017] In another aspect, the present disclosure provides a composition comprising an adenosine, arginine, carnosine, niacinamide, and a magnesium ion source. Application of the composition to a skin region increases cutaneous blood flow in the skin region compared to cutaneous blood flow in a similar skin region not treated with the composition, as measured by doppler ultrasound.

[0018] In some embodiments, the ratio of arginine to adenosine in the composition may be between about 10:1 and about 100:1. By way of non-limiting example, the ratio of arginine to adenosine is about 25:1.

[0019] In some embodiments, the ratio of arginine to carnosine may be between about 2:1 and about 20:1. By way of non-limiting example, the ratio of arginine to carnosine is about 5:1. In some specific examples, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1 in the composition.

[0020] In some embodiments, the composition may comprise about 0.1-1 wt% arginine, about 0.01-0.06 wt% adenosine, about 0.05-0.2 wt% carnosine, and/or about 0.5-6 wt% niacinamide.

[0021] In some embodiments, the composition described above and herein may further comprise an extracellular matrix component selected from the group consisting of collagen, elastin, hyaluronic acid, and sodium hyaluronate. In certain embodiments, the sodium hyaluronate may be hydrolyzed sodium hyaluronate.

[0022] In some embodiments, the composition may further comprise an antioxidant. Any gallate type or catechin type antioxidant can be used. By way of non-limiting example, in an embodiment, the antioxidant is epigallocatechin gallate (*i.e.*, an antioxidant most commonly derived from green tea).

[0023] In some embodiments, arginine may be L-arginine.

[0024] In some embodiments, a magnesium ion source may be a magnesium salt. In certain embodiments, the magnesium salt may be selected from the group consisting of magnesium gluconate,

magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, and magnesium oxide.

[0025] In some embodiments, the composition described above and herein may further comprise an extra cellular matrix component. In certain embodiments, the extracellular matrix component may be selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid and lectin. In certain embodiments, the extracellular matrix component has an average molecular weight of about 1,000-60,000 daltons, or about 1,000-40,000 daltons, or about 1,000-30,000 daltons, or about 1,000-25,000 daltons, or about 1,000-20,000 daltons, or about 1,000-15,000 daltons, or about 1,000-10,000 daltons.

[0026] In still another aspect, the present disclosure provides a composition comprising arginine, carnosine, an adenosine, a skin firming agent, a skin hydration agent, a skin barrier agent, and an antioxidant.

[0027] In some embodiments, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1 in the composition.

[0028] In some embodiments, the ratio of arginine to adenosine is between about 10:1 and about 100:1 or between about 15:1 and about 50:1 in the composition.

[0029] In some embodiments, the ratio of arginine to carnosine is between about 2:1 and about 20:1 in the composition. In certain embodiments, the ratio of arginine to carnosine is between about 2:1 about 10:1 in the composition.

[0030] In some embodiments, the composition may comprise about 0.1-1 wt% arginine, about 0.01-0.06 wt% adenosine, and/or about 0.05-0.2 wt% carnosine.

[0031] In some embodiments, the skin firming agent comprised in the composition described above and herein may be selected from the group consisting of heptapeptide-7, magnesium gluconate, nicotiana benthamiana hexapeptide-40sh-polypeptide-76, and hydrolyzed eragrostis Tef seed extract.

[0032] In some embodiments, the skin hydration agent may be selected from the group consisting of fruit extract complex, hydrolyzed sodium hyaluronate, niacinamide, and jojoba esters.

[0033] In some embodiments, the skin barrier agent may be selected from the group consisting of jojoba esters, linoleic acid, linolenic acid, and squalane.

[0034] In some embodiments, the antioxidant may be selected from the group consisting of tocopheryl acetate, aminopropyl ascorbyl phosphate, and epigallocatechin gallate.

[0035] In yet another aspect, the present disclosure provides a method for treating skin. Such method comprises providing any one of the compositions described above and herein, and applying or instructing to apply the composition to skin.

[0036] In some embodiments, the composition may be applied to the skin once daily or twice daily. In some embodiments, the composition may be applied to the skin for a period of at least about 2 weeks or at least about 1 month. In some embodiments, the composition is applied to skin on a person's neck, décolleté, face, and/or back of hands.

[0037] In any of the methods described above and herein, applying the composition to skin achieves a beneficial change in the skin. In certain embodiments, the beneficial change may be one or more of the following: a reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, and/or an improved skin tone.

[0038] In any of the methods described above and herein, nitric oxide is produced or generated after application of the composition to skin, as evidenced by one or more of the following: an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region not treated with the composition; an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region treated with a composition identical in all respects except for the absence of one or more of arginine, adenosine, and/or carnosine; and/or an increased cutaneous blood flow in the treated skin region for a period of about 2-48 hours after application to skin.

[0039] In addition to the exemplary aspects and embodiments described above, further aspects and embodiments will become apparent by reference to the drawings and by study of the following descriptions.

[0040] Additional embodiments of the present methods and compositions, and the like, will be apparent from the following description, drawings, examples, and claims. As can be appreciated from the foregoing and following description, each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present disclosure

provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment of the present disclosure. Additional aspects and advantages of the present disclosure are set forth in the following description and claims, particularly when considered in conjunction with the accompanying examples and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0041] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0042] **FIG. 1** is a standard curve used to determine the concentration of test proteins.

[0043] **FIG. 2** illustrates increased nitric oxide availability to *ex vivo* skin tissue treated with an exemplary topical composition, Composition T-1.

[0044] **FIGS. 3A-3B** illustrate evaluation of cutaneous blood flow in the neck skin between baseline (untreated control; **FIG. 3A**) and 2.5 hours following application of the exemplary topical composition T-1 (**FIG. 3B**).

[0045] **FIG. 4** illustrates mean percent improvement in appearance of key measurements of skin (lines/winkles, skin texture, and skin tone) over the course of 8 weeks following application of the exemplary topical composition T-1.

[0046] **FIG. 5** illustrates mean percent improvement in appearance of key measurements of décolleté (lines/winkles, skin texture, skin tone, and photodamage) over the course of 12 weeks following application of the exemplary topical composition T-1 in the morning and evening in combination with application of AlphaRet® Overnight Cream in the evening.

DETAILED DESCRIPTION

[0047] Levels of natural nitric oxide in the body decline as skin ages or is damaged by environmental stressors, the time when skin cells need access to nitric oxide the most. The present disclosure provides a targeted solution that supplements and supports nitric oxide availability to draw from and addresses skin aging and damage. With this target solution, nitric oxide is ready for access

and deployment to skin when needed. That is, nitric oxide is made available on demand as the skin needs it to achieve balance.

[0048] I. Definitions

[0049] Various aspects now will be described more fully hereinafter. Such aspects may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey its scope to those skilled in the art.

[0050] Where a range of values is provided, it is intended that each intervening value between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. For example, if a range of 1 μm to 8 μm is stated, it is intended that 2 μm, 3 μm, 4 μm, 5 μm, 6 μm, and 7 μm are also explicitly disclosed, as well as the range of values greater than or equal to 1 μm and the range of values less than or equal to 8 μm.

[0051] The singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to a "polymer" includes a single polymer as well as two or more of the same or different polymers, reference to an "excipient" includes a single excipient as well as two or more of the same or different excipients, and the like.

[0052] As used herein, "about" will be understood by persons of ordinary skill in the art and will vary to some extent depending upon the context in which it is used. If there are uses of the term which are not clear to persons of ordinary skill in the art given the context in which it is used, "about" will mean up to plus or minus 10% of the particular term.

[0053] The term "active agent" is used herein to refer to a chemical material or compound that induces a desired beneficial effect when administered topically or subcutaneously, and includes agents that are therapeutically and/or prophylactically effective as pharmaceuticals ("pharmacologically active agents"), as well as agents that are cosmeceutically effective ("cosmeceutically active agents"). Also included are derivatives and analogs of those compounds or classes of compounds specifically mentioned that also induce the desired effect. By an "effective" amount of an active agent is meant a nontoxic but sufficient amount of an active agent to provide the desired beneficial effect. More specifically, by a "therapeutically effective," "prophylactically effective," or "cosmeceutically

effective" amount is meant a nontoxic but sufficient amount of a beneficial agent to provide the desired therapeutic, prophylactic, or cosmeceutical effect.

[0054] The term "aging-related skin condition" relates to any skin condition or disorder associated with, caused by, or affected by, intrinsic aging and/or extrinsic aging. Aging-related skin conditions that may be treated using the present methods and formulations include, but are not limited to, wrinkles, age spots, sun damage (particularly UV radiation-induced oxidative stress), blemishes, hyperpigmented skin, age spots, increased skin thickness, loss of skin elasticity and collagen content, dry skin, lentigines, melasmas, as well as scars.

[0055] The terms "buffer" or "buffering agents" refer to materials which when added to a solution, cause the solution to resist changes in pH.

[0056] "Carriers" or "vehicles" as used herein refer to carrier materials suitable for incorporation in a topically or subcutaneously applied composition. Carriers and vehicles useful herein include any such materials known in the art, which are nontoxic and do not interact with other components of the formulation in which it is contained in a deleterious manner.

[0057] The terms "chelator" or "chelating agent" refer to any materials having more than one atom with a lone pair of electrons that are available to bond to a metal ion.

[0058] By "cosmeceutically effective" and “cosmetic effect” is meant a nontoxic agent that when applied to the surface of skin beneficially affects and/or changes the appearance of the skin, e.g., reducing fine lines and wrinkles of the skin, without changing the structure of skin.

[0059] The terms "cosmeceutically active agent" and "cosmeceutically active base" are used interchangeably herein to refer to a cosmeceutically effective basic compound or composition of matter which, when topically administered to a human patient, is effective to treat one or more aging-related skin conditions. Also included are derivatives and analogs of those compounds or classes of compounds that also induce the desired effect, e.g., an application for improving the appearance of an aging-related skin condition.

[0060] The terms "treating" and "treatment" as used herein refer to reduction in severity and/or elimination of skin related conditions resulting from intrinsic and/or extrinsic aging processes of the skin, or other trauma to the skin resulting in, e.g., a scar. The present method of "treating" a skin condition related to aging, as the term is used herein, refers to the prevention of aging-related skin

conditions as well as the treatment of aging-related skin conditions in affected individuals. That is, the present method of "treating" includes improving the appearance of or mitigating the onset of future damage to skin, and/or improving the structure and function of skin. Aging-related skin conditions include, but are not limited to, photodamage, pigmentation, wrinkles, fine lines.

[0061] By "cosmeceutically acceptable," such as in the recitation of a "cosmeceutically acceptable carrier," or a "cosmeceutically acceptable derivative," is meant a compound that is not biologically or otherwise undesirable, i.e., the compound may be incorporated into a cosmeceutical formulation and topically administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the cosmeceutical formulation in which it is contained. The term "pharmaceutically acceptable" is used in an analogous manner, to refer to a compound or composition that may be incorporated into a pharmaceutical formulation herein (i.e., a formulation containing one or more pharmacologically active agents) without causing undesirable biological effects or unwanted interaction with other components of the formulation.

[0062] The term "stable" as in a stable emulsion means that the composition retains its structure as an emulsion. A desired emulsion structure, for example, may be characterized by a desired size range, macroscopic observations of emulsion science (is there one or more layers visible, is there visible precipitate), pH, and a stable concentration of one or more the components.

[0063] The term "subject" as used herein refers to organisms to be treated by the compositions. Such organisms include animals (domesticated animal species, wild animals), and humans.

[0064] The term "surfactant" refers to any molecule having both a polar head group, which energetically prefers solvation by water, and a hydrophobic tail which is not well solvated by water. The term "cationic surfactant" refers to a surfactant with a cationic head group. The term "anionic surfactant" refers to a surfactant with an anionic head group.

[0065] As used herein, the term "topically" refers to application of the compositions to the surface of the skin and tissues.

[0066] The compositions of the present disclosure can comprise, consist essentially of, or consist of, the components disclosed.

[0067] All percentages, parts and ratios are based upon the total weight of the topical compositions and all measurements made are at about 25°C, unless otherwise specified.

[0068] By reserving the right to proviso out or exclude any individual members of any such group, including any sub-ranges or combinations of sub-ranges within the group, that can be claimed according to a range or in any similar manner, less than the full measure of this disclosure can be claimed for any reason. Further, by reserving the right to proviso out or exclude any individual substituents, analogs, compounds, ligands, structures, or groups thereof, or any members of a claimed group, less than the full measure of this disclosure can be claimed for any reason.

[0069] Throughout this disclosure, various patents, patent applications and publications are referenced. The disclosures of these patents, patent applications and publications in their entireties are incorporated into this disclosure by reference in order to more fully describe the state of the art as known to those skilled therein as of the date of this disclosure. This disclosure will govern in the instance that there is any inconsistency between the patents, patent applications and publications cited and this disclosure.

[0070] For convenience, certain terms employed in the specification, examples and claims are collected here. Unless defined otherwise, all technical and scientific terms used in this disclosure have the same meanings as commonly understood by one of ordinary skill in the art to which this disclosure belongs.

[0071] II. Compositions

[0072] Compositions that supplement and support nitric oxide availability to skin (e.g., neck or décolleté skin) are provided herein. The compositions generate nitric oxide *in situ* after topical application to the skin, providing nitric oxide locally in the skin and in response to the local skin environment. The compositions harness the power of the body's natural process of nitric oxide creation to visibly improve aging skin. Upon application of these compositions to skin, one or more of the following effects are achieved: skin looked firmer and more resilient; the appearance of crepiness, laxity and texture improved; and/or skin was intensely hydrated thus the suppleness of the skin improved.

[0073] In one aspect, the present disclosure provides a composition comprising a first agent that is a precursor of nitric oxide, a second agent that facilitates production of nitric oxide by the first agent, and a third agent that antagonizes (i) nucleotide activation of skeletal muscle ryanodine receptors or (ii) cellular calcium influx. The first, second and third agents are mixed to form a composition suitable for topical application to skin. The composition may further comprise stabilizing ingredients.

[0074] The agent that is a precursor of nitric oxide may be arginine, citrulline, nitroglycerin, amyl nitrite, and/or sildenafil. In an embodiment, the nitric oxide precursor is present in the composition in an amount of between about 0.05-2 wt%, 0.075-1.5 wt%, 0.1-1.5 wt%, 0.1-1.25 wt%, 0.1-1 wt%, 0.2-0.8 wt% or 0.3-0.6 wt%. In an embodiment, the first agent is L-arginine, which is a natural ingredient that metabolizes to nitric oxide. It can be processed by the skin and deployed as nitric oxide, on demand and as needed, when delivered to the skin in a composition as detailed herein, where the precursor is stable in the composition (e.g., as evidenced by minimal degradation of the precursor when stored at room temperature for 6 months or 12 months), and where complimentary ingredients in the composition facilitate its penetration through the stratum corneum and into the epidermal and/or dermal layers.

[0075] Nitric oxide is a signaling molecule and functions to dilate blood vessels. The precursor of nitric oxide is instrumental in generation of nitric oxide in the skin, and also facilitates migration of the other ingredients in the composition into the skin, at least in part by dilation of blood vessels. In addition, the precursor of nitric oxide is capable of stimulating the cells to produce proteins, such as collagen and elastin, which facilitates the beneficial change in the skin achieved by the composition.

[0076] The composition, in an embodiment, also comprises an agent that facilitates activation of nitric oxide. Exemplary agents include niacinamide and/or carnosine. For instance, the composition may comprise between about 0.5-6 wt%, 1-6 wt%, 1.5-6 wt%, 1.5-5 wt%, 2-5 wt%, 2-4 wt%, 2.5-4.5 wt%, or 2.5-3.5 wt% niacinamide and/or between about 0.05-0.2 wt%, 0.08-0.2 wt%, 0.09-0.2 wt%, 0.075-0.15 wt%, 0.08-0.15 wt%, 0.09-0.15 wt%, 0.09-0.125 wt% carnosine. Niacinamide (nicotinamide) plays a role in the arginine conversion to nitric oxide *via* NADPH (reduced nicotinamide adenine dinucleotide phosphate). Carnosine facilitates nitric oxide (NO) production *via* endothelial NO synthase (eNOS) activated by released from intracellular Ca^{2+} pathway.

[0077] As used herein, the term "carnosine" includes and encompasses the di-peptides beta-alanyl-histidine and all related compounds such as anserine (beta-alanyl-1-methyl-histidine) and homocarnosine (gamma-amino-butyryl-histidine). As used herein, the term "carnosine" also includes D, L-carnosine, D-carnosine, L-carnosine, as well as salts thereof and modified carnosine.

[0078] The third agent may antagonize nucleotide activation of skeletal muscle ryanodine receptors. An example of the third agent is adenosine. By way of non-limiting example, the composition may

comprise adenosine in any of the ranges mentioned above. Adenosine acts as a competitive antagonist that reversibly inhibited ATP- and AMP-activated ryanodine receptors (RyRs).

[0079] Alternatively, the third agent may antagonize cellular calcium influx. An example of such an agent is a source of magnesium, e.g., a magnesium salt. Magnesium acts as competitive antagonists for the Ca^{2+} influx channel, inhibiting muscle contraction. By way of non-limiting example, the magnesium salt may be magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, or magnesium oxide. In certain embodiments, the composition may comprise about 0.1-1 wt%, 0.2-1 wt%, 0.3-0.9 wt%, 0.4-0.8 wt%, or 0.4-0.6 wt% of a source of magnesium or a source of magnesium ion.

[0080] Any one of the compositions disclosed above and herein may further comprise an extracellular matrix component. The extracellular matrix may be selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid, sodium hyaluronate (e.g., hydrolyzed sodium hyaluronate), and lectin. In certain embodiments, the extracellular matrix component has an average molecular weight of about 1,000-60,000 daltons, or about 1,000-40,000 daltons, or about 1,000-30,000 daltons, or about 1,000-25,000 daltons, or about 1,000-20,000 daltons, or about 1,000-15,000 daltons, or about 1,000-10,000 daltons.

[0081] Any one of the compositions described above and herein may further comprise a cosmeceutically acceptable vehicle. The vehicle or carrier may be incorporated into a cosmeceutical formulation of the present disclosure and topically administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the cosmeceutical formulation in which it is contained.

[0082] Any one of the compositions described above and herein may be an emulsion. In exemplary embodiments, the emulsion may be an oil-in-water emulsion.

[0083] In another aspect, the present disclosure provides a composition comprising an adenosine, arginine (e.g., L-arginine), carnosine, niacinamide, and a magnesium ion source. Application of the composition to a skin region increases cutaneous blood flow in the skin region compared to cutaneous blood flow in a similar skin region not treated with the composition, as measured by doppler ultrasound.

[0084] In some embodiments, the composition may comprise between about 0.1-1 wt% arginine, between about 0.01-0.06 wt% adenosine, between about 0.05-0.2 wt% carnosine, and/or between about 1.5-6 wt% niacinamide. In some embodiments, the ratio of arginine to adenosine in the composition may be between about 10:1 and about 100:1, e.g., the ratio of arginine to adenosine is about 25:1. In some embodiments, the ratio of arginine to carnosine may be between about 2:1 and about 20:1, e.g., the ratio of arginine to carnosine is about 5:1. In exemplary embodiments, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1 in the composition.

[0085] The compositions described above and herein may further comprise an antioxidant, such as epigallocatechin gallate (*i.e.*, green tea antioxidant).

[0086] A magnesium ion source may be a magnesium salt selected from the group consisting of magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, and magnesium oxide. Such magnesium ion source antagonizes cellular calcium influx.

[0087] In still another aspect, the present disclosure provides a composition comprising arginine, carnosine, an adenosine, a skin firming agent, a skin hydration agent, a skin barrier agent, and an antioxidant. In some embodiments, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1 in the composition. In some embodiments, the ratio of arginine to adenosine is between about 10:1 and about 100:1 or between about 15:1 and about 50:1 in the composition. In some embodiments, the ratio of arginine to carnosine is between about 2:1 and about 20:1 in the composition. In certain embodiments, the ratio of arginine to carnosine is between about 2:1 about 10:1 in the composition.

[0088] In some embodiments, the composition may comprise about 0.1-1 wt% arginine, about 0.01-0.06 wt% adenosine, and/or about 0.05-0.2 wt% carnosine. The combination of these agents, along with stabilizing ingredients, facilitates nitric oxide production when applied to skin and support nitric oxide availability to the skin.

[0089] The skin firming agent comprised in the composition disclosed above and herein may be selected from the group consisting of heptapeptide-7, magnesium gluconate, nicotiana benthamiana hexapeptide-40sh-polypeptide-76, and hydrolyzed eragrostis Tef seed extract. heptapeptide-7 is a targeted peptide that supports the extracellular matrix (ECM). Magnesium gluconate is skin-

supporting mineral that helps soften and reduce the appearance of expression lines and wrinkles. Nicotiana benthamiana hexapeptide-40sh-polypeptide-76 is a plant-derived growth factor that helps to smooth the appearance of wrinkles and texture, enhancing the appearance of skin radiance. Hydrolyzed eragrostis Tef seed extract is a superfood extract that helps support collagen, resulting in an improvement in the appearance of neck lines, creating smoother and firmer-looking skin.

[0090] The skin hydration agent comprised in the composition disclosed above and herein may be selected from the group consisting of fruit extract complex, hydrolyzed sodium hyaluronate, niacinamide, and jojoba esters. Fruit extract complex hydrates the skin by supporting key Natural Moisturizing Factor (NMF) components such as sodium lactate, sodium PCA and citrulline. Hydrolyzed sodium hyaluronate helps provide skin with high moisture retention and free-radical defense. Niacinamide is a moisturizing source of Vitamin B3. Jojoba esters provides hydration and supports the skin's barrier. That is, jojoba esters is both a skin hydration agent and a skin barrier agent.

[0091] The skin barrier agent comprised in the composition disclosed above and herein may be selected from the group consisting of jojoba esters, linoleic acid, linolenic acid, and squalane. Linoleic and linolenic acids are essential fatty acids that support the skin's barrier (Omega-6 and Omega-3). Squalane is a naturally occurring emollient in the skin that supports moisture retention to improve skin suppleness and flexibility.

[0092] The antioxidant comprised in the composition disclosed above and herein may be selected from the group consisting of tocopheryl acetate, aminopropyl ascorbyl phosphate, and epigallocatechin gallate (EGCG). Tocopheryl acetate is a form of Vitamin E that provides skin conditioning and antioxidant protection. Aminopropyl ascorbyl phosphate is a form of Vitamin C that supports hyaluronic acid, increases skin smoothness and helps improve the appearance of dark patches. EGCG is a potent antioxidant found in green tea that provides antioxidant support while delivering soothing and calming benefits.

[0093] Any one of the compositions disclosed above and herein can be in the form of any pharmaceutically acceptable dosage form, including but not limited to, liquids, ointments, creams, oils, emulsions, lotions, gels, liquids, bioadhesive gels, sprays, shampoos, aerosols, pastes, foams, sunscreens, capsules, microcapsules, or in the form of an article or carrier, such as a bandage, insert, syringe-like applicator, pessary, powder, talc or other solid, shampoo, cleanser (leave on and wash off product), day creams, night creams, make-up removal creams, foundation creams, make-up removal

formulations, protective or skin care body milks, skin care lotions, gels, or foams (such as cleansing or disinfecting lotions), bath compositions, deodorant compositions, aftershave and pre-shave gels or lotions, and agents that favor penetration within the epidermis, the dermis and keratin layers. The composition is capable of effectively treating, preventing, and/or minimizing the dermatological conditions described herein, without being systemically absorbed and without significantly irritating the skin.

[0094] In an exemplified embodiment, the composition is a topical composition comprising a naturally produced protein building block selected from adenosine, carnosine and arginine, and a stabilizing ingredient. An exemplary topical composition comprises arginine, carnosine, an adenosine, a skin firming agent, a skin hydration agent, a skin barrier agent, and an antioxidant. The exemplary composition achieves delivery of arginine into the skin; arginine is a precursor to nitric oxide and is processed in situ in the skin to generate nitric oxide on demand, i.e., in response to the local in situ need for nitric oxide. This topical composition uniquely supports and delivers arginine topically, where it contributes to a skin depot or an in situ reservoir of arginine. From the deport, skin enzymes access and deploy arginine to convert to nitric oxide on demand and as needed in the local environment.

[0095] Thus, the topical composition described herein supports the skin's natural process of nitric oxide creation and is supplemented with purposeful and targeted ingredients to address the specific needs of skin, and more particularly, the specific needs of skin in specific body areas, *e.g.*, the skin of the neck. The topical composition is enhanced, optionally, with extracts, antioxidant systems and skin-supporting ingredients. In an embodiment, the composition comprises a firming complex that supports collagen to leave skin looking and feeling firmer and tighter and/or a skin barrier agent that supports the skin barrier to deliver supple, more youthful-looking skin.

[0096] In some embodiments, water is present in the composition in a range from about 1% to about 95%, or from about 10% to about 80%, or from about 25% to about 75%, or from about 50% to about 65%, or any suitable combination, sub-combination, range, or sub-range thereof by weight, based on the weight of the cleansing composition. In some embodiments, water is present in the composition in an amount from at least about 10%, or 25%, or 50%, or 75% by weight, based on the weight of the composition. One of ordinary skill in the art, however, will appreciate that other ranges are within the scope of the invention. Thus, water is present, by

weight, based on the total weight of the cleansing composition, from about 1, 2, 3, 4, 5, 6,7, 8,9,10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 to about 95 weight percent, including increments and ranges therein and there between.

[0097] Generally, in various embodiments, the composition includes any one or more of the herein described ingredients, as well as any optional ingredients, present within the ranges as stated herein, and more broadly within a range from about 0.001% to about 95%, by weight, or any suitable combination, sub-combination, range, or sub-range thereof by weight, based on the total weight of the composition.

[0098] Thus, any one or a combination of actives and additives may be present, by weight, based on the total weight of the cleansing composition, each one or the combination present from about 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 to about 20 weight percent, including increments and ranges therein and there between.

[0099] In a representative embodiment, the composition includes Arginine, Adenosine, Carnosine, magnesium salt including magnesium gluconate, Heptapeptide-7, Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-76, Citrullus Lanatus (Watermelon) Fruit Extract, Lens Esculenta (Lentil) Fruit Extract, Epigallocatechin Gallate, Pyrus Malus (Apple) Fruit Extract, Hydrolyzed Sodium Hyaluronate, Niacinamide, Linoleic and Linolenic Acids, Squalane, Aminopropyl Ascorbyl Phosphate, Epigallocatechin Gallate (EGCG) and water. According to the representative embodiment, the ingredients may be present in the composition in the following amounts or ranges: Arginine (0.5%), Adenosine (0.02%), Carnosine (0.10%), magnesium salt including magnesium gluconate (0.50%), Heptapeptide-7 (0.0125%), Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-76 (0.000002%), Citrullus Lanatus (Watermelon) Fruit Extract (0.4%), Lens Esculenta (Lentil) Fruit Extract (0.3%), Epigallocatechin Gallate (0.01%), Pyrus Malus (Apple) Fruit Extract (0.2%), Hydrolyzed Sodium Hyaluronate (0.25%, or in a range from about 0.05% to about 1%), Niacinamide (3%), Linoleic and Linolenic Acids (~1%), Squalane (0.15%), Aminopropyl Ascorbyl Phosphate (0.5%), Epigallocatechin Gallate (EGCG) (0.01%), and water (~55-65%), wherein each of one or more of Ethylhexyl Olivate, Jojoba Esters, Glycerin, Cetearyl Olivate, Isocetyl Stearoyl Stearate, Sodium Lactate, Sorbitan

Olivate, Coco-Caprylate/Caprate, Polyglycerin-6, Cetearyl Alcohol, Butylene Glycol, Magnesium Gluconate, Hydrolyzed Eragrostis Tef Seed Extract, Lens Esculenta (Lentil) Fruit Extract, Tocopheryl Acetate, Sodium PCA, Tremella Fuciformis (Mushroom) Extract, Tocopherol, Cetyl Palmitate, Sorbitan Palmitate, Cetyl Alcohol, Pentaerythrityl Tetra-di-t-butyl Hydroxyhydrocinnamate, Sodium Stearoyl Glutamate, Propanediol, Silica, C9-12 Alkane, Pullulan, Xanthan Gum, Triheptanoin, Sclerotium Gum, Sorbitan Oleate, Ethylhexylglycerin, Ammonium Polyacryloyldimethyl Taurate, Myristyl Alcohol, Stearyl Alcohol, Calcium Gluconate, Polyurethane-100, Lecithin, Gluconolactone, Phenoxyethanol, Sodium Benzoate, Phytic Acid, Potassium Sorbate, Citric Acid, and Sodium Hydroxide are each present in a range from about 0.001% to about 95%, and more particularly from about 0.001% to about 5%, or from about 0.1% to about 3%, by weight, or any suitable combination, sub-combination, range, or sub-range thereof by weight, based on the total weight of the composition.

[00100] Thus, any one or a combination of actives and additives may be present, by weight, based on the total weight of the cleansing composition, each one or the combination present from about 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70, 0.80, 0.90, 1.0, 2, 3, 4, to about 5 weight percent, including increments and ranges therein and there between. Of course, it will be appreciated that in other formulations and embodiments, the composition may include any one of the foregoing in a range as otherwise described herein.

[00101] According to the representative embodiment, the composition may also include one or more of each of : an emulsifier, a surfactant, a preservative, a gelling agent, a thickening agent, a skin firming agent, a skin hydration agent, a skin barrier agent, an antioxidant, and combinations thereof.

[00102] III. Methods of Use

[00103] Methods for treating, preventing, and/or minimizing wrinkles, signs of aging skin, and/or skin imperfections are provided herein. Examples of signs of aging skin and/or skin imperfections which can be treated, prevented, and/or minimized with the compositions described herein, and the methods of use of the compositions, include, but are not limited to, (1) fine to moderate wrinkles, (2) liver spots or age spots (lentigines or solar lentigines), (3) uneven skin tone and/or texture, (4) sun-damaged skin or photodamaged skin (particularly UV

radiation-induced oxidative stress), (5) blemishes, (6) hyperpigmented skin, (7) increased skin thickness, (8) dry skin, (9) loss of skin elasticity and collagen content, (10) melasmas (atypical pigmentation or hyper-pigmentation of the skin), (11) reduced skin clarity and/or radiance, (12) reduced skin smoothness and/or softness, (13) enlarged pore size (e.g., larger pore can make an individual appear older), (14) reduced hydration, (15) reduced skin tightness, and any combination thereof. Collectively the signs of aging skin, skin imperfections and scars are referred to as "dermatological conditions."

[00104] In an exemplary embodiment, a method for treating, reducing and/or minimizing dermatological conditions in a region of skin comprises applying a composition as described above and herein to the region of skin. In some embodiments, the composition is applied topically, which is a non-invasive administration technique. The composition can be applied to any skin region of a subject. In one embodiment, the composition is applied to the facial tissue of a subject. In another embodiment, the composition is applied to the neck tissue of a subject. It has been surprisingly found that the compositions of the present disclosure can be used to substantially treat, minimize, and/or diminish the dermatological conditions described above.

[00105] A method of treating skin is provided herein, which comprises providing any one of the compositions described above and herein, and applying or instructing to apply the composition to skin. In some embodiments, the composition may be applied to the skin once daily or twice daily. In some embodiments, the composition may be applied to the skin for a period of at least about 2 weeks or at least about 1 month. In some embodiments, the composition is applied to skin on a person's neck, décolleté, face, and/or back of hands. In some embodiments, the method of treating is a method of cosmetically treating. In some embodiments, the method of treating is a method of therapeutically (pharmaceutically) treating.

[00106] In any of the methods described above and herein, applying the composition to skin achieves a beneficial change in the skin. In certain embodiments, the beneficial change may be one or more of the following: a reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, and/or an improved skin tone.

[00107] In any of the methods described above and herein, nitric oxide is produced or generated after application of the composition to skin, as evidenced by one or more of the following: an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region not treated with the composition; an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region treated with a composition identical in all respects except for the absence of one or more of arginine, adenosine, and/or carnosine; and/or an increased cutaneous blood flow in the treated skin region for a period of between about 2-48 hours, 2-36 hours, 2-24 hours, 2-12 hours or 2-8 hours after application to skin.

[00108] IV. Examples

[00109] The following examples are illustrative in nature and are in no way intended to be limiting.

[00110] EXAMPLE 1

[00111] Exemplary Composition T-1

[00112] A topical composition, referred to herein as Composition T-1, in the form of an emulsion was prepared by creating an oil phase and a water phase, each with certain of the ingredients listed below. The oil phase was added to the water phase with stirring to create an oil in water emulsion.

[00113] Composition T-1 contained between about 0.1-1 wt% arginine, about 0.01-0.06 wt% adenosine, about 0.05-0.2 wt% carnosine, about 0.5-6 wt% niacinamide, and about 0.4-0.6 wt% of a magnesium ion source.

[00114] Composition T-1 also contained other ingredients, including, but not limited to, an emulsifier, a surfactant, a preservative, a gelling agent, a thickening agent, a skin firming agent, a skin hydration agent, a skin barrier agent, and an antioxidant. The other ingredients included one or more of the following: Ethylhexyl Olivate, Jojoba Esters, Glycerin, Cetearyl Olivate, Isocetyl Stearoyl Stearate, Sodium Lactate, Sorbitan Olivate, Coco-Caprylate/Caprate, Polyglycerin-6, Cetearyl Alcohol, Butylene Glycol, Cetyl Alcohol, Triheptanoin, Phenoxyethanol, Cetyl Palmitate, Linoleic Acid, Propanediol, Sorbitan Palmitate, Aminopropyl Ascorbyl Phosphate, Xanthan Gum, Ammonium Polyacryloyldimethyl Taurate, C9-12 Alkane, Citrullus Lanatus (Watermelon) Fruit Extract, Polyurethane-100, Pentaerythrityl Tetra-di-t-butyl Hydroxyhydrocinnamate, Sodium Stearoyl Glutamate, Lens Esculenta (Lentil) Fruit

Extract, Hydrolyzed Eragrostis Tef Seed Extract, Hydrolyzed Sodium Hyaluronate, Tocopheryl Acetate, Linolenic Acid, Lecithin, Sclerotium Gum, Pyrus Malus (Apple) Fruit Extract, Sorbitan Oleate, Squalane, Pullulan, Ethylhexylglycerin, Gluconolactone, Tremella Fuciformis (Mushroom) Extract, Sodium Benzoate, Phytic Acid, Silica, Potassium Sorbate, Sodium PCA, Citric Acid, Heptapeptide-7, Tocopherol, Epigallocatechin Gallate, Myristyl Alcohol, Stearyl Alcohol, Calcium Gluconate, Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-76, and Sodium Hydroxide.

[00115] EXAMPLE 2

[00116] Protein Analysis of *ex vivo* Skin Tissues and Nitric Oxide Availability

[00117] A study was conducted utilizing *ex vivo* skin tissues in order to investigate influence of the compositions of the present disclosure on protein expression or activity in tissues treated topically with the composition. The *ex vivo* tissues maintained a normal skin barrier function, a mature stratum corneum, a functional basal layer, all cell types and skin appendages of *in vivo* human skin.

[00118] *Ex* vivo skin tissues were obtained from donor and used for treatment and protein expression analysis within 72 hours of skin collection. Tissues were equilibrated in an incubator at 37°C, 5% CO_2, and ~95% humidity for two hours before applying the treatments. The equilibration medium was removed and replaced with 1.0 mL of maintenance media.

[00119] Following equilibration, 15 µL of the test topical composition of Example 1 (Composition T-1) was applied to the topical surface of each culture; a sterile glass spreader was used to distribute the composition across the surface. Each culture was visually inspected to ensure the even distribution of topical treatment. For the control group, 0.9% saline was used. Four tissue replicates (n = 4) were included in each of the testing group ("Composition T-1") and control group ("Saline").

[00120] Following application of the test topical composition, the cultures were returned to 37°C with 5% CO_2 for 24 hours. After the end of first day, treatment was removed using a cotton-swab soaked in phosphate buffered saline (PBS) and fresh composition was reapplied and the cultures were returned to 37°C with 5% CO_2 for additional 24 hours.

[00121] After 48 hours of exposure, the topical composition was removed using a cotton-swab soaked in PBS. An 8 mm punch was taken of each sample to punch out the part of skin biopsy inside the silicon ring. Each skin biopsy was then cut in half, placed into tubes and snap frozen in liquid nitrogen to preserve the native state of enzymes and protein.

[00122] A standard assay was then performed to determine nitric oxide availability, which measured changes in target protein concentration using Enzyme-Linked Immunosorbent Assays (ELISAs) (*e.g.*, Nitrate/Nitrite Colorimetric Assay (Cayman; Cat# 780001)). A standard curve used to determine the concentration of test proteins is shown in **FIG. 1**. Statistical analysis was performed using a Student's t-test (unpaired t-test). Test groups with $p \leq 0.05$ were considered statistically significant.

[00123] The results show that more nitric oxide was present in *ex vivo* skin tissues that were treated with the test topical composition than in those that were treated with saline (*see*, **FIG. 2**). In fact, the availability of nitric oxide was increased by more than 200%. This suggests that test topical composition significantly increased nitric oxide availability when applied to *ex vivo* skin tissues.

[00124] EXAMPLE 3

[00125] Evidence of Nitric Oxide Availability

[00126] Doppler ultrasound measurements were obtained to evaluate and visualize the difference in cutaneous blood flow in the neck between baseline (untreated control) and 2.5 hours following a single, thin layer application of the test topical composition (Composition T-1). Testing was conducted using LOGIQ E10 Ultrasound Series (GE Healthcare).

[00127] The results show an increased cutaneous blood flow in the neck skin treated with the test topical composition (*see*, **FIG. 3B**) compared to cutaneous blood flow in a similar skin region not treated with the composition (*see*, **FIG. 3A**).

[00128] Since youthful-acting skin cells require nitric oxide to open up nutrient and oxygen channels in the skin, the flow of nutrients and oxygen to skin cells is connected to dermal health and the body's natural healing process. The increased cutaneous blood flow in the neck skin treated with the test topical composition demonstrates the increased availability of nitric oxide in the treated neck skin.

[00129] EXAMPLE 4

[00130] Efficacy and Tolerability Study

[00131] The efficacy and tolerability of twice-daily application of the test topical composition (Composition T-1) were evaluated in female subjects (n = 26) of varying skin tones with mild-to-moderate lines/wrinkles on the neck. The evaluation was done in a clinical trial conducted by two Board-Certified dermatologists over the course of 12 weeks.

[00132] The subjects reported high levels of tolerability on the neck throughout the duration of the study. The results show early, statistically significant mean percent improvement in appearance of key measurements (e.g., lines/wrinkles, skin texture, and skin tone) at all timepoints (*see*, **FIG. 4**).

[00133] Exceptional subject satisfaction was reported after 8 weeks of use. 96% of subjects agreed that the skin on their neck looked firmer and tighter, and less crepey and saggy. They also felt their neck skin was more hydrated.

[00134] The results demonstrate that the test topical composition is effective in treating aging skin and improving appearance of the skin.

[00135] EXAMPLE 5

[00136] Clinical Study of Combination Treatment

[00137] The efficacy and tolerability of twice-daily application of the test topical composition (Composition T-1) and nightly (PM) application of a retinoid and alpha hydroxy composition, AlphaRet® Overnight Cream, were evaluated in subjects (n = 10) with mild-to-moderate lines/wrinkles on the neck and photodamage on the décolleté. The evaluation was done in a clinical trial conducted by two Board-Certified dermatologists over the course of 12 weeks. AlphaRet® Overnight Cream is a commercially available composition that includes a retinoid combined with an alpha hydroxy acid (AHA) to provide visible skin rejuvenation with little-to-no irritation. In one example, AlphaRet may include Aqua/Water, Glycolic Acid, Sodium Glycolate, Cetearyl Olivate, Pentylene Glycol, Cyclopentasiloxane, Isohexadecane, Sorbitan Olivate, Tetrahexyldecyl Ascorbate, Glycerin, Ceresin, Glycol Palmitate, Butylene Glycol, Niacinamide, Squalane, Methyl Gluceth-20, Ethyl Lactyl Retinoate, Palmitoyl Tripeptide-1, Palmitoyl Tetrapeptide-7, Ceramide NP, Hyaluronic Acid, Ubiquinone, Tocopherol, Bisabolol,

Tocopheryl Acetate, Superoxide Dismutase, Butyrospermum Parkii (Shea) Butter, Allantoin Glycyrrhetinic Acid, Sodium PCA, Panthenol, Camellia Sinensis Leaf Extract, Portulaca Oleracea Extract, Linoleic Acid, Cholesterol, Linolenic Acid, Magnesium Aluminum Silicate, Dimethyl Isosorbide, Dimethicone, Glyceryl Stearate, PEG-100 Stearate, Steareth-2, Hydroxyethylcellulose, Acacia Senegal Gum, Xanthan Gum, Hydrated Silica, Carbomer, Polysorbate 20, Tromethamine, Disodium EDTA, Ethylhexylglycerin, Phenoxyethanol, Titanium Dioxide.

[00138] The subjects reported high levels of tolerability of the combination of Composition T-1 and on the neck and décolleté throughout the duration of the study. The results show statistically significant mean percent improvement in appearance of key measurements (lines/wrinkles, skin texture, skin tone, and photodamage) at all timepoints (*see*, **FIG. 5**).

[00139] Exceptional subject satisfaction was reported after 8 weeks of the combination use. 100% of subjects agreed that the skin on their neck looked smoother, brighter, and more even, and overall looked better. 100% of subjects agreed that the skin on their neck and décolleté looked firmer, tighter and brighter, and less crepey and saggy. They also felt their neck and décolleté skin was more hydrated.

[00140] EXAMPLE 6

[00141] Exemplary Composition

[00142] An exemplary topical composition comprising the following ingredients was prepared: Aqua/Water, Niacinamide, Ethylhexyl Olivate, Jojoba Esters, Glycerin, Cetearyl Olivate, Isocetyl Stearoyl Stearate, Sodium Lactate, Sorbitan Olivate, Coco-Caprylate/Caprate, Polyglycerin-6, Cetearyl Alcohol, Butylene Glycol, Magnesium Gluconate, Arginine, Heptapeptide-7, Nicotiana Benthamiana Hexapeptide-40 sh-Polypeptide-76, Hydrolyzed Eragrostis Tef Seed Extract, Adenosine, Carnosine, Aminopropyl Ascorbyl Phosphate, Citrullus Lanatus (Watermelon) Fruit Extract, Lens Esculenta (Lentil) Fruit Extract, Epigallocatechin Gallate, Pyrus Malus (Apple) Fruit Extract, Hydrolyzed Sodium Hyaluronate, Linoleic Acid, Tocopheryl Acetate, Linolenic Acid, Squalane, Sodium PCA, Tremella Fuciformis (Mushroom) Extract, Tocopherol, Cetyl Palmitate, Sorbitan Palmitate, Cetyl Alcohol, Pentaerythrityl Tetra-di-t-butyl Hydroxyhydrocinnamate, Sodium Stearoyl Glutamate, Propanediol, Silica, C9-12 Alkane, Pullulan, Xanthan Gum, Triheptanoin, Sclerotium Gum, Sorbitan Oleate,

Ethylhexylglycerin, Ammonium Polyacryloyldimethyl Taurate, Myristyl Alcohol, Stearyl Alcohol, Calcium Gluconate, Polyurethane-100, Lecithin, Gluconolactone, Phenoxyethanol, Sodium Benzoate, Phytic Acid, Potassium Sorbate, Citric Acid, and Sodium Hydroxide.

[00143] In various embodiments, the disclosure provides:

[00144] A composition, comprising:

an adenosine,

arginine,

carnosine,

niacinamide, and

a magnesium ion source,

wherein application of the composition to a skin region increases cutaneous blood flow in the skin region compared to cutaneous blood flow in a similar skin region not treated with the composition, as measured by doppler ultrasound.

[00145] In some embodiments, the ratio of arginine to adenosine is between about 10:1 and about 100:1, or wherein the ratio of arginine to adenosine is about 25:1.

[00146] In some embodiments, the ratio of arginine to carnosine is between about 2:1 and about 20:1 or wherein the ratio of arginine to carnosine is about 5:1.

[00147] In some embodiments, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1.

[00148] In some embodiments, the composition comprises about 0.1-1 wt% arginine.

[00149] In some embodiments, the composition comprises about 0.01-0.06 wt% adenosine.

[00150] In some embodiments, the composition comprises about 0.05-0.2 wt% carnosine.

[00151] In some embodiments, the composition comprises about 0.5-6 wt% niacinamide.

[00152] In some embodiments, epigallocatechin gallate (green tea antioxidant).

[00153] In some embodiments, arginine is L-arginine.

[00154] In some embodiments, a magnesium ion source is a magnesium salt.

[00155] In some embodiments, magnesium salt is selected from the group consisting of magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, and magnesium oxide.

[00156] In some embodiments, the composition comprises an extracellular matrix component.

[00157] In some embodiments, the extracellular matrix component is selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid, sodium hyaluronate, and lectin.

[00158] In some embodiments, the extracellular matrix component has an average molecular weight of about 1,000-60,000 daltons, or about 1,000-40,000 daltons, or about 1,000-30,000 daltons, or about 1,000-25,000 daltons, or about 1,000-20,000 daltons, or about 1,000-15,000 daltons, or about 1,000-10,000 daltons.

[00159] A composition, comprising:

a first agent that is a precursor of nitric oxide;

a second agent that facilitates production of nitric oxide by the first agent;

a third agent that antagonizes (i) nucleotide activation of skeletal muscle ryanodine receptors or (ii) cellular calcium influx; and

wherein the first agent, the second agent, and the third agent are mixed to form a composition suitable for topical application to skin.

[00160] In some embodiments, the first agent is selected from the group consisting of arginine, citrulline, nitroglycerin, amyl nitrite, and sildenafil.

[00161] In some embodiments, the first agent is L-arginine.

[00162] In some embodiments, L-arginine is in an amount of about 0.1-1 wt% in the composition.

[00163] In some embodiments, the second agent is niacinamide, carnosine, or both.

[00164] In some embodiments, the composition comprises about 1.5-6 wt% niacinamide and/or about 0.05-0.2 wt% carnosine.

[00165] In some embodiments, the third agent antagonizes nucleotide activation of skeletal muscle ryanodine receptors and is an adenosine.

[00166] In some embodiments, the composition comprises about 0.01-0.5 wt% adenosine.

[00167] In some embodiments, the third agent antagonizes cellular calcium influx and is a source of magnesium.

[00168] In some embodiments, the source of magnesium is a magnesium salt.

[00169] In some embodiments, magnesium salt is selected from the group consisting of magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, and magnesium oxide.

[00170] In some embodiments, the composition comprises about 0.1-1 wt% magnesium.

[00171] In some embodiments, the composition comprises an extracellular matrix component.

[00172] In some embodiments, the extracellular matrix component is selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid and lectin.

[00173] In some embodiments, the extracellular matrix component has an average molecular weight of about 1,000-60,000 daltons, or about 1,000-40,000 daltons, or about 1,000-30,000 daltons, or about 1,000-25,000 daltons, or about 1,000-20,000 daltons, or about 1,000-15,000 daltons, or about 1,000-10,000 daltons.

[00174] In some embodiments, the composition comprises a cosmeceutically acceptable vehicle.

[00175] In some embodiments, the composition is an emulsion.

[00176] In some embodiments, the emulsion is an oil-in-water emulsion.

[00177] A topical composition, comprising:

arginine;

carnosine;

an adenosine;

a skin firming agent;

a skin hydration agent;

a skin barrier agent; and

an antioxidant.

[00178] In some embodiments, the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1.

[00179] In some embodiments, the ratio of arginine to adenosine is between about 10:1 and about 100:1 or between about 15:1 and about 50:1.

[00180] In some embodiments, the ratio of arginine to carnosine is between about 2:1 and 20:1 or between about 2:1 and about 10:1.

[00181] In some embodiments, the composition comprises about 0.1-1 wt% arginine.

[00182] In some embodiments, the composition comprises about 0.01-0.06 wt% adenosine.

[00183] In some embodiments, the composition comprises about 0.05-0.2 wt% carnosine.

[00184] In some embodiments, the skin firming agent is selected from the group consisting of heptapeptide-7, magnesium gluconate, nicotiana benthamiana hexapeptide-40sh-polypeptide-76, and hydrolyzed eragrostis Tef seed extract.

[00185] In some embodiments, the skin hydration agent is selected from the group consisting of fruit extract complex, hydrolyzed sodium hyaluronate, niacinamide, and jojoba esters.

[00186] In some embodiments, the skin barrier agent is selected from the group consisting of jojoba esters, linoleic acid, linolenic acid, and squalane.

[00187] In some embodiments, the antioxidant is selected from the group consisting of tocopheryl acetate, aminopropyl ascorbyl phosphate, and epigallocatechin gallate.

[00188] A method for treating skin, comprising:

providing a composition according to any one of the foregoing embodiments; and

applying or instructing to apply the composition to skin.

[00189] In some embodiments, applying or instructing to apply comprises applying once daily or instructing to apply once daily.

[00190] In some embodiments, applying or instructing to apply comprises applying twice daily or instructing to apply twice daily.

[00191] In some embodiments, applying or instructing to apply is for a period of at least about 2 weeks or at least about 1 month.

[00192] In some embodiments, applying or instructing to apply is to skin on a person's neck, décolleté, face, and/or back of hands.

[00193] In some embodiments, applying achieves a beneficial change in skin to which the composition was applied.

[00194] In some embodiments, applying achieves or wherein the beneficial change is one or more of the following: a reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, and/or an improved skin tone.

[00195] In some embodiments, nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region not treated with the composition.

[00196] In some embodiments, nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region treated with a composition identical in all respects except for the absence of one or more of arginine, adenosine, and/or carnosine.

[00197] In some embodiments, nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the treated skin region for a period of about 2 - 48 hours after application to skin.

[00198] The articles "a" and "an," as used herein, mean one or more when applied to any feature in embodiments of the present invention described in the specification and claims. The use of "a" and "an" does not limit the meaning to a single feature unless such a limit is specifically stated. The article "the" preceding singular or plural nouns or noun phrases denotes a particular specified feature or particular specified features and may have a singular or plural connotation depending upon the context in which it is used. The adjective "any" means one, some, or all indiscriminately of whatever quantity.

[00199] "One or more," as used herein, means at least one, and thus includes individual components as well as mixtures/combinations.

[00200] The transitional terms "comprising," "consisting essentially of," and "consisting of," when used in the appended claims, in original and amended form, define the claim scope with respect to what unrecited additional claim elements or steps, if any, are excluded from the scope of the claim(s). The term "comprising" is intended to be inclusive or open-ended and does not exclude any additional, unrecited element, method, step or material. The term "consisting of" excludes any element, step or material other than those specified in the claim and, in the latter instance, impurities ordinary associated with the specified material(s). The term "consisting essentially of" limits the scope of a claim to the specified elements, steps or material(s) and those that do not materially affect the basic and novel characteristic(s) of the claimed invention. All materials and methods described herein that embody the present invention can, in alternate embodiments, be more specifically defined by any of the transitional terms "comprising," "consisting essentially of," and "consisting of."

[00201] In some embodiments, the inventive composition may include one or more additional optional ingredients, for example, selected from but not limited to, silicones, including but not limited to silicone polymers, silicon elastomers, and dimethicone and other silicone oils, sulfates including but not limited to sulfate based surfactants, alcohols, polyols, UV filters, oils, chelating agents, antimicrobial agents, neutralizing/pH-adjusting agents, vitamins, fragrances, pearlescent agents, odor absorbers, coloring materials, essential oils, fruit extracts, and combinations thereof.

[00202] In some embodiments, the inventive composition may exclude, be free from or devoid of one or more ingredients, including ingredients as described herein and other ingredients, for example, selected from but not limited to, silicones, including but not limited to silicone polymers, silicon elastomers, and dimethicone and other silicone oils, sulfates including but not limited to sulfate based surfactants, alcohols, polyols, UV filters, oils, chelating agents, antimicrobial agents, neutralizing/pH-adjusting agents, vitamins, fragrances, pearlescent agents, odor absorbers, coloring materials, essential oils, fruit extracts, and combinations thereof. In some particular embodiments "free" means that the composition is free, essentially free or devoid specifically of an excluded ingredient. Those of skill in the art will appreciate that an excluded ingredient may be present in a composition via its presence in

one or more of the formulation components but is otherwise not deliberately added to the composition; thus, in some embodiments a composition may be “essentially -free” wherein an excluded ingredient is present at a concentration that does not exceed 5% by weight, and in some instances is present not more than 3% by weight, and in some instances is present not more than 1% by weight, based on the weight of the composition.

[00203] Other than in the operating examples, or where otherwise indicated, all numbers expressing quantities of ingredients and/or reaction conditions may modified in all instances by the term "about," meaning within 10% of the indicated number (e.g. “about 10%” means 9% – 11% and “about 2%” means 1.8% - 2.2%).

[00204] All percentages and ratios are calculated by weight unless otherwise indicated. All percentages are calculated based on the total composition unless otherwise indicated. Generally, unless otherwise expressly stated herein, “weight” or “amount” as used herein with respect to the percent amount of an ingredient refers to the amount of the raw material comprising the ingredient, wherein the raw material may be described herein to comprise less than and up to 100% activity of the ingredient. Therefore, weight percent of an active in a composition is represented as the amount of raw material containing the active that is used, and may or may not reflect the final percentage of the active, wherein the final percentage of the active is dependent on the weight percent of active in the raw material.

[00205] All ranges and amounts given herein are intended to include subranges and amounts using any disclosed point as an end point. Thus, a range of “1% to 10%, such as 2% to 8%, such as 3% to 5%,” is intended to encompass ranges of “1% to 8%,” “1% to 5%,” “2% to 10%,” and so on. All numbers, amounts, ranges, etc., are intended to be modified by the term “about,” whether or not so expressly stated. Similarly, a range given of “about 1% to 10%” is intended to have the term “about” modifying both the 1% and the 10% endpoints. Further, it is understood that when an amount of a component is given, it is intended to signify the amount of the active material unless otherwise specifically stated.

[00206] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, unless otherwise indicated the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard

deviation found in their respective testing measurements. The example that follows serves to illustrate embodiments of the present disclosure without, however, being limiting in nature.

[00207] While a number of exemplary aspects and embodiments have been discussed above, those of skill in the art will recognize certain modifications, permutations, additions and sub-combinations thereof, and it will be understood by those skilled in the art that various changes may be made, and equivalents may be substituted for elements thereof without departing from the scope of the invention. In addition, many modifications may be made to adapt a particular situation or material to the teachings of the invention without departing from the essential scope thereof. It is therefore intended that the invention is not limited to the particular embodiments disclosed herein and that the following appended claims and claims hereafter introduced are interpreted to include all such modifications, permutations, additions and sub-combinations as are within their true spirit and scope.

IT IS CLAIMED:

1. A composition, comprising:

an adenosine,

arginine,

carnosine,

niacinamide, and

a magnesium ion source.

2. The composition of claim 1, wherein the composition is further characterized by one or more features selected from: application of the composition to a skin region increases cutaneous blood flow in the skin region compared to cutaneous blood flow in a similar skin region not treated with the composition, as measured by doppler ultrasound; a ratio of arginine to adenosine is between about 10:1 and about 100:1, a ratio of arginine to adenosine is about 25:1; a ratio of arginine to carnosine is between about 2:1 and about 20:1; a ratio of arginine to carnosine is about 5:1; a ratio of arginine to carnosine is about 5:1 and a ratio of arginine to adenosine is about 25:1; or combinations thereof.

3. The composition of claim 1, wherein the composition is further characterized by one or more features selected from: the composition comprises about 0.1-1 wt% arginine; the composition comprises about 0.01-0.06 wt% adenosine; the composition comprises about 0.05-0.2 wt% carnosine; the composition comprises about 0.5-6 wt% niacinamide; or combinations thereof.

4. The composition of claim 1, wherein the composition is further characterized by one or more features selected from: the composition comprises epigallocatechin gallate (green tea antioxidant); arginine is L-arginine; the magnesium ion source is a magnesium salt selected from the group consisting of magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, magnesium oxide, or combinations thereof; or combinations thereof.

5. The composition of claim 1, comprising an extracellular matrix component selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid, sodium hyaluronate, and lectin, and wherein the extracellular matrix component has an average molecular weight selected from the group consisting of about 1,000-60,000 daltons, about 1,000-40,000 daltons, about 1,000-30,000 daltons, about 1,000-25,000 daltons, about 1,000-20,000 daltons, about 1,000-15,000 daltons, about 1,000-10,000 daltons, or a combination thereof.

6. A composition, comprising:

a first agent that is a precursor of nitric oxide;

a second agent that facilitates production of nitric oxide by the first agent;

a third agent that antagonizes (i) nucleotide activation of skeletal muscle ryanodine receptors or (ii) cellular calcium influx; and

wherein the first agent, the second agent, and the third agent are mixed to form a composition suitable for topical application to skin.

7. The composition of claim 6, wherein the first agent is selected from the group consisting of arginine, citrulline, nitroglycerin, amyl nitrite, sildenafil, and combinations thereof.

8. The composition of claim 6, wherein the first agent is L-arginine, present in an amount of about 0.1-1 wt% in the composition.

9. The composition of any one of claims 6, wherein the composition is further characterized by one or more features selected from: the second agent is niacinamide, carnosine, or both, and, when present, the composition comprises about 1.5-6 wt% niacinamide and about 0.05-0.2 wt% carnosine; the third agent antagonizes nucleotide activation of skeletal muscle ryanodine receptors and is an adenosine, present from about 0.01-0.5 wt% of the composition; the third agent antagonizes cellular calcium influx and is a source of magnesium selected from the group consisting of magnesium gluconate, magnesium glycinate, magnesium citrate, magnesium carbonate, magnesium malate, magnesium taurate, magnesium hydroxide, magnesium sulfate, magnesium hydroxide, magnesium oxide, and combinations thereof; the third agent is a source of magnesium present from about 0.1-1 wt% of the composition; the composition comprises an

extracellular matrix component selected from the group consisting of collagen, elastin, fibronectin, hyaluronic acid and lectin, wherein the extracellular matrix component has an average molecular weight and wherein the extracellular matrix component has an average molecular weight selected from the group consisting of about 1,000-60,000 daltons, about 1,000-40,000 daltons, about 1,000-30,000 daltons, about 1,000-25,000 daltons, about 1,000-20,000 daltons, about 1,000-15,000 daltons, about 1,000-10,000 daltons, or a combination thereof; the composition comprises a cosmeceutically acceptable vehicle; the composition is an emulsion; the composition is is an oil-in-water emulsion; or combinations thereof.

10. A topical composition, comprising:

arginine;

carnosine;

an adenosine;

a skin firming agent;

a skin hydration agent;

a skin barrier agent; and

an antioxidant.

11. The composition of claim 10, wherein the composition is further characterized by one or more features selected from: the ratio of arginine to carnosine is about 5:1 and the ratio of arginine to adenosine is about 25:1; the ratio of arginine to adenosine is between about 10:1 and about 100:1; the ratio of arginine to adenosine is between about 15:1 and about 50:1; the ratio of arginine to carnosine is between about 2:1 and 20:1; the ratio of arginine to adenosine is between about 2:1 and about 10:1; or combinations thereof.

12. The composition of claim 10, wherein the composition is further characterized by one or more features selected from: the composition comprises about 0.1-1 wt% arginine; the composition comprises about 0.01-0.06 wt% adenosine; the composition comprises about 0.05-0.2 wt% carnosine; the skin firming agent is selected from the group consisting of heptapeptide-7, magnesium gluconate, nicotiana benthamiana

hexapeptide-40sh-polypeptide-76, hydrolyzed eragrostis Tef seed extract, and combinations thereof; the skin hydration agent is selected from the group consisting of fruit extract complex, hydrolyzed sodium hyaluronate, niacinamide, jojoba esters, and combinations thereof; the skin barrier agent is selected from the group consisting of jojoba esters, linoleic acid, linolenic acid, squalane and combinations thereof; the antioxidant is selected from the group consisting of tocopheryl acetate, aminopropyl ascorbyl phosphate, epigallocatechin gallate and combinations thereof; or combinations thereof.

13. A method for treating skin, comprising:

providing a composition according to claim 1; and

applying or instructing to apply the composition to skin.

14. The method of claim 13, wherein wherein the method is further characterized by one or more steps selected from: applying or instructing to apply comprises applying once daily or instructing to apply once daily; applying or instructing to apply comprises applying twice daily or instructing to apply twice daily; applying or instructing to apply is for a period of at least about 2 weeks or at least about 1 month; applying or instructing to apply is to skin on a person's neck, décolleté, face, and/or back of hands; applying achieves a beneficial change in skin to which the composition was applied; or combinations thereof.

15. The method of claim 13, wherein wherein the method is further characterized by one or more features selected from: applying achieves or wherein the beneficial change is one or more of the following: a reduced appearance of fine lines or fine wrinkles, an improved appearance of sun damage, an improved appearance of skin firmness, an improved skin appearance, a perceived improvement in skin appearance, an improved evenness in skin tone, an improved hydration of skin, a reduction in redness, an improved skin tone, or a combination thereof; nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the treated skin region compared to cutaneous blood flow in a similar skin region not treated with the composition; nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the

treated skin region compared to cutaneous blood flow in a similar skin region treated with a composition identical in all respects except for the absence of one or more of arginine, adenosine, and/or carnosine; nitric oxide is produced or generated after application of the composition to skin, as evidenced by an increased cutaneous blood flow in the treated skin region for a period of about 2 - 48 hours after application to skin; or combinations thereof.

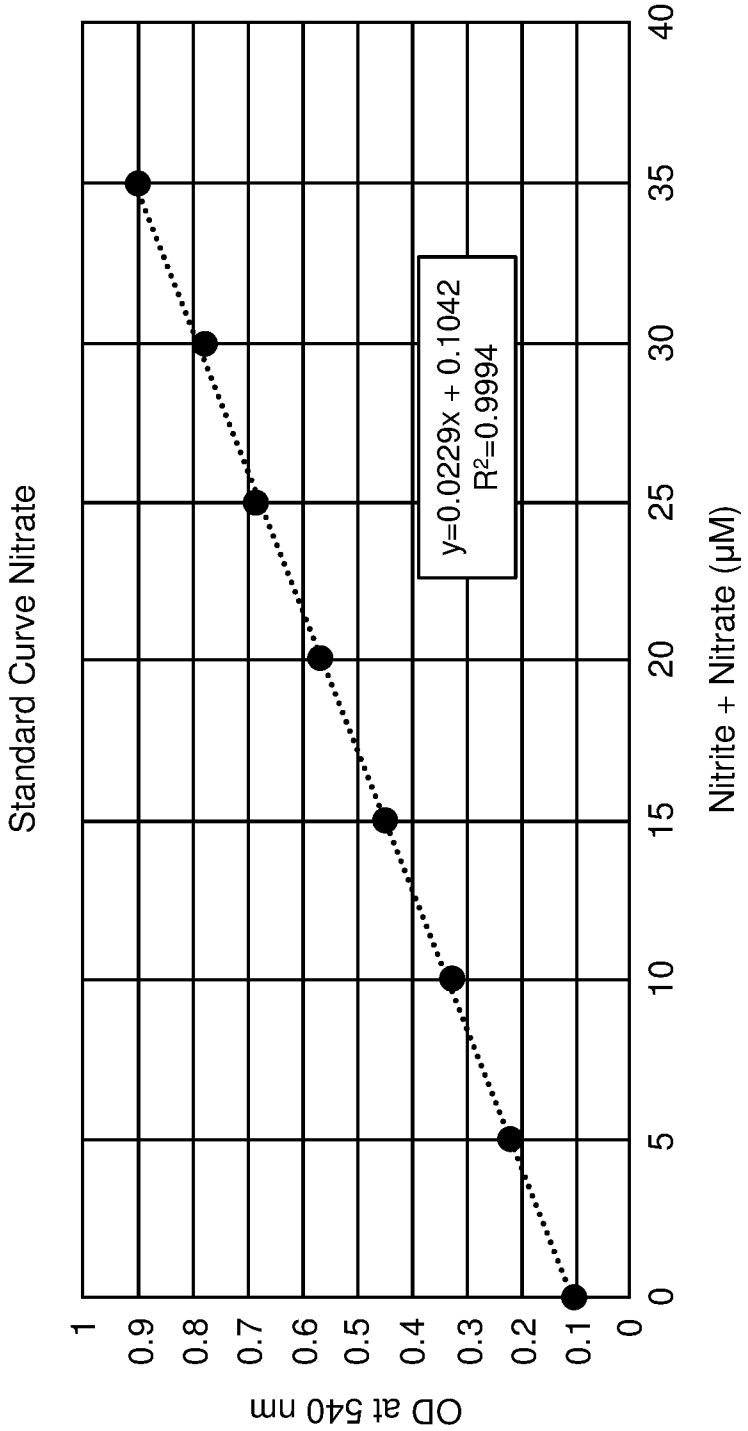

Standard Curve Nitrate
y=0.0229x + 0.1042
R²=0.9994
OD at 540 nm
Nitrite + Nitrate (µM)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
0
5
10
15
20
25
30
35
40

FIG. 1

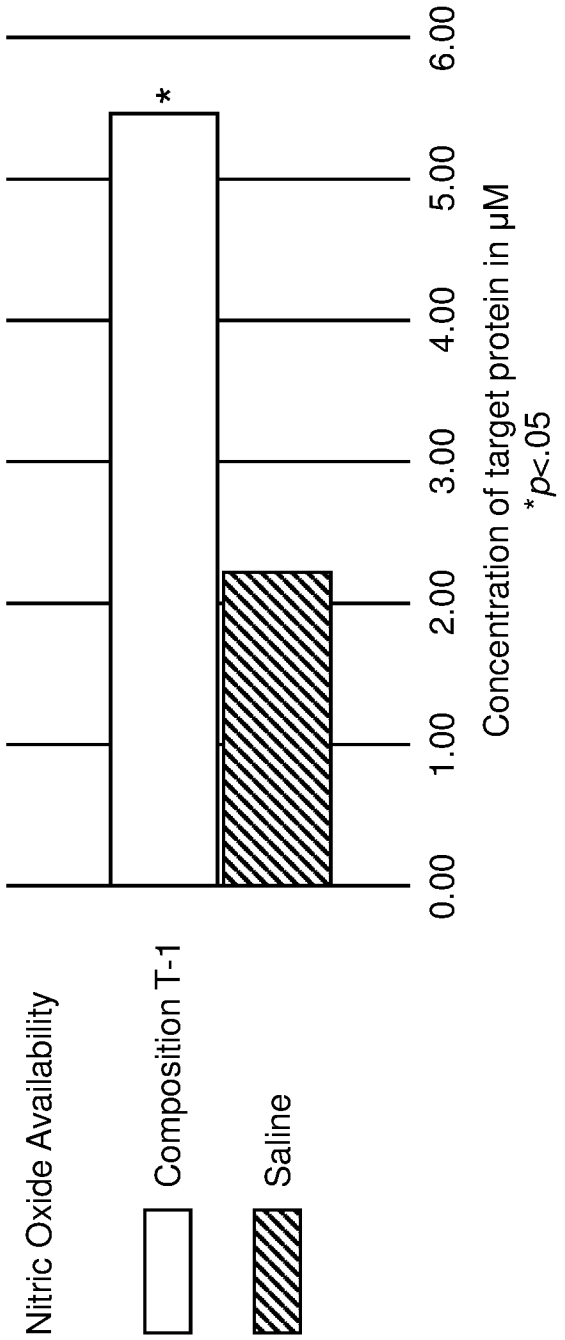
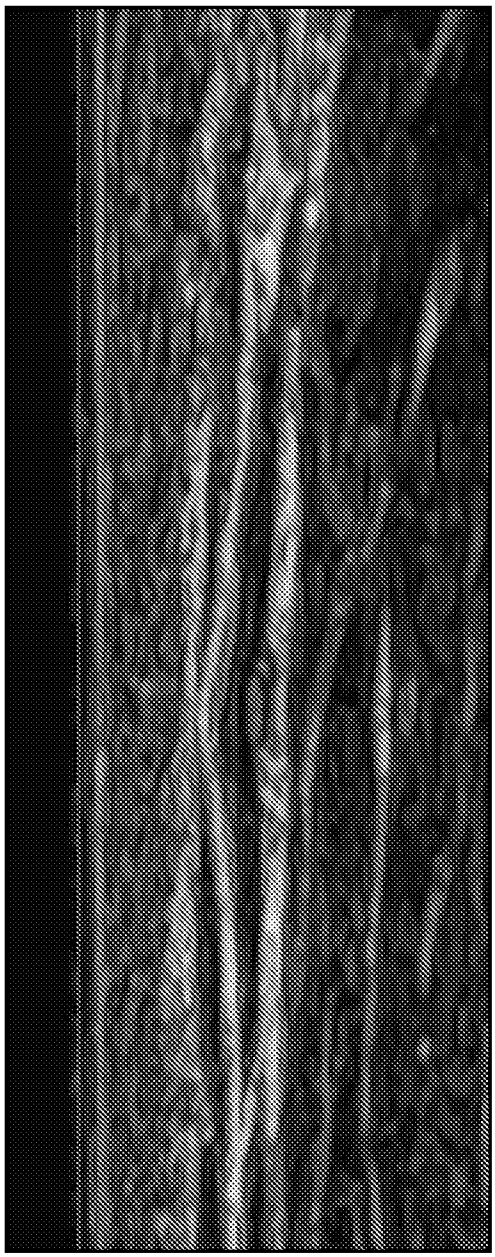
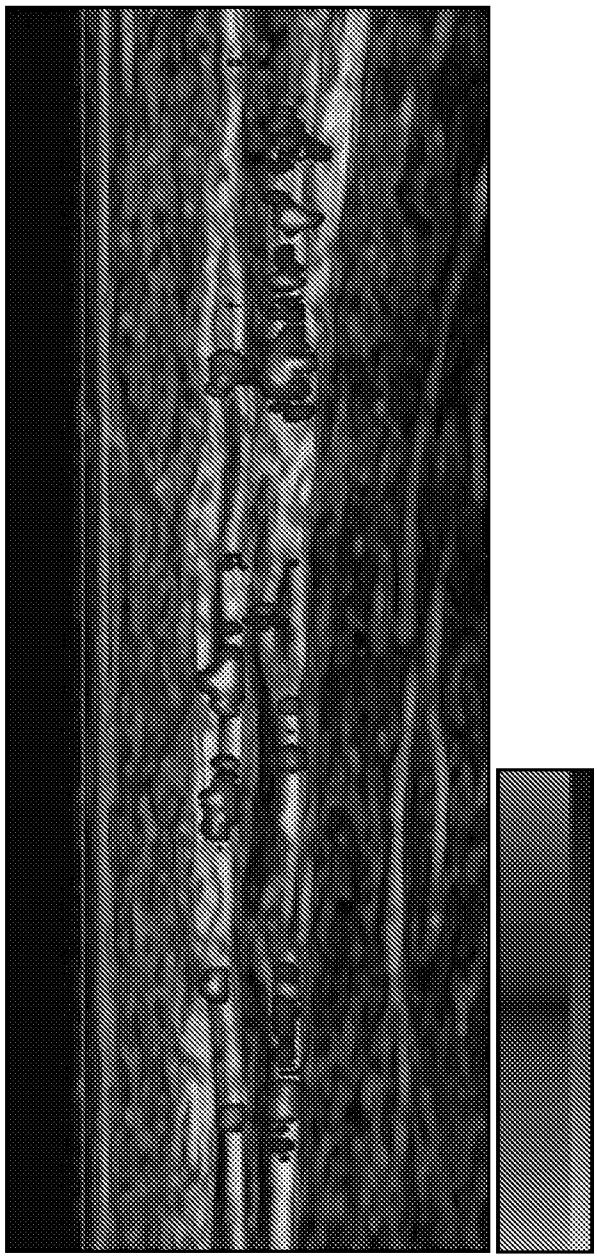

Nitric Oxide Availability
Composition T-1
Saline
0.00
1.00
2.00
3.00
4.00
5.00
6.00
*
Concentration of target protein in µM
*p<.05

FIG. 2



Untreated Control

FIG. 3A



2.5 hrs post application of Composition T-1

Visual change in blood flow

FIG. 3B

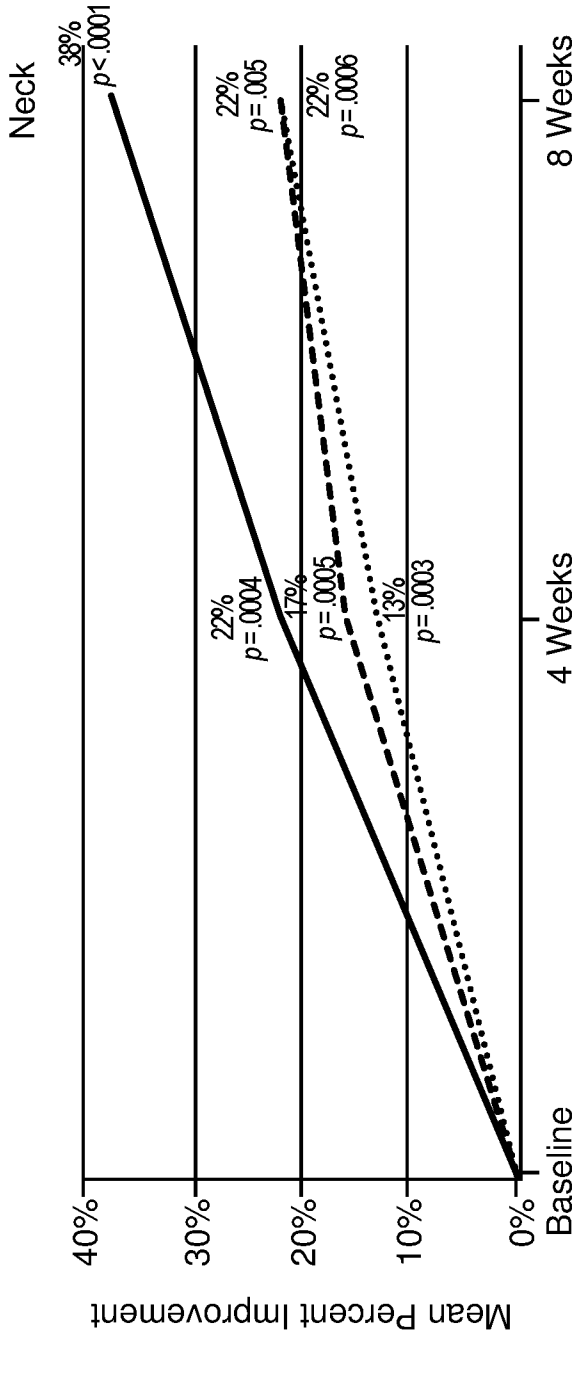

Neck
38%
p<.0001
22%
p=.005
22%
p=.0006
22%
p=.0004
17%
p=.0005
13%
p=.0003
40%
30%
20%
10%
0%
Baseline
4 Weeks
8 Weeks
Mean Percent Improvement
Lines/Wrinkles
Skin Texture
Skin Tone

FIG. 4

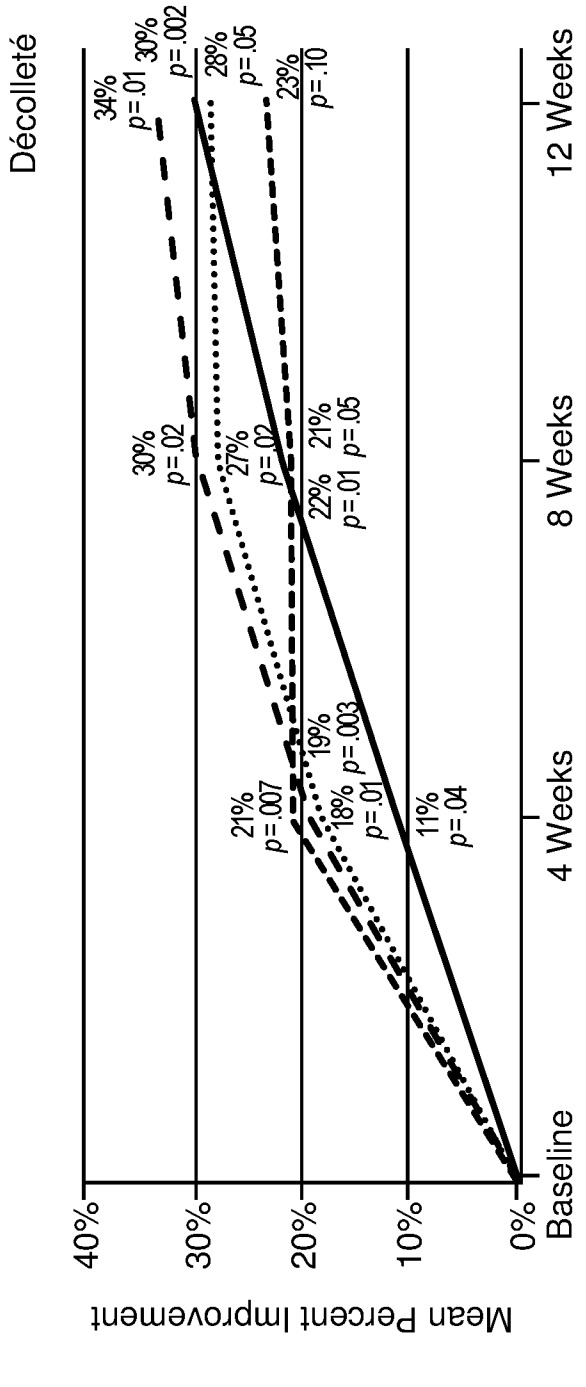

Décolleté
Mean Percent Improvement
40%
30%
20%
10%
0%
Baseline
4 Weeks
8 Weeks
12 Weeks
34%
p=.01
30%
p=.002
28%
p=.05
23%
p=.10
30%
p=.02
27%
p=.02
22%
p=.01
21%
p=.05
21%
p=.007
19%
18%
p=.01
p=.003
11%
p=.04
Lines/Wrinkles
Skin Texture
Skin Tone
Photodamage

FIG. 5